Brosseau, G. E., Jr. University of Iowa, Iowa City, Iowa. Some aspects of ring chromosome behavior in Drosophila.

The somatic behavior of ring chromosomes in corn is well known from the genetic and cytological studies of McClintock (1938). Comparable studies of ring chromosomes in Drosophila are primarily restricted to genetical analysis because of the relatively unfavorable cytological situation found in Drosophila. Rings in corn show mitotic instability which is manifested cytologically by the formation of double bridges, interlocked rings, loss of the ring and changes in ring size. The bridges and interlocks probably result from sister strand crossing over, a single crossover producing a double bridge, 2 crossovers yielding an interlock (sister union as suggested by Hinton, 1959, would produce double bridges but not interlocks). McClintock attributes loss primarily to lagging of the ring chromosome during anaphase. Large rings yield more bridges and changes in size; small rings are more prone to loss by lagging. Changes in size result from breakage of the bridges and fusion of the broken ends. Rings in Drosophila show a greater somatic stability than ring chromosomes in corn. However, both small and large rings in Drosophila show a small frequency of mitotic loss which is manifested by the occurrence of mosaic patches when suitable markers are used. Maternal ageing prior to the introduction of X^{c2} increases the somatic instability of this ring (Brown and Hannah, 1952) and the unusual ring X, $X^{c2}w^{vc}$, shows a high frequency of spontaneous mitotic loss (Hinton, 1955). The few cytological studies which have been done on ring chromosome behavior have not completely elucidated the mechanism of ring loss in Drosophila nor do they permit direct comparisons of ring behavior in corn and Drosophila. The results reported here represent another attempt to study the mitotic behavior of ring chromosomes in Drosophila. While the results are inconclusive, they are presented here for the benefit of others who may be interested in this problem.

The rings studied were the ring X's, X^{c2} and $X^{c2}w^{vc}$, del(1)X^{c2} (an X-ray induced deletion of most of the euchromatin of X^{c2}, cytologically it is about 1/2 the size of the ring x) and the ring Y chromosome of Oster, MYR. The w^{vc} stock had been selected for instability just prior to this study. The X^{c2} series was divided into 2 groups, the mothers were aged as virgins for 12 days prior to introducing the ring in one while they were unaged in the other. Larval brain squash preparations were made by dissecting out the brains in hypotonic citrate and staining in lacto-orcein. The preparations were examined using phase contrast. Prophase and metaphase cells were examined for presence of the ring and for changes in size of the ring. Anaphase and telophase were scored for double bridges, interlocked rings, lagging of the rings and any other abnormalities. There was little difficulty in distinguishing between a double bridge and an interlock. No concommitant studies of somatic mosaicism were made. The data obtained are presented in Table 1.

In general, the frequency of abnormal cells in prophase and metaphase was very low. The 6 XO cells in the w^{vc} series were all on a single slide and probably represented an XO sector arising from an early elimination of the ring. Anaphases and telophases were not scored on this slide. Four instances of changes in ring size were recorded, 2 large rings and 2 small rings. Determination of these changes in size was very subjective as a number of factors such as the compactness of coiling of the ring could give erroneous interpretations. Evidence for change in size of the ring must be considered ambiguous. The frequency of mitotic loss of these rings as measured by XO prophase and metaphase figures is apparently very low.

Examination of anaphase and telophase gave more definite indications of ring chromosome instability. X^{c2}/Y w dl-49 progeny from unaged mothers yielded more interlocks than double bridges indicating that 2 sister strand exchanges are more frequent than a single exchange, if indeed sister strand exchange is the cause of these configurations. Ageing of the mother increases the frequency of both interlocks and double bridges, indicating a correlation between these configurations and ring chromosome loss. This suggestion is not borne out by the results with w^{vc} or MYR. Although w^{vc} yields a high frequency of somatic loss, the frequency of bridges and interlocks was about the same as X^{c2} except that bridges and interlocks were about equally frequent. These observations do not agree with those of Braver and Blount (1949) or of Hinton (1955). No explanation of this discrepancy is a available at this time. In the present case there does not seem to be a good correlation between bridges, interlocks and somatic loss. This conclusion is supported by the MYR results. MYR is generally a mitotically stable chromosome, no mosaics for bw^{+} (the marker on MYR) being observed in eye tissue. Thus the bridge configurations seen with MYR are

unaccompanied by any detectable loss of the ring. These bridges also mean that sister strand crossing over, or whatever event causes them, occurs in heterochromatin as well as euchromatin.

The data reported here do not lend support to the conclusion that bridges and interlocks frequently lead to ring chromosome loss in larval brain tissue. Of course, the fate of bridges in brain tissue may be quite different from their fate in the rapidly dividing cleavage divisions where most of the losses actually occur. As in corn, the loss may be the result of lagging of the ring in anaphase. A few lagging rings were observed with all of the rings except MYR. These cases could be squashing artifacts although the author feels that the observed lagging chromosomes are bona fide instances of chromosome lagging.

In the last case, del(1)X^{c2}, no bridges or interlocks were found. There were 3 instances of lagging of the small ring; in one of these both rings were found at the same pole. The column headed other in table 1 includes instances of stickyness (5), single bridges of undefined origin, but not involving the ring (2), and 4 bridges of undefined nature in the MYR series.

These observations on ring chromosome behavior in Drosophila indicate that these rings act much like the rings in corn. The difference in the observed frequencies of mosaicism in these 2 species is probably due to different fates of the bridges and interlocks. In corn (as in other plants) the growth of the cell wall ruptures the bridge and fusion of the broken ends occurs. In Drosophila there is no cell wall and the bridges may often fail to break. Changes in the size of rings, if they in fact occur, would be evidence for breakage of at least some of the bridges. Hinton (1959) presents cytological evidence that some bridge breakage may occur in cleavage divisions. No clear evidence of bridge breakage was seen in the present study. The fate of cells with either broken or unbroken bridges is uncertain. This condition may cause the death of the cells or perhaps prevents their further division.

The mechanism of ring chromosome loss in Drosophila remains to some extent unclear. Hinton's (1959) finding that one or both ends of a bridge is occasionally not included in the late anaphase or telophase group lead him to conclude that anaphase bridges were the main cause of ring chromosome loss. In the present case, most of the bridges were observed to have both ends at the poles. The few exceptions may have been squashing artifacts; there is no way to be certain. Since loss of ring chromosomes by lagging and by bridge formation are not mutually exclusive events, they may both contribute to loss. It remains to be demonstrated whether one or the other of these events is the principal cause of ring chromosome loss in Drosophila. (Supported by research grant GM-06508 from NIGMS, USPHS)

References: Braver, G. and J. L. Blount, 1949, Rec. Genet. Soc. Amer. 18:78
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Table 1

The frequency of cytologically apparent abnormalities associated with ring chromosomes

Genotype	Prophase + metaphase: Number of cells	Number XO	Large rings	Small rings	% normal cells
1. X^{c2}/y w dl-49 ♀ (unaged)	196	0	1	0	99.5
2. X^{c2}/y w dl-49 ♀ (aged)	143	0	1	0	99.3
3. $X^{c2}w^{vc}$/y w lz dl-49 ♀	150	6	0	2	94.9
4. del(1)X^{c2}/y:= ♀	51	1	0	0	98.1
5. y v/MYR ♂	198	1	0	0	99.5

Genotype	Number of cells	Anaphase + telophase: Double bridges	Inter-locks	Total bridges	% bridges	Lagging	Other
1. X^{c2} (unaged)	150	1	8	9	6.0	1	1
2. X^{c2} (aged)	161	9	18	27	16.8	2	1
3. $X^{c2}{}_{w}{}^{vc}$	317	6	4	10	3.1	3	5
4. $del(1)x^{c2}$	118	0	0	0	0	3	0
5. MYR	225	7	2	9	4.0	0	4

Nagle, James J. North Carolina State University, Raleigh. A study of intra- and interspecific polymorphism.

Chromosomal polymorphism has recently been found in populations of Race B of D. mojavensis which occur in Baja, California and Sonora, Mexico (DIS 38:58). This polymorphism involves Chromosome pairs 2 and 3 of the six constituting the karyotype of the species. In Chromosome 2 a simple paracentric inversion distinguishes the Standard (ST-2) from the LaPaz (LP) banding sequence, and a simple paracentric inversion in Chromosome 3 distinguishes the Standard (ST-3) from the Mulege (MU) arrangement. Analyses made from recent collections (courtesy of Dr. W. B. Heed) and from laboratory populations established from previous collections revealed ST-2 to be in low frequency (5-10%), while ST-3 and MU occurred in about equal numbers.

Two cage populations were initiated with equal proportions of males and females of D. mojavensis (Race B) and D. arizonensis, a closely related species. Cytological analyses indicate that mojavensis is replacing arizonensis, although limited hybridization (2-5% recombinant types per generation) is occurring. The intraspecific polymorphism of mojavensis has been maintained over the period of competition with arizonensis. The mean percentage of the second and third chromosome types within mojavensis, based on thirteen samples of each population (over 750 days), is given in Table 1.

Table 1. Mean percentages of the second and third chromosome types of mojavensis.

Chromosome Number	Banding Sequence	Percentage: Population 1	Population 2
2	ST-2	4.7	9.6
2	LP	95.3	90.4
3	ST-3	47.8	48.5
3	MU	52.2	51.5

A third population was initiated with male and female F_1 interspecific hybrids. In this case the chromosomes of mojavensis were obligatorily in heterozygous combinations with those of arizonensis at the beginning of the population. Table 2 gives the percentage of mojavensis second and third chromosomes observed in ten samples of the "hybrid" population. The proportions of ST-2 and LP have remained near the equilibrium frequencies observed in the mojavensis stocks, as given above. On the other hand, the percentages of ST-3 and MU deviate significantly from the 50-50 proportions observed in the laboratory populations of mojavensis (Table 1). Specifying no selection, the F_1 was expected to consist of equal amounts of ST-3/AR-3 combinations. The large deviation from this expectation, especially in the earlier samples, indicates that the combination ST-3/AR-3 has a much higher adaptive value than MU/AR AR-3. These data support the concept of fitness relativity, as put forth by Levene, Pavlovsky and Dobzhansky (1954, 1958). The intraspecific polymorphism, presumably based on heterosis of the ST-3/MU heterokaryotype, is greatly upset when subjected to a new genetic milieu; a novel chromosomal homologue, AR-3, is superimposed upon the intraspecific polymorphic system through hybridization. A new polymorphic condition is now being approached, seemingly due to ST-3/AR-3 heterokaryotypic superiority, with the apparent elimination of the MU arrangement despite any advantage it has in combination with the ST-3 chromosome.