

SOMATIC CROSSING-OVER AND ELIMINATION OF RING X CHROMOSOMES OF *DROSOPHILA MELANOGASTER*¹

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THE present study had its origin in the observation of DR. ALOHA HANNAH-ALAVA that combinations of ring and rod X chromosomes would, if appropriately marked, yield frequencies of somatic mosaicism very much greater than those obtained with somatic crossing-over of two rod chromosomes. Later BROWN and WELSHONS (1955) reported a frequency of mosaicism of about five spots per abdomen of flies heterozygous for a marked rod and a ring, $\gamma ac sn^s/X^{c2} + + +$; this frequency was eight to ten times greater than that observed with two rods, $\gamma ac sn^s/+ + +$, in the same series.

Ring chromosomes are expected to give rise to mosaicism in *Drosophila* in two ways. Loss of the ring will give X0 tissue which may be recognizable as male tissue if occurring in a sexually dimorphic region. The production of gynandromorphs by this process has been reviewed and described by BROWN and HANNAH (1952) and HANNAH (1953). Somatic crossing-over between a rod and ring was first reported by STERN (1936) who used the ring chromosome, $X^{c\gamma}$; in this case both yellow spots and twin spots were observed, proving that crossing-over rather than elimination of $X^{c\gamma}$ was responsible for much of the mosaicism.

In the experiment of BROWN and WELSHONS (1955), most of the mosaicism observed with the ring and rod combination consisted of small spots which included only one bristle. Only the end of the abdomen is sexually dimorphic and even there the small spots could not be classified with certainty as to whether they were male, X0, or female, XX tissue, as would be expected following elimination of the ring X, or somatic crossing-over between the rod and the ring, respectively.

The present work was undertaken to determine the process responsible for the high frequency of mosaicism in the rod-ring combination. Either the ring was being eliminated at a very high frequency, or somatic crossing-over was occurring at an unexpectedly high rate, or both processes were making large contributions.

The present experiments were thus designed to partition the yield between the two processes. The same culture and technical methods as described previously

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were used (BROWN and HANNAH 1952; BROWN and WELSHONS 1955). No attempt was made to control variability through special control of the environment or use of isogenic lines (BROSSEAU 1957; WEAVER 1960). The results obtained from a variety of stocks would be either internally consistent or they would not, and further refinement would be necessitated for the present purposes only if they were not.

RESULTS

The percent mosaicism of several trials with rod/rod combinations forms the best basis of comparison for the rod/ring series and a summary of all the results from this laboratory will be so used. These summaries include both those results cited by BROWN and WELSHONS (1955) and those made at the time of the present experimental work (Table 1) as well as a number of others. They also include results from progeny of mothers aged and unaged before mating as such aging has little or no effect on the incidence of somatic crossing-over (BROWN and WELSHONS 1955).

A total of 468 abdomens of females heterozygous for $\gamma ac sn^3/+++$ yielded 232 spots, or a percent mosaicism of 49.6. A total of 11 cultures, from which were taken 30 to 100 abdomens each, were included. The percentages for the individual cultures ranged from 13.3 to 70.0 and their unweighted average was 47.0 percent.

A total of 490 abdomens of females heterozygous for $\gamma ac +++ sn^3$, or reciprocal, yielded 338 spots, or 69.0 percent, including 11 percent twin spots.

TABLE 1

Mosaicism in various combinations of rod and ring chromosomes

Series number		Percent mosaics Unaged	Aged	Average for series	Components†
Rod-rod					
1. Coupling	$\gamma ac sn^3/+++$	52	61
2. Repulsion	$\gamma ac +++ sn^3$	43	51
Rod-ring					
Coupling					
3. Recessives on rod	$\gamma ac sn^3/X^{c2}+++$	524	584	554	e + c
4. Recessives on ring (reciprocal)	$X^c \gamma sn^3/+++$ $+++/X^c \gamma sn^3$	241 261	200 225	} 232	c
Repulsion					
*5. With X^{c2} (reciprocal)	$\gamma ac +++/X^{c2}+++ sn^3$ $X^{c2} +++ sn^3/\gamma ac +++$	478 (286)	524 (340)	} 506	e + 2c
*6. With $X^c \gamma$	$+++ sn^3/X^c \gamma +++$	467 (213)	484 (208)		
Ring-ring					
7. Coupling (reciprocal)	$X^c \gamma sn^3/X^{c2}+++$ $X^{c2} +++/X^c \gamma sn^3$	775 1107	927 739	} 887	e + 2c
8. Repulsion (reciprocal)	$X^c \gamma +++/X^{c2}+++ sn^3$ $X^{c2} +++ sn^3/X^c \gamma +++$	1395 1748	1243 1460		

* Percentages of mosaicism due to elimination, e, were calculated for these series and cited in parentheses; see text for further explanation.

† e = percent mosaicism due to elimination of ring. c = percent mosaicism due to somatic crossing-over in coupling phase in rod/ring heterozygotes; see text for further explanation.

There were a total of 14 cultures, again with 30 to 100 abdomens each. The percentages for the individual cultures ranged from 43.0 to 156.7 and their unweighted average was 72.9 percent.

A variety of corrections can be made for these percentages, some of which cancel out for comparative purposes. For example, not all bristles on sn^s/sn^s abdomens show the singed phenotype sufficiently well to be recognizable as such if they were present singly or in small groups as a mosaic spot among otherwise wild-type bristles. Also, γ is not completely autonomous (HANNAH 1953) and those yellow bristles with darker than expected coloration might well not be detected in a mosaic patch surrounded by wild-type bristles. In the coupling series, the presence of either a detectably yellow or singed condition would assure the recognition of the spot while in the repulsion series the presence of a bristle of either sort would increase the likelihood of the detection of the companion type if these were occurring together in a twin spot.

In general, the repulsion series should yield about twice as many spots as the coupling series since both products would be detectable in the former but only one in the latter. More precisely, the percentages for coupling and repulsion, C and R , can be separated into two components, $C = C' + t$ and $R = R' + t$ in which C' and R' are the percentages of simple mosaic spots and t = percent mosaics in which both products of the cross-over lead to cell lineages in the hypoderm. The " t " mosaics would appear as twin spots in the repulsion phase but would not be distinguishable from the remainder in the coupling phase. Thus $2C' = R'$, and $2C = R' + 2t = R + t$. The latter expression would then give a total of 80 percent (69 percent plus 11 percent twin spots) for the repulsion series, and half of this value, 40 percent, may be compared with the 49.6 percent for the coupling series. Considering the possible sources of error mentioned in the preceding paragraph and the wide range of frequency, the agreement between the two series is fairly good. Combining these two sources of information, we may therefore conclude that two rod chromosomes will yield about 50 percent mosaicism in the coupling, 100 percent in the repulsion phase.

Crosses were made, and frequently their reciprocals, to give the various combinations of rod and ring chromosome heterozygotes in coupling and repulsion phase. In addition to other common laboratory stocks, two different ring chromosomes were used, X^c , in which γ is completely linked with the ring structure, and X^{c2} , with similar linkage of γ^+ . Parallel series were run of matings with unaged females and those that had previously been aged 10–12 days; the total tally included 2300 mounted abdomens which were then scored for mosaicism.

The results of these crosses are summarized in Table 1. The effects of aging involved only the larger spots which formed only a small fraction of the total number of mosaic patches; consideration of these will be given below.

The rod/rod cultures set up for this experiment were both well within the range of variation reported above; that for the coupling phase was somewhat higher, for the repulsion phase considerably lower than the overall averages.

When both genetic markers are placed on the ring (Table 1, no. 4), elimination of the ring will not yield mosaicism for the genetic markers; mosaicism

should result only from somatic crossing-over. The observed values for crossing-over, therefore, varied from 200 to 261 percent, outside the range of those for the coupling phase rod/rod combinations and the average for the four cultures was 232 percent.

When both genetic markers are placed on the rod, series 3, mosaicism can arise from both crossing-over and elimination of the ring, and 554 percent mosaicism appeared in these cultures. For these two series, no. 4 has only the component, *c*, expected from crossing-over while no. 3, has both component *e* from elimination and *c* from crossing-over.

When one genetic marker is on the ring and one on the rod, series 5 and 6, the mosaicism due to elimination of the ring would remain the same whereas that due to crossing-over would be doubled since both products of a cross-over would be detectable. The components of mosaicism would thus be $e + 2c$. The correction for twin spots has been omitted since these occurred in only a small percentage in the ring heterozygotes. In these cases, the markers on the ring would appear only after somatic crossing-over while those on the rod would appear after both somatic crossing-over and elimination of the ring. As expected, the mosaic spots showed a great excess of the rod markers. Doubling the percentage of mosaicism of the ring markers thus gave an approximation of that due to crossing-over, and subtracting this from the total gave the percentage due to elimination of the ring (Table 1, series 5 and 6, parentheses).

When two rings are used with the markers in coupling phase (series 7), elimination of only one of the rings will yield mosaicism and thus there will be but a single *e* component. However, if *c* is the component attributable to crossing-over of one ring with a rod, the combination of two rings might be expected to give twice this value, or a component due to crossing-over of $2c$. With two rings in repulsion phase (series 8), the mosaicism due to elimination would be doubled to $2e$ since loss of either ring could produce a spot; that attributable to crossing-over should be twice that of the same chromosomes in coupling phase or $4c$ (the correction for twin spots again omitted).

Estimates of the amount of somatic crossing-over were calculated in several ways and all gave about the same order of magnitude, with a considerably higher average and no overlap of range when compared in the equivalent phase, coupling or repulsion, with the rod/rod combinations. Table 2 presents the results of the simplest method of making the calculations which was to use the average value of *e* as derived from series 5 and 6 as a constant for all the series. On the basis of the calculated values of *c*, mosaicism due to somatic crossing-over of the ring varied from 95 to 301 percent in the coupling phase with an average of 205 percent. For the coupling phase the rod/rod combinations varied from 13 to 70 percent and their average was slightly less than 50 percent. For the repulsion phases with the ring, the above values may be doubled, 190 to 602 percent, average, 410 percent, and compared with a range of 43 to 157 and an average of 73 percent for the rods (or 100 percent if the value for the coupling phase is doubled). There is thus about four or five times more mosaicism produced by somatic crossing-over with a ring than with a rod.

TABLE 2

Calculated percent crossing-over for ring chromosome combinations

Series	Avg. total percent mosaicism	Components*	Percent elim. †	Percent c.o.	c*
3	554	e + c	284	270	270
4	232	c	...	232	232
5	506	e + 2c	284	222	111
6	475	e + 2c	284	191	95
7	887	e + 2c	284	603	301
8	1461	2e + 4c	568	893	223
avg.					205

* e = percent mosaicism due to elimination of ring, c = percent mosaicism due to somatic crossing-over in coupling phase in rod/ring heterozygote; see text for further explanation.

† Held constant at the average value calculated from series 5 and 6, Table 1.

Although the total mosaicism was not apparently influenced by aging the mother prior to mating, the proportion of large patches attributable to ring loss during early development was greater in the aged than the unaged series. The unaged ring series produced 1.9 percent spots with 32 or more bristles while the aged yielded 3.5 percent. These results border on statistical significance and fall within the range of 0–1.6 percent gynandromorphs from unaged mothers and 2.0–8.0 percent from aged mothers reported by BROWN and HANNAH (1952). The above results also conform to those of BROWN and WELSHONS (1955) who reported that maternal aging, as might be expected, increased the frequency only of eliminations occurring relatively early in development.

DISCUSSION

As mentioned above, ordinary laboratory stocks were used in the present experiments. It has been shown by STERN (1936) that Minutes will influence somatic crossing-over and by WEAVER (1960) that probably most chromosomes of *Drosophila* carry genes which will tend to increase or decrease the frequency of somatic crossing-over. Genes of the latter sort would be expected to be floating at random in laboratory populations. Further control of the process can be obtained by very strict attention to the environment (BROSSEAU 1957). Results obtained by narrow control of the genotype and environment are valid only under the specified circumstances. Because the frequency of somatic crossing-over has been long noted for its erratic fluctuations, work with a typical series of laboratory stocks seemed to offer the best promise of obtaining results of general reliability. The extent of fluctuation of the estimated values for ring chromosome somatic crossing-over was roughly equivalent to that observed for the rods.

As previously reported by BROWN and WELSHONS (1955), most of the mosaicism with the ring chromosome combinations consisted of small spots, the great majority of which contained but a single bristle. Although the frequency of mosaicism with rings was much higher than with rods alone, the average size of the spots was quite reduced. STERN (1936) has diagrammed some of the possible consequences of crossing-over with a ring chromosome. In general, such

events lead to the formation of dicentric chromosomes and subsequent anaphase bridging. If bridging were to interfere mechanically with the mitotic process, or broken bridges lead to reduced viability of the recipient cells, then the resultant mosaics might be reduced in size or confined to terminal phases in development. In this case, estimates of the potentiality of ring chromosomes for somatic crossing-over would be minimal. On the other hand, the ring chromosomes may carry determiners, perhaps in the centric heterochromatin, which shift the time in development at which somatic crossing-over occurs; a shift from an early to a later developmental stage would change the pattern of mosaicism from fewer, larger spots, to more numerous, smaller spots (see also BROSSEAU 1957, for discussion of developmental relationships).

In the calculations of the c values, it was assumed that no interaction occurred, that a ring chromosome would give about the same frequency of crossing-over whether combined with a rod or with another ring. This assumption is undoubtedly invalid because a crossover involves two chromosomes and the nature of each would be expected to influence the process. Furthermore, the amount of mosaicism yielded by a normal rod chromosome can be drastically reduced by combining it with certain altered rods (WALEN, unpublished); therefore, the percentage of mosaicism is not, as was assumed in the calculations of c , always that of the more effective member of the pair of homologues. Other estimates of c , based on the assumption of an average effectiveness in the rod/ring heterozygotes, were somewhat higher than those reported above and the average c value of series 3-6 agreed better with that of 7 and 8. This procedure was itself also unfair because rods yield larger mosaic patches than do rings or ring combinations. Undoubtedly a further understanding of the process of somatic crossing-over itself will be necessary before an accurate estimate may be made of the rod/ring interaction. Suffice it to say for the present that the assumption of no interaction leads to the more conservative, or minimal estimates of c herewith reported.

The major question arising from these results is that of the reason ring chromosomes should show an enhanced frequency of crossing-over, roughly four or five times that of the rods. Two alternatives may be suggested. Ring chromosomes may be under extra tension because of their shape and this extra tension may lead to a greater likelihood of the formation of unions with homologous chromosomes. Such internal forces have long been suggested as playing a role in the mechanism of crossing-over. On the other hand, the ring chromosomes used in these experiments have more extensive centric heterochromatin than the structurally normal rod X chromosome. STERN (1936) found that the greater part of somatic crossing-over occurred adjacent to the centromere. If somatic crossing-over does take place largely in heterochromatin then increasing the amount of heterochromatin, as in the rings, would be expected to increase its frequency. The present data do not allow discrimination between these alternatives but experiments specifically designed to test this point are being performed by one of us, WALEN, and the results will be reported later.

SUMMARY

Ring X chromosomes yield somatic mosaicism either by somatic crossing-over or by their elimination to reveal recessives carried on the homologous chromosomes.

Crosses of various combinations of appropriately marked ring and rod chromosomes enabled estimates to be made of the frequency of elimination and crossing-over of the rings. In addition to frequent elimination, a ring chromosome will produce from somatic crossing-over about five times as much mosaicism as a rod. A maximum average mosaicism of 15 spots per abdomen resulted from the combination of two ring chromosomes each appropriately marked.

The ring shape itself, which might lead to greater internal tension in the chromosome, and the extra heterochromatin carried by the ring are suggested as two possible reasons for the increased incidence of somatic crossing-over with the ring.

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LITERATURE CITED

- BROSSEAU, G. E., JR., 1957 The environmental modification of somatic crossing-over in *Drosophila melanogaster* with special reference to developmental phase. J. Exptl. Zool. **136**: 567-593.
- BROWN, S. W., and ALOHA HANNAH, 1952 An induced maternal effect on the stability of the ring-X-chromosome of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **38**: 687-693.
- BROWN, S. W., and W. WELSHONS, 1955 Maternal aging and somatic crossing-over of attached-X chromosomes. Proc. Natl. Acad. Sci. U.S. **41**: 209-215.
- HANNAH, ALOHA, 1953 Non-autonomy of yellow in gynandromorphs of *Drosophila melanogaster*. J. Exptl. Zool. **123**: 523-560.
- STERN, C., 1936 Somatic crossing-over and segregation in *Drosophila melanogaster*. Genetics **21**: 625-730.
- WEAVER, ELLEN, 1960 Somatic crossing-over and its genetic control in *Drosophila*. Genetics **45**: 345-357.