THE BEHAVIOR OF AN UNSTABLE RING CHROMOSOME IN *DROSOPHILA MELANOGASTER*

Thesis by

Claude Willey Hinton

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1954

ACKNOWLEDGEMENTS

It has been an especial privilege to pursue my graduate studies under the direction of Professor A. H. Sturtevant; I am indebted to his ideal example combining the naturalist's spirit and the scholar's responsibility. Dr. E. Novitski, now at the University of Missouri, introduced the problem of ring chromosome instability to me, and his continued interest in my work is sincerely appreciated. The critical approach of Dr. E. B. Lewis has proven most valuable in the interpretation of various results.

I have received generous financial support as provided by the Atomic Energy Commission Predoctoral Fellowship (three years) and the United States Public Health Service Research Fellowship (one year).

ABSTRACT

The behavior of "Catcheside's ring," an unstable closed-X chromosome (w^{vc}) of _Drosophila melanogaster_, has been analyzed with the purpose of identifying the factors controlling the variable level of w^{vc} instability and of determining the mechanism of w^{vc} elimination. The Y chromosome and the structure of the homologous rod-X chromosome were shown to have no influence on the frequency of w^{vc} elimination. Since no segregating autosomal or sex-linked modifiers could be detected, the primary control of w^{vc} instability must be limited to the w^{vc} chromosome itself. The behavior of unstable small duplications derived from the w^{vc} chromosome suggests that the locus of w^{vc} instability must be in or near the w^{vc} centromere region.

The frequency of w^{vc} elimination is directly related to developmental temperature and to age of maternal w^{vc} parents. Certain preliminary results incurred the speculation that a cytoplasmic factor is operative in w^{vc} elimination.

Either anaphase lagging or the production of anaphase bridges by the w^{vc} chromosome will account for its loss. The occurrence of w^{vc} derivatives, deficient for either extensive or small euchromatic segments, suggests that anaphase bridges composed of continuous dicentric rings may be formed; however, such bridges do not necessarily constitute the exclusive means of elimination.

TABLE OF CONTENTS

Introduction

From their analyses of gynandromorphs in Drosophila melanogaster, Morgan and Bridges (1919) and L. V. Morgan (1929) concluded that, with few exceptions, such individuals could be explained by loss of one of the X chromosomes during development of female zygotes to produce adult mosaics of XO (male) and XX (female) tissues. Loss of the normal rod shaped X chromosome occurs only to the extent that 2.8 gynandromorphs per 10^4 females were recovered by Bonnier and Lüning (1952). Ring shaped or closed-X chromosomes, on the other hand, are much more susceptible to loss since Mrs. Morgan (1926) found, in the case of X^{c1}, a "high percentage of gynandromorphs." For X^{c2}, the incidence of gynandromorphs varies between 5.7 and 6.8 per 10^3 heterozygous females (Battacharya, 1950, Braver and Blount, 1949), and this frequency may be increased tenfold by aging maternal rod-X parents prior to mating to X^{c2} males (Brown and Hannah, 1952). Mrs. Morgan suggested that because of its shape, anaphase movement of the ring chromosome is retarded so that it is sometimes omitted from a daughter nucleus. However, lagging of the ring chromosome would not be expected to produce the anaphase bridges observed in divisions of larval neuroblasts by Braver and Blount (1949). Anaphase bridges leading to ring loss might be produced by uncompensated twists in the plane of ring chromosome reproduction as postulated by Griffen and Lindsley (1946), or by sister strand crossing over as proposed by Brown and Hannah (1952) following McClintock's cytogenetic study (1938) of somatic ring chromosome behavior in maize.

The present investigation concerns an X chromosome of Drosophila *melanogaster* known as "Catcheside's ring" whose stability is highly variable. In some lines of Catcheside's ring, the frequency of elimination is no higher than that characteristic of X^{c2} from which it was derived; in other lines, as many as 50 percent of the female zygotes experience ring loss during development. Thus the nature of the variable controlling the rate of ring chromosome loss must be added to the problem of the mechanism of ring chromosome loss, and the results describing the behavior of Catcheside's ring will be presented from this viewpoint.

The Origin and Probable Structure of Catcheside's Ring

In considering the origin and structure of Catcheside's ring, it is first necessary to recall the probable origin of attached-X chromosomes, namely, by exchange between the arms of the Y chromosome and the homologous basal region of the X, so that the attached-X carries the Y centromere (L. V. Morgan, 1938). The structure of X^{c2}, as observed in the salivary gland chromosomes by Schultz and Catcheside (1937), is consistent with the hypothesis that it arose by union of the distal end of one member of an attached-X pair with the basal region of the other member producing a minute deficiency for the tip of the X chromosome and a duplication for the basal heterochromatic region.

After X-raying X^{c2}, Catcheside (unpublished; but see Catcheside and Lea, 1945) recovered a chromosome which retained the ring structure but, in addition, exhibited variegation for the white, roughest and Notch loci (figure 1). This new variegated phenotype indicated that an inversion had been produced in X^{c2} removing these loci from their normal position to a new position adjacent to heterochromatin, and Catcheside's ring has therefore received the designation $In(1)X^{c2}, w^{vc}$. It may be

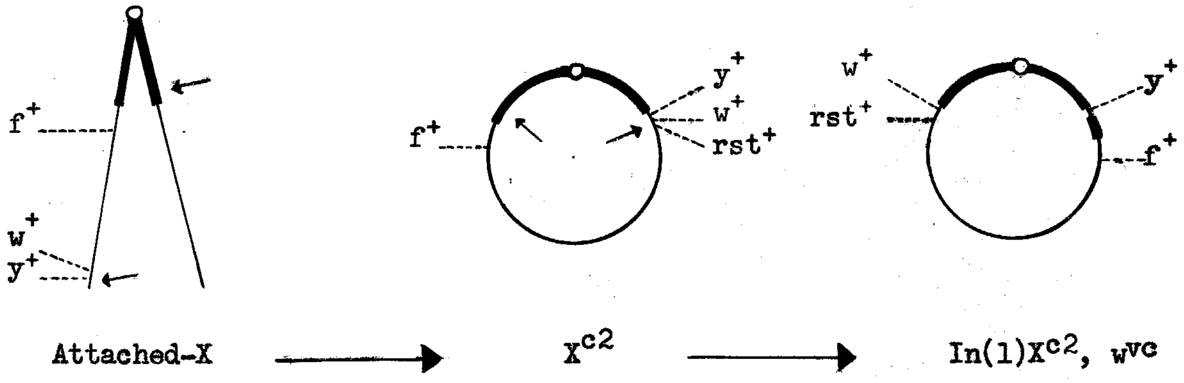

Figure 1. Diagrammatic representation of the origin and probable structure of Catcheside's ring. The centromeres are denoted by small open circles, and the heavy and light lines represent heterochromatin and euchromatin, respectively. Small arrows indicate the approximate points of breakage.

pointed out that cytological determination of the exact breakpoints is virtually precluded because of the heterochromatic complexity; however, the variegation pattern suggests that one break occurred just to the left of the white locus and the other occurred in heterochromatin to produce the heterochromatic type of position effect on w, rst, and N, but not on loci normally to the left of w.

Unfortunately, it is not known when Catcheside's ring became unstable. After its recovery at the California Institute of Technology, Catcheside's ring was maintained in the Carnegie Institution of Washington stocks at Cold Spring Harbor, New York. The first recorded instance of instability appears in the report of Griffen and Lindsley (1946; Lindsley, personal communication). It may be supposed that the unstable condition of Catcheside's ring arose as an unrecognized concomitant of its structure;

however, the fact that Catcheside's ring stabilizes without apparent changes in its structure obviates the necessity of this supposition.

The Crossing Behavior of Catcheside's Ring

Except where specific references are made, the mutants and chromosomes employed in this study are described by Bridges and Brehme (1944). The symbolic designation $In(1)X^{c2}$, w^{vc} for Catcheside's ring will be abbreviated hereafter as w^{vc}.

The matings and results presented in table 1 have been chosen to illustrate the typical crossing behavior of w^{vc} under conditions of low and high instability. Each culture represents the production of a single female during a standard 7 day egg-laying period at 25°C. The P_1 w^{vc} f/dl-49, y w lz^s females were obtained as virgins from a stock culture and outcrossed to dl-49, y Hw m^2 g^4 males. The F_2 and F_3 generations were produced by inbreeding w^{vc} females by their rod-X sibs. The regular male classes of offspring are not enumerated since they provide no useful information; it is pertinent to note, however, that viability of w^{vc} males was extremely erratic, varying with the level of instability and Notch variegation. The following discussion of the data of table 1 includes methods of analysis and general considerations of w^{vc} elimination.

Loss of the w^{vc} chromosome from part of a w^{vc} f/dl-49, y Hw m^2 g^4 zygote (F_1 and F_3 of table 1) was registered by the recovery of a gynandromorph (G) whose male tissues displayed the phenotype of the recessives yellow, miniature and garnet in contrast to the wild type phenotype of the female parts. The mutant yellow, which affects all hypodermal structures, was used extensively in conjunction with

Table 1

Comparison of the progenies of single females in lines of low and high w^{vc} instability. The cultures grouped in brackets represent the progenies of sister females. Both the y w lz^s and y Hw m^2 g^4 chromosomes carried the dl-49 inversion.

	Low Instability						High Instability						
	C#	y♀	w^{vc}♀	G	P	%GP	C#	y♀	w^{vc}♀	G	P	%GP	
P_1 w^{vc} f/y w lz^s x y Hw m^2 g^4													
F_1	4500	51	39	3	0	7.1	4497a	91	27	15	7	44.9	
F_1 w^{vc} f/y Hw m^2 g^4 ⓧ y w lz^s							4562	84	45	23	2	35.7	
							4564	111	48	23	21	47.8	
F_2	4573	98	99	1	1	2.0	4566	48	15	10	2	44.4	41.4
							4565	83	30	19	11	50.0	
							4584	100	42	11	5	27.6	
							4785	103	72	7	5	14.3	
F_2 w^{vc} f/y w lz^s ⓧ y Hw m^2 g^4							4786	112	41	14	8	34.9	27.1
							4787	127	44	13	3	26.7	
	4804	102	80	0	0	-	4788	108	42	14	10	36.4	
F_3	4805	107	92	1	2	3.1							
	4806	78	105	5	1	5.4	4775	101	21	11	12	52.3	
	4807	56	40	1	1	4.8	4776	162	55	11	15	32.9	
							4777	111	20	13	9	52.4	41.5
							4778	109	46	33	14	50.5	
							4779	111	53	11	8	26.4	
Sum		492	455	11	5			1561	599	228	132		
(w^{vc}♀ + G + P)/y♀			.96						.61				

various other recessive markers to identify gynandromorphs. Although the expected extremes of a single "male" bristle on a female and a single "female" bristle on a male were occasionally encountered, in practice only those gynandromorphs which were recognized on careful examination at 9-18X magnifications were so classified. The overall distribution of male and female areas in gynandromorphs appeared consistent with the analyses of Sturtevant (1929), Parks (1936) and Patterson and Stone (1938) showing that chromosome loss occurs primarily during the early indeterminate cleavages of the embryo. Gynandromorphs with noncontiguous mosaic areas were relatively rare, and those resulting from loss of the rod-X chromosome were exceedingly rare. Some mosaics which seemed to be of especial interest were recorded in semi-diagrammatic form.

In addition to gynandromorphs, a second class of exceptional individuals was recovered; these individuals were sterile XO males comparable to the exceptional males produced by claret females in Drosophila simulans (Sturtevant, 1929). Examination of the data of table 1 shows that these patroclinous males (P) were recovered in frequencies positively correlated with the incidence of gynandromorphs, suggesting a similar origin of the two classes.

Some patroclinous males undoubtedly arise from sources other than complete elimination of the w^{vc} chromosome from the zygote. Exceptional females and gynandromorphs arising from primary nondisjunction constitute less than one percent of the female progeny of unstable w^{vc} f/dl-49, y Hw m^2 g^4 females (table 2). If this proportion should also apply to exceptional males resulting from primary nondisjunction, then only about one twelfth of the patroclinous males recorded in table 2 could be of that origin. A four strand double crossover between a ring

Table 2

The distribution of exceptional offspring from the mating of unstable w^{vc} f/dl-49, y Hw m^2 g^4 females by dl-49, y w lz^s males.

C#	Regular Females y	w^{vc}	G	Exceptional Females w^{vc}	G	Exceptional Males P	%GP
4930-38	376	122	86	4	5	53	54.1
4940-44	287	90	45	2	3	55	52.8
4947-56	341	141	56	2	2	31	38.4
4957-69	376	86	52	1	2	29	48.8
4985-5009	1295	137	99	3	7	181	67.2
5015-29	427	66	37	2	2	59	59.0
Total		4119			35	408	

and a rod chromosome forms a double dicentric chromatid structure and a Nullo-X egg which, fertilized by an X-bearing sperm, results in a patroclinous male. In the present case where both the w^{vc} and rod-X chromosomes carry dissimilar inversions, the frequency of double crossing over is strongly reduced due to structural interference of synapsis, and patroclinous males arising from this source may be considered negligible. Finally an experiment was designed to show directly that complete elimination of the w^{vc} chromosome occurs. Homozygous y w spl sn females were mated to unstable w^{vc} f/sc^8:Y males; since the sc^8:Y chromosome carries a y^+ allele (Muller, 1948), the regular male offspring which received the sc^8:Y chromosome from their fathers could be distinguished from the XO offspring which received, but subsequently lost, the w^{vc} chromosome (table 3). Nondisjunction and crossing over in the y w spl sn females would have no effect on the results and can therefore be disregarded; similarly, the possibility of nondisjunction in the w^{vc} male parent can be eliminated since no gynandromorphs were recovered which carried the

Table 3

Production of XO males by complete elimination of the w^{vc} chromosome from zygotes of the mating y w spl sn females by w^{vc} f/sc^{8}:Y males.

Regular Males	Regular Females		Exceptional Males	Exceptional Females
y^{+} w spl sn	w^{vc}	G	y w spl sn	y^{+} w spl sn
4269	5551	85	90	7

sc^{8}:Y chromosome. The occurrence of y w spl sn males in numbers approximating those of the gynandromorph class indicates that w^{vc} loss must occur frequently prior to completion of the first cleavage division and supports the interpretation that most of the patroclinous males produced in outcrosses of unstable w^{vc} females are the result of complete elimination of the w^{vc} chromosome.

Nonmosaic w^{vc} females, gynandromorphs, and most patroclinous males all arise from unstable w^{vc}/rod-X zygotes; therefore, the combined incidence of gynandromorphs (G) and patroclinous males (P) among this class measures the frequency of w^{vc} loss assuming one elimination event per patroclinous male or gynandromorph. This index of w^{vc} instability has been calculated on a percentage basis: $\%GP = (G + P)100/G + P + w^{vc}$ ♀. The inclusion of patroclinous males from sources other than w^{vc} elimination introduces an error in %GP which becomes significant only at the lowest levels of w^{vc} instability. Exceptional females and gynandromorphs are added to the appropriate regular classes before calculation of %GP; this procedure probably introduces an error of opposite sign in %GP since complete elimination of w^{vc} from a nondisjunctional zygote would produce a male indistinguishable from the regular male class. It will be evident on examination of the values listed in table 1 that %GP is a highly variable quantity even among sister females. However, these limited data also illustrate two related generalizations based on extensive tests of sister females; the total %GP for a group of sister females tends to approximate that of their mother, and, consequently, the variability in %GP is less within groups of sister females than between such groups.

Comparison of the zygotic types rod-X/rod-X and w^{vc}/rod-X in the lines of low and high instability (table 1) gives ratios of .94 and .61, respectively. Although this strong reduction in the proportion of w^{vc}/rod-X zygotes recovered is not invariably observed, it is usually associated with higher levels of w^{vc} instability.

The variability characteristic of w^{vc} instability as measured by the frequency of w^{vc} loss suggested that either the unstable condition or the process of elimination might be susceptible to modification by a multiplicity of factors, both genetic and environmental. The studies to be described in the following sections were based on the premise that the identification and analysis of any such modifiers would provide information regarding the nature of w^{vc} instability and the mechanism of elimination.

Tests for Genetic Modifiers of w^{vc} Instability.

One of the more obvious possibilities of modifiers of w^{vc} behavior might be expected in autosomal genes which would segregate on repeated outcrossing of initially unstable w^{vc} lines. The ordinary procedure of autosome substitution to localize modifiers did not seem feasible after preliminary trials failed because of the lowered viability and fertility accompanying the introduction of autosomal inversions and dominant markers into the unstable w^{vc} genome. Instead, the simpler procedure of comparing the behavior of sister w^{vc} females after inbreeding and outcrossing was utilized to detect the presence or absence of autosomal modifiers. A single P_1 w^{vc} f/dl-49, y w lz^s female from an unstable stock culture was outcrossed to dl-49, y Hw m^2 g^4 males from a stabilized w^{vc} stock; these parents produced two successive seven day subcultures, 4497a and b of table 4 (certain aspects of table 4 will be discussed in a subsequent section). From 4497a 5 F_1 w^{vc} females were separately inbred (x_1) by their dl-49, y w lz^s brothers while 5 others were outcrossed (x_2) by dl-49, y w lz^s males from a stabilized w^{vc} stock. The difference in the total %GP for each of the two sets is not significant. Similarly, comparative matings of w^{vc} females from selected F_2 cultures produced an F_3 generation; as a check on the possibility of modifiers present in the stabilized stocks, F_2 females from cultures 4656-57 were either inbred or outcrossed (x_3) to dl-49, y Hw m^2 g^4 males from a source unrelated with any w^{vc} stocks. The results reveal no evidence for segregating autosomal modifiers in these crosses since 1) there is striking agreement in the total %GP between sets of sister females, and 2) the x_2 and x_3 matings of females from cultures 4507-8 and 4656-7, representing two successive outcrosses, produced F_3 values as high as or higher than that of the F_1. Since the behavior of the w^{vc} chromosome has exhibited no significant variation correlated with any of the different rod-X chromosomes used, it is also apparent that no sex-linked modifiers of w^{vc} instability exist.

Brown and Hannah (1952) pointed out that ring chromosome loss could not be explained by somatic crossing over between the ring and its

Table 4

Comparison of w^{vc} instability between the progenies of individual sister females after outcrossing and inbreeding. In(1)dl-49 was present in both the rod-X chromosomes. Cultures 4497a and b represent the first and second week subcultures of the P_1 female.

F_1 C#	F_1 %GP	Mating	F_2 C#	F_2 %GP	Mating	F_3 Number of Cultures	F_3 Total %GP
4497a	44.9 ± 7.0	x_1	4562	35.7			
			4564	47.8			
			4565	50.0	x_1	5	41.5 ± 2.7
					x_2	5	40.8 ± 2.6
			4566	44.4			
			4584	27.6	x_1	2	27.3 ± 3.4
					x_2	4	27.1 ± 2.7
			Total	41.4 ± 2.8			
		x_2	4505	43.5			
			4506	56.6			
			4507	31.7	x_1	4	57.4 ± 3.1
					x_2	6	58.3 ± 2.6
			4508	32.2	x_1	4	49.5 ± 3.6
					x_2	4	48.6 ± 3.2
			4509	33.7			
			Total	38.1 ± 2.7			
4497b	56.3 ± 7.2	x_2	4655	41.7			
			4656	44.3	x_1	5	74.3 ± 2.9
					x_3	7	64.2 ± 2.5
			4657	53.7	x_1	3	55.8 ± 5.4
					x_3	4	65.9 ± 3.0
			4658	46.7			
			4659	57.7			
			4660	54.5			
			Total	47.6 ± 2.5			

P_1 w^{vc} f/y w lz^s (unstable $) x y Hw m^2 g^4 (stabilized $)

F_1 w^{vc} f/y Hw m^2 g^4 — x_1 y w lz^s (brothers); x_2 y w lz^s (stabilized $)

F_2 w^{vc} f/y w lz^s — x_1 y Hw m^2 g^4 (brothers); x_2 y Hw m^2 g^4 (stabilized $); x_3 y Hw m^2 g^4 (unrelated $)

Table 5

Instability of the w^{vc} chromosome over rod-X homologs of the standard (+) and dl-49 inversion (In) sequences. Cross A compares the zygotic homologs while Cross B refers to the maternal homologs.

Cross A

Cross	Females		Gynandromorphs		%G	
	$+/w^{vc}$	In/w^{vc}	$+/w^{vc}$	In/w^{vc}	$+/w^{vc}$	In/w^{vc}
$\frac{(+)\,y\ w\ spl\ sn}{(In)\,y\ Hw\ m^2\ g^4}$ x w^{vc} f/Y	2714	2612	431	492	13.7 ± 0.6	15.9 ± 0.7

Cross B

Cross	w^{vc}♀	G	P	%GP
$\frac{(+)\,y\ w\ spl\ sn}{w^{vc}\ f}$ sisters x $(In)\,y\ w\ lz^s/Y$	156	78	58	46.6 ± 2.9
$\frac{(In)\,y\ Hw\ m^2\ g^4}{w^{vc}\ f}$ sisters x $(In)\,y\ w\ lz^s/Y$	225	80	81	41.7 ± 2.5

rod-X homolog since the expected twin male areas are not observed in gynandromorphs. This evidence does not deny the possibility that the structure of the rod-X may have some influence on ring loss mediated through the processes of synapsis or disjunction either at meiosis or in somatic mitoses. Accordingly, Crosses A and B of table 5 were designed to compare w^{vc} instability over the standard rod-X (+) with that in the presence of the dl-49 inversion sequence (In) which involves approximately the middle third of the X chromosome. Cross A consisted of 2 y w spl sn/dl-49, y Hw m^2 g^4 females and a single unstable w^{vc} f/Y male per culture. The F_1 daughters and gynandromorphs were classified according to whether they carried the standard or the dl-49 chromosome from their mothers. Although the difference in %G for the two classes approaches statistical significance at the .01 level of probability, this result is not considered significant since the frequency of XO males resulting from complete elimination could not be determined in this cross; in any event, the small %G difference might be attributed in part to differences in the two rod-X chromosomes other than the inversion. From one culture of Cross A, sister F_1 w^{vc} females were selected which carried either the (+) or the (In) chromosomes; comparison of their progenies (Cross B) shows that the structure of the maternal rod-X chromosome is also without effect on the level of w^{vc} instability.

The relative incidence of gynandromorphs among the regular and exceptional female classes of table 2 indicates that the probability of w^{vc} elimination is increased in nondisjunctional zygotes; there were 36.9 and 60.0 percent gynandromorphs among the regular and exceptional classes, respectively. This may mean that an unstable w^{vc} chromosome which has undergone nondisjunction is more liable to subsequent loss or, alternatively, that the presence of a Y chromosome in such nondisjunctional zygotes increases the likelihood of w^{vc} loss. One might also suspect some relation of the Y chromosome and w^{vc} instability because of the heterochromatic complexity of the w^{vc} chromosome constitution; this suspicion, however, cannot be sustained when gynandromorph production by regular and primary exceptional w^{vc} sisters is compared (table 6). Use of the sc^8:Y chromosome was of particular advantage in these crosses since gynandromorphs carrying it could be identified by their y^+ male phenotype. On the other hand, the numerous patroclinous males expected to arise from secondary nondisjunction and those resulting from complete elimination of w^{vc} could not be distinguished in Crosses D and F, and these data have been omitted. Phenotypic distinction of the female classes $\frac{y\ v\ f\ car}{y\ w\ lz^s}/sc^8{:}Y$ and $\frac{w^{vc}\ f}{y\ w\ lz^s}/sc^8{:}Y$ of Cross F was likewise impossible; in this case, it was possible to make a reasonable estimate of the former class based on the distribution of the sc^8:Y chromosome among the comparable regular male classes and by subtraction, the incidence of the latter female class was obtained. Despite these limitations, the results of the two experiments are consistent in showing that total gynandromorph production is the same for w^{vc} females possessing sc^8:Y and their sisters having no Y chromosome. Secondly, analysis of the distribution of gynandromorphs among the zygotic classes of Crosses D and F also leads to the conclusion that the sc^8:Y chromosome has no influence on w^{vc} instability. It is assumed that these conclusions for the sc^8:Y chromosome would also be applicable in the case of the standard Y chromosome, and the lack of significant differences in %G for the three zygotic classes of Cross F agrees with this assumption.

Table 6

Loss of w^{vc} in zygotes with and without a Y chromosome. The y v f car and y w lz^s chromosomes carry In(1)dl-49. The numbers in parentheses (Cross F) are estimates.

Cross C: w^{vc} f/y v f car, D: w^{vc} f/y v f car/sc^8:Y } sisters x y v f car/sc^8:Y

	y v f car / y v f car		w^{vc} f / y v f car			total %G
			Females	Gynandromorphs		
	--	sc^8:Y	(± sc^8:Y)	--	sc^8:Y	
C	1654	0	783	89	2	10.4 ± 1.0
D	166	182	202	12	12	10.6 ± 2.0

Cross E: w^{vc} f/y v f car, F: w^{vc} f/y v f car/sc^8:Y } sisters x y w lz^s/Y

	y v f car/Y		y v f car / y w lz^s		w^{vc} f / y w lz^s		w^{vc} f / y w lz^s/sc^8:Y		w^{vc} f / y v f car/Y		total %G
	--	sc^8:Y	--	sc^8:Y	♀	G	♀	G	♀	G	
E	663	0	758	0	289	20	0	0	12	2	6.8 ± 1.4
F	233	276	252	(299)	160	13	(121)	11	82	10	(8.6 ± 1.4)
					7.5 %G		(8.3 %G)		10.9 %G		

Table 7

The incidence of primary exceptional females in lines of high and low w^{vc} instability. Mating: w^{vc} f/dl-49, y Hw m^2 g4 x dl-49, y w lz^s.

C#	Regular Females				Exceptional Females		%GP	Percent Exceptional Females
	y	w^{vc}	G	P	w^{vc}	G		
(table 2)	3102	642	375	408	14	21	55.1	0.77 ± .13
							(High Instability)	
3607-15	1518	1350	27	16	0	0		
4191-4215	2362	1939	26	25	5	1		
4216-40	1555	1390	23	14	5	0		
4260-84	1984	1725	21	22	17	0		
4330-52	1683	1349	25	12	3	0		
4458-74	1335	1066	19	26	20	1	(Low Instability)	
total	10437	8819	141	115	50	2	2.8	0.27 ± .04

Returning to the question of the higher incidence of gynandromorphs among nondisjunctional zygotes, some evidence has been found relative to the suggestion that an unstable w^{vc} chromosome which has undergone nondisjunction is more liable to subsequent loss. This evidence is that the frequency of exceptional females is significantly higher in lines of high w^{vc} instability than in those of low instability (table 7). Although the observed frequency of exceptional females does not reflect accurately the frequency of primary nondisjunction (all patroclinous males were assumed to arise from regular w^{vc}/rod-X zygotes and complete elimination of w^{vc} from nondisjunctional w^{vc}/rod-X zygotes could not be detected), the conclusion that primary nondisjunction is likewise higher in lines of high w^{vc} instability seems justified. Therefore, if nondisjunction and elimination are considered related results of w^{vc} instability, their joint probability of occurrence (as nondisjunctional gynandromorphs) would be higher than that for independent events.

In summary, the results of these tests show that genetic control of w^{vc} instability must be entirely inherent in the w^{vc} chromosome since no evidence was obtained which suggested any influence of autosomal or sex-linked modifiers, of the structure of the homologous X chromosome, or of the Y chromosome on the frequency of w^{vc} elimination. Certain results also indicated that the frequency of primary nondisjunction in w^{vc}/rod-X females is associated with the level of w^{vc} instability.

The Locus of w^{vc} Instability

If the primary cause of w^{vc} instability were to be thought of as being confined to a single genetic locus, then this locus must be limited to the w^{vc} chromosome itself, as disclosed by the various tests already described. Although no concerted effort has yet been made to localize such a factor by crossing over, certain preliminary observations may be mentioned here. Six w^{vc} chromosomes were recovered in which the region including the f locus had been exchanged with the y w spl sn or In(1)w^{m4}, y w sn m chromosomes by double crossing over; similarly, one crossover ring was obtained which carried the m allele, but not sn or f^+, from w^{m4}. All seven substituted w^{vc} chromosomes were unstable, and comparison of their instability with that of w^{vc} chromosomes carried by non-recombinant sibs revealed no conspicuous differences; therefore, if w^{vc} instability is determined by a specific gene, its locus must lie outside the m-f interval of the chromosome.

Further delimitation of the region of the w^{vc} chromosome responsible for its instability has been suggested by the behavior of a second kind of unstable w^{vc} derivative. These derivatives consisted of small fragments of the w^{vc} chromosome which were recognized by the

Table 8

Tabulation of duplications derived from unstable w^{vc} chromosomes.

Presumptive Zygotic Genotypes	Number and Type of Individuals Carrying Duplication	Duplication Distribution in Male Areas	Duplication Constitution	
			Covered	Not Covered
w^{vc} f / dl-49, y Hw m^2 g^4	G	partial	y	m^2
	G	complete	y	
	2 G	complete	y	m^2 g^4
	2 P	partial	y	
	P	partial	y	m^2 g^4
w^{vc} f / dl-49, y w lz^s (Y)	G	partial	y w	lz^s
	G	partial	y	
	P*	partial	y w	lz^s
w^{vc} f / y w spl sn	G	complete	y	sn
w^{vc} f / w^{m4}, y w sn m	P	partial	y	sn
y w spl sn (bb) / w^{vc} f	G	partial	y	w spl sn bb
	2 G	complete	y w	spl sn
	2 P	partial	y	sn
	P	complete	y w	spl sn
y w spl sn/sc^8:Y / w^{vc} f	P**	?	w spl	sn
$Y^S.XY^L$, y v f car / w^{vc} f	G	complete	y	car
y f:=/w^{vc} B/Y	$\widehat{XX}$ ♀ Dp(w^{vc})4097	partial	y w N	f B
y w/w^{vc} f/O	$\widehat{XX}$ ♀ Dp(w^{vc})5279	partial	y w	spl sn, v f car

*This male transmitted the fragment to one sterile son.
**The duplication carried by this male was not observed among 1580 offspring.

appearance of either or both y and w-variegated phenotypes in male areas of gynandromorphs which were expected to display the y and w phenotypes of the mutant alleles in the uneliminated rod-X chromosome. Patroclinous males also occurred which evidently carried a duplication covering the y and/or w mutant loci of their X chromosomes. Such individuals were rarely observed; table 8 comprises 22 cases which arose from a variety of matings with the w^{vc} chromosome being of either maternal or paternal origin. The mechanism producing these duplications will be discussed in a subsequent section.

The observation most pertinent to this discussion is that in over one-half of the cases, the duplication was present in only part of the male areas of gynandromorphs, or in only part of a patroclinous male. Since the majority of these duplications occurred in unbreedable gynandromorphs or sterile XO males, they were not available for further analysis. On the other hand, Dp(w^{vc})4097 and Dp(w^{vc})5279 were recovered in fertile attached-X females and were transmitted to about one-half of the y f:= or y w female progeny of each generation; nearly every attached-X female carrying either of the duplications had at least one and usually several areas of y tissue which varied in size from small patches to over half the hypodermis. Careful examination of a group of 183 Dp(w^{vc})4097; y f:=/Y females showed that all of them were y-y^+ mosaics; the presence of a Y chromosome was confirmed by the production of fertile sons from crosses of some of these females to rod-X/Y males. Other females of this group were crossed to males bearing the attached XY chromosome but no free Y (Lindsley and Novitski, 1950); from this cross, a group of Dp(w^{vc})4097; y f:=/0 females was inspected. All 69 of these females were also found to be mosaics, and there was no noticeable difference in the number or size of y areas between females of the two groups. The failure to detect variation in mosaicism related to the presence or absence of a Y chromosome suggests that the y-y^+ mosaicism is not the result of a heterochromatic variegation process accompanying the duplication. Dp(w^{vc})4097 exhibited a dominant phenotype, known as Confluens, causing irregularities in wing venation, but this effect was absent in y wing tissue of mosaic Dp(w^{vc})4097; y f:= females. The eyes of Dp(w^{vc})5279; y w females occasionally showed w and w-variegated tissue in sharply delineated sectors bordered by y and y^+ setae, respectively.

It is apparent from these results that the duplications frequently undergo somatic elimination, and the inherent conclusion is that the control of elimination is the same in the case of the duplications as in the unstable w^{vc} chromosome from which they were derived. Accordingly, the constitution of the duplications is of significance in localizing the cause of instability. The occurrence of duplications in male tissue requires that their euchromatic content be of limited extent. This assumption is supported by the combined evidence from the duplications which, in one case or another, did not cover the mutant loci sn, lz^s, m^2, v, g^4, f, B, car, or bb (table 8). In only one case where a test was possible did the duplication lack the w locus, although this locus was obviously included in 8 other cases, and similarly, spl was included in 2 cases and excluded in 4 cases. Dp(w^{vc})4097, which produced in females the Confluens phenotype characteristic of duplications for the Notch loci, rarely survived as a duplication male; Dp(w^{vc})5279, on the other hand, frequently lived as a duplication male, and both duplications were viable in combination with the base of T(1;4)w^{m5}. Since Dp(w^{vc})4097 and Dp(w^{vc})5279 were regularly transmitted, and since the other 20 duplications were present in more or less extensive areas of tissue representing many cell divisions, it is evident that they all possessed the centric and some part of the associated heterochromatic regions of the w^{vc} chromosome. It follows from these observations that the primary control of w^{vc} instability must be a property of either the y-w, the heterochromatic, the centromere, or some particular combination of these regions of the w^{vc} chromosome.

$Dp(w^{vc})4097$ proved to be a small ring shaped chromosome as seen in larval ganglion mitoses. For purposes of comparison, reference may be made to $Dp(1;f)X^{c2}$, a small ring derivative of the stable X^{c2} chromosome including the y-pn loci. Schultz (Bridges and Brehme, 1944) reported that $Dp(1;f)X^{c2}$ exhibits two types of variegation; one type is supressed by the addition of Y chromosomes, and the other, consisting of a "few yellow hairs" (per individual ?) is insensitive to Y changes. While this latter type of variegation may be explained by elimination of $Dp(1;f)X^{c2}$, it seems clear that the pattern of mosaicism is essentially different from that produced by duplications derived from the unstable w^{vc} chromosome.

The implication of the general region of the centromere as the site of w^{vc} instability control finds parallel situations in unusual chromosome behavior in other organisms. Thus in Sciara coprophila and S. reynoldsi, Crouse (1943) showed that interchanges combining the proximal portion of the sex chromosomes with the distal segments of certain autosomes continued to undergo somatic elimination and nondisjunction at the secondary spermatocyte division which characterize the "normal" sex chromosomes of this genus, whereas the reciprocal interchanges behaved normally. Similarly, Roman (1947) found nondisjunction of B-type translocations of maize to be determined by the centromere regions of the B chromosomes which frequently undergo nondisjunction during the microspore mitoses.

Temperature and Maternal Effects on w^{vc} Behavior

Incidental observations at the beginning of these studies indicated that the frequency of w^{vc} elimination was in some degree dependent upon the age of w^{vc} females and upon the developmental temperature. These variables were therefore routinely controlled by selecting newly hatched w^{vc} females for experiments conducted at 25 ± 1°C. The experiments described below were undertaken to ascertain the extent and mode of action of the temperature and maternal effects.

From stock cultures reared at 25°C., virgin w^{vc} f/dl-49, y w lz^s females were selected and mated 2 per culture to y w spl sn males; these matings were divided into two groups which were maintained either at 18 or 26°C. for the duration of the cultures. Random samples of the F_1 w^{vc} f/y w spl sn females from each group were mated 3-4 per culture to y^2 cv v f males, and each group was subdivided; one subgroup remained at the original temperature while the other was transferred to the alternative temperature. The results, summarized in table 9, clearly demonstrate that the %GP is higher for both generations raised at 25° than for those at 18°. One might reasonably suppose the temperature sensitive stage of development to be localized during the early cleavages when elimination occurs. Other results have shown that cold shocks of -10°C. for 8 minutes (the desemination process of Novitski and Rush, 1948) administered to unstable w^{vc} females have no effect on %GP among

Table 9

The effect of temperature on w^{vc} elimination.

Developmental Temperature	P_1 $\frac{w^{vc}\ f}{dl\text{-}49,\ y\ w\ lz^{s}}$ x y w spl sn: w^{vc}♀	G	P	%GP	F_1 $\frac{w^{vc}\ f}{y\ w\ spl\ sn}$ x y^2 cv v f: w^{vc}♀	G	P	%GP
					2465	751	718	37.3 ± .8
26°C.	205	158	50	50.4 ± 2.5				
					2478	600	634	33.2 ± .8
					3488	444	354	18.6 ± .6
18°C.	973	317	140	32.0 ± 1.2				
					2648	192	288	15.3 ± .6

their offspring as compared with the offspring of their untreated sisters.

With increasing age of an unstable w^{vc} female, the incidence of gynandromorphs and patroclinous males among her offspring increases. Examples of this maternal age effect are provided by the results of the assorted matings G-K of table 10 where the second subcultures of each mating produced significantly higher %GP values than the first. This effect was not manifested by the matings L and M where the initial level of instability was low, although subculturing was extended as long as

Table 10

The effect of maternal age on w^{vc} elimination frequency. The y w lz^s and y Hw m^2 g^4 chromosomes carry the dl-49 inversion; other rod-X chromosomes are of standard sequence.

	Mating	Maternal Age in Days	w^{vc}♀	G	P	%GP
G	$\frac{w^{vc}}{y\ sc\ lz^g\ v\ f}$ x sn^{36a}	1-7	664	84	73	19.1 ± 1.4
		8-14	151	63	36	39.6 ± 3.1
H	$\frac{w^{vc}\ f}{y\ w\ lz^s}$ x y sc lz^g v f	1-4	79	14	13	25.5 ± 4.2
		5-11	179	44	53	35.1 ± 2.9
J	$\frac{w^{vc}\ f}{y\ w\ lz^s}$ x y Hw m^2 g^4	1-7	390	157	131	42.5 ± 1.9
		8-14	74	62	82	66.1 ± 3.2
K	$\frac{w^{vc}\ f}{y\ w\ lz^s}$ x y Hw m^2 g^4	1-7	145	72	66	48.8 ± 3.0
		8-14	20	20	29	71.0 ± 5.5
L	$\frac{w^{vc}\ f}{y\ w\ lz^s}$ x y Hw m^2 g^4	1-7	334	8	14	6.2 ± 1.3
		8-14	258	0	9	3.4 ± 1.1
		15-21	245	3	8	4.3 ± 1.3
		22-28	100	0	5	4.8 ± 2.1
M	$\frac{w^{vc}\ f}{y\ Hw\ m^2\ g^4}$ x y w lz^s	1-8	582	8	7	2.5 ± 0.6
		9-16	390	7	7	3.5 ± 0.9
		17-24	94	7	1	7.8 ± 2.7

28 days. The following observations assign the role of the increase in elimination frequency to the w^{vc} female rather than the rod-X male parent. In this experiment, groups of sister w^{vc} females were selected, and some of them from each family were mated immediately while others were stored 12 days prior to mating to young males (table 11). Aging caused significant increases in %GP in families 3572 and 3588, but again the females with low initial w^{vc} instability (family 3565) either failed to respond or actually gave reduced %GP values after aging. Additional comment may be made relative to matings J and K (table 10) which consisted of singly mated sister w^{vc} females; each of these 20 females exhibited increased w^{vc} elimination in the second subculture although the numbers were too small to be of individual significance. It is also noteworthy that these matings are characterized by a larger proportion of patroclinous males relative to gynandromorphs in the second subculture.

Table 11

The effect of aging w^{vc} f/y w spl sn females for 12 days prior to mating to y sc lz8 v females.

Family	Number of Sisters Tested		w^{vc}♀	G	P	%GP
3572	Control	10	417	144	153	41.6 ± 1.8
	Aged	6	219	126	88	49.4 ± 2.4
3588	Control	4	191	71	69	42.3 ± 2.7
	Aged	4	93	52	66	55.9 ± 3.4
3565	Control	10	888	9	35	4.7 ± 0.7
	Aged	6	375	2	4	1.6 ± 0.6

This observation, accompanied by an increase (not significant) in the average size of male areas included in tergites 2-5 of gynandromorphs from the later subculture, suggests that w^{vc} elimination occurs earlier as well as more frequently after aging.

Reference to culture 4497 of table 4 also provides a typical example of the maternal age effect on comparison of the a and b subcultures; but the unique feature of these data is that the F_1 females selected from 4497b produced higher %GP values than those selected from 4497a, indicating that the maternal age effect may be transmitted from generation to generation. Unfortunately, no other reliable data from similar crosses are available. It may also be interjected here that no decisive information has been obtained relative to the operation of an age effect in the case of unstable w^{vc} males.

The existence of temperature and maternal age effects on w^{vc} elimination presented the possibility that some of the variation in the level of w^{vc} instability might be mediated through the cytoplasm of the egg rather than directly on the w^{vc} chromosome. This possibility was examined by obtaining y w spl sn/dl-49, y Hw m^2 g^4 daughters of a) unstable w^{vc}/y w spl sn females and b) y w spl sn females and comparing gynandromorph production of the two groups after mating to w^{vc} f/Y brothers from different sources. The results of these crosses (table 12) leave no doubt that the egg cytoplasm of the females derived from unstable w^{vc} mothers provided more favorable conditions for elimination of unstable paternal w^{vc} chromosomes; at the same time, however, elimination of stabilized paternal w^{vc} chromosomes (family 3376) was not enhanced. In the strict sense, appropriate reciprocal crosses to distinguish the possibilities of maternal and genetic modifier effects

Table 12

The dependence of paternal w^{vc} elimination upon the source of maternal rod-X/rod-X females. The symbols (+) and (In) refer to chromosomes carrying the standard and dl-49 inversion sequences.

Paternal w^{vc} Male Family	P_1 $\frac{w^{vc}\ f}{(+)y\ w\ spl\ sn}$ x (In)y Hw m^2 g^4 (74.3 %GP)			P_1 $\frac{(+)y\ w\ spl\ sn}{(+)y\ w\ spl\ sn}$ x (In)y Hw m^2 g^4 (No elimination)		
	F_1 $\frac{(+)y\ w\ spl\ sn}{(In)y\ Hw\ m2\ g^4}$ x w^{vc} f/Y			F_1 $\frac{(+)y\ w\ spl\ sn}{(In)y\ Hw\ m^2\ g^4}$ x w^{vc} f/Y		
	Number of Cultures	%G/(In)	%G/(+)	Number of Cultures	%G/(In)	%G/(+)
3362	2	53.1	*	3	16.6	14.3
3369	2	44.0	45.1	2	13.4	13.6
3385	2	45.2	44.2	2	12.4	9.4
3386	2	33.1	36.4	1	13.7	10.3
3391	1	48.4	36.6	1	21.6	13.2
	total	41.8 ± 1.7		total	13.4 ± 0.8	
3376	5	0.4	0.6	3	0.6	0.6

*A lethal was present in the y w spl sn chromosome of one culture.

were not performed, but the data from similar crosses (table 4) revealed no effect of the source of rod-X parents on maternal w^{vc} elimination. If this evidence for the lack of genetic modifiers of w^{vc} instability is admissible in the present case, then the differences in gynandromorph production by females from the two sources may be attributed to some cytoplasmic condition elicited by the unstable w^{vc} chromosome in the previous generation.

Despite the definitely preliminary nature of these observations, it already seems apparent that the maternal age and cytoplasmic effects on w^{vc} elimination are both manifestations of the same phenomenon. Provisionally, this phenomenon may be viewed as a system in which the unstable w^{vc} chromosome initiates the production of some cytoplasmic "principle" which, in turn, provides the requisite conditions for elimination of the unstable w^{vc} chromosome. The age effect on elimination would reflect either an increased reaction time between the two components of the system or a quantitative increase of the cytoplasmic component. The differential response of unstable paternal w^{vc} chromosomes to cytoplasms of different sources would indicate that the cytoplasmic principle is either autonomous or at least stable for one generation in the absence of the unstable w^{vc} chromosome. The failure of stabilized w^{vc} chromosomes to exhibit age effects and to respond to cytoplasmic differences would suggest that such chromosomes are not susceptible to, and may not elicit, the cytoplasmic principle. With reference to the mitotic mechanism, such a scheme may be thought of as operating between the spindle apparatus and the centromere region of the w^{vc} chromosome or between the cytoplasm and the chromosome at the time of reduplication.

The relationship, if any, between the maternal effects discussed above and the induced maternal age effect on paternal X^{c2} elimination reported by Brown and Hannah (1952) is obscure. In fact, an attempt to repeat their experiment using stabilized w^{vc} f/Y male parents failed to show any significant increase in gynandromorph production by y w spl sn females which were aged for 7 days prior to mating; the %G values were 1.2 ±0.2 and 0.8 ±0.1 for the aged and control groups, respectively. It is possible that technical differences in the aging procedures would account for the failure to confirm Brown and Hannah's results. Bonnier and Lüning (1952) have presented data on the elimination of "normal" rod-X chromosomes from which they conclude that the response of certain centromere regions of paternal X chromosomes to different cytoplasms varies according to the maternal genotype controlling the cytoplasm. In implicating an interaction between the cytoplasm and the X chromosome to enhance elimination, these cases may possibly provide precedents for the w^{vc} instability system.

The Mechanism of w^{vc} Elimination

Loss of ring chromosomes is most readily explained on the basis of impaired polar movement of the chromosome; on this view, the chromosome is simply excluded from one or both daughter nuclei at telophase. It is not necessary to assume, as did L. V. Morgan (1926, 1929), that retarded anaphase movement is a consequence of the ring chromosome's shape; indeed, in view of the results suggesting that w^{vc} instability is a property of the centromere region, anaphase lagging might be directly related to centromere activity. Such a simple mechanism of elimination appears to be denied by the cytological evidence presented by Braver and Blount (1949) who examined larval ganglion cells of stable X^{c2}/dl-49 and unstable w^{vc}/dl-49 females and found 12 and 22 percent bridges in the anaphases of each strain, respectively. Clearly, lagging ring chromosomes would not be expected to produce genuine anaphase bridge configurations. However, there is reason to question the significance of mitotic abnormalities appearing in smear preparations of this tissue since, despite the appearance of bridges, there is no evidence that elimination actually occurs in ganglion cells—Braver and Blount did not record the occurrence of XO cells; furthermore, the high frequency of bridges involving X^{c2} is of doubtful meaning when related to the low incidence of gynandromorphs (0.68%) found in this same material.

Braver and Blount state that "the bridges consist of interlocked rings in most cases and of unmistakable long dicentric rings in occasional cells" of the larval ganglia. The preponderance of interlocking rings is taken to favor the speculation of Griffen and Lindsley (1946) that ring loss is the consequence of "uncompensated spiralization of at least 360 degrees during interphase reduplication." The basis for the distinction between this mechanism and McClintock's (1938) theory, that bridges formed by somatic ring chromosomes in maize arise from exchanges between the two chromonemata during or after splitting, is vague. In any event, the results are comparable in the two cases; according to McClintock's discussion, a single exchange (spiralization of 180°) would produce a continuous dicentric ring, two progressive exchanges (spiralization of 360°) would produce interlocked rings, and the relative frequency of the two types of bridges would depend upon the size of the ring chromosome. The failure of either type of bridge to experience breakage would lead to ring loss. One should recall that the cleavage mitoses of Drosophila occur in a syncytium; this may be a factor of importance in determining whether or not bridges, if present, do undergo breakage.

Essentially, only two alternative causes need be considered in order to account for ring chromosome loss in Drosophila melanogaster: anaphase lagging, and the production of anaphase bridges. It has been tacitly assumed that the elimination mechanism of the unstable w^{vc} chromosome is the same as that for "stable" closed-X chromosomes; correlatively, the variation in the frequency of loss must be viewed as an attribute of some factor controlling the loss mechanism rather than of the mechanism itself.

The duplications derived from the w^{vc} chromosome (table 8) may provide a clue to the mechanism of w^{vc} loss if it is supposed, in the absence of critical information to the contrary, that the fragments were produced as concomitants of w^{vc} elimination. The production of a free fragment from a ring chromosome requires two breaks, one on either side of the centromere if the fragment is to include this organelle; if the breaks are followed by fusion of the broken ends, the fragment would assume a ring structure as found in the case of Dp(w^{vc})4097. The necessary configuration for induction of two breaks by opposing centromere forces is presented by anaphase bridges composed of dicentric continuous rings; bridges formed by interlocked rings do not satisfy the required conditions since a single break in either member would relieve the stress.

McClintock's (1938) comparison of the behavior of large and small ring chromosomes in maize revealed that the small rings are more frequently eliminated although they form fewer continous (or interlocking) ring configurations as expected on the basis of their size. The fact that small rings are more frequently eliminated appears to be explained by exclusion of double sized small rings from the telophase nuclei; cytologically, such configurations are typically situated more or less equidistant from either of the late anaphase congregations at the poles of the spindle, whereas double sized or interlocked large rings extend from pole to pole. A similar comparison may be made between Dp(w^{vc})4097 and the unstable w^{vc} chromosome; whereas the frequency of elimination of this small ring was such that almost every y f:= female bearing it had at least one mosaic area, the combined frequency of gynandromorphs and patroclinous males resulting from w^{vc} elimination rarely exceeded 50% of the recovered w^{vc}/rod-X zygotes. However, this correlation in the behavior of small ring chromosomes in maize and Drosophila may be spurious. It seems more probable that this apparent higher frequency of small ring elimination in Drosophila is the result of a difference in the time of elimination, for the mosaic areas produced by Dp(w^{vc})4097 elimination were distinctly smaller on the average than male areas of gynandromorphs.

One might anticipate the recovery of certain other viable products of breakage in continuous ring anaphase bridges, including ring chromosomes deficient for a small euchromatic segment. Obviously, detection of such deleted chromosomes requires coincidence of the deficient segment and some mutant marker of the particular rod-X homolog present in the zygote. During the course of these studies, 8 w^{vc}/w females were recovered whose eyes, instead of being white variegated, were pure white, and one other w^{vc} female was found having a small sector of variegated tissue in otherwise white eyes (table 13); there was no evidence of sexual mosaicism or other phenotypic irregularities in any of these individuals, and all of them occurred in separate cultures. When these "white" w^{vc} females were backcrossed to w males, 4 of them produced only typical variegated w^{vc} offspring, but the other 4 fertile ones trans-

Table 13

The occurrence and behavior of "white" w^{vc} females.

Presumptive Zygotic Genotype	"white" w^{vc} Females	Case Number	F_1 w^{vc} Behavior			
			Phenotype	Viability	Stability	Structure
y w spl sn / w^{vc} f	7	1068	sterile			
		1069	variegated			
		1089*	variegated			
		1150	white	male lethal	stable	ring
		1667	white	male viable	unstable	
		5641	variegated			
		5779	white	male viable	unstable	ring
dl-49, y w lz^s / w^{vc} f	1	3186	variegated			
w^{vc} f / dl-49, y w v f car	1	5514	white	male lethal	stable	ring

*This female had a sector of variegated tissue in otherwise white eyes.

mitted only "white" w^{vc} chromosomes. In subsequent generations of the latter cases, it was determined that the mutant condition did not segregate from the w^{vc} chromosome; in one case (1150), the addition of a Y chromosome did not affect the white phenotype; the ring structure of the mutant w^{vc} chromosome persisted in larval ganglion metaphases of three cases (case 1667 was not examined). It is not possible to interpret these changes on the basis of crossing over. Germinal crossing over is excluded by the fact that in 8 of the 9 cases, the w^{vc} chromosome was of paternal origin, and the maternal w^{vc} parent of the remaining case did not carry the w allele; furthermore, in at least 4 cases, the change was evidently a somatic event. Somatic exchange can also be excluded with reasonable assurance since double exchanges within a very restricted region (y-spl) between homologs heterozygous for inversions would be required. It is therefore postulated that these "white" w^{vc} chromosomes represent either point mutations at the w^{+} locus or small deficiencies including that locus. While it has not been possible to distinguish these alternatives, the deficiency hypothesis is indicated in cases 1150 and 5514 where the mutant w^{vc} chromosome, although stable, is hemizygous lethal; on the other hand, cases 1667 and 5779 can be more easily explained by point mutation since the "white" w^{vc} chromosome remained viable in the male even in the unstable condition.

If it is assumed that the duplications and some of the "white" w^{vc} chromosomes are bridge breakage products, then these rarely observed cases probably represent only a minority of those actually produced; others may have survived undetected, but the majority would have been lethal if breaks occurred at random in the two members of the bridge. As a guess, this latter class may account for the reduced recovery of unstable w^{vc}/rod-X as compared with rod-X/rod-X zygotes in lines of high w^{vc} instability (table 1). It should be emphasized that even if the continuous ring anaphase bridges were established as the source of the w^{vc} derivatives discussed here, this evidence would not justify the argument that such configurations, either with or without breakage, constitute the exclusive means of w^{vc} elimination.

In an attempt to distinguish between the profferred mechanisms of ring chromosome elimination, the premise that elimination is dependent on ring structure should be examined. Such an experiment has been designed for the unstable w^{vc} chromosome, but because of the preliminary and negative nature of the results, no detailed description is warranted. It was possible to obtain, from a special crossover situation, two w^{vc} derivatives both of which are approximately metacentric chromosomes carrying the centromere and heterochromatic arrangement of the original w^{vc} chromosome. On testing, it was found that these derivatives were essentially stabilized, producing less than 1 %GP. In addition to the original w^{vc} components, the metacentric chromosomes also carried a small duplication which paired in normal sequence with its homologous region in the other arm; as a result of a single crossover in this region, newly reformed rings were recovered. Tests of these reformed w^{vc} chromosomes showed that they also were stabilized. Consequently, the failure of the metacentric chromosomes to undergo elimination can only be considered a negative result since there is no proof that they were derived from unstable rather than stabilized w^{vc} chromosome.

Summary

1. The major consequence of instability of the w^{vc} chromosome is somatic elimination producing gynandromorphs (G) and XO males (P). The incidence of these individuals is positively correlated, and their combined frequency (%GP) among w^{vc}/rod-X zygotes provides a measure of w^{vc} instability which is highly variable from family to family. This study sought to identify the factors controlling the level of w^{vc} instability and to determine the mechanism of elimination.

2. Tests for genetic modifiers of w^{vc} instability proved entirely negative. There was no difference in %GP produced by unstable w^{vc} females which were outcrossed and their sisters which were inbred for two generations. Neither the genetic constitution nor the structure of rod-X homologs caused significant variation in the frequency of w^{vc} loss. Comparisons of sister w^{vc} females with and without a marked Y chromosome showed that this chromosome had no effect on w^{vc} instability.

It is concluded that the primary control of w^{vc} instability is limited to the w^{vc} chromosome.

3. The behavior of small duplications derived from the unstable w^{vc} chromosome suggests that the locus of w^{vc} instability must be in or near the centromere region. Of 20 such duplications observed in XO males or in male areas of sterile gynandromorphs, 11 were present as mosaics. Two duplications which arose in fertile females continued to undergo somatic elimination in succeeding generations.

4. Nondisjunctional female zygotes experience w^{vc} loss more frequently than their regular sisters; this observation, plus the occurrence of more primary exceptional females in lines of high than low w^{vc} instability, suggested that the frequency of primary nondisjunction might also be related to w^{vc} instability.

5. The frequency of w^{vc} elimination is temperature dependent; comparison of %GP values produced by cultures reared at 18 and 26°C. shows an approximate twofold increase at the higher temperature.

6. With increasing age of unstable w^{vc} females, the %GP among their offspring increases; this increase is also demonstrated by unstable w^{vc} females aged prior to mating. However, stabilized w^{vc} females do not exhibit age increases in w^{vc} instability.

7. A cytoplasmic effect on w^{vc} elimination is indicated by the results from crosses of unstable w^{vc} males to rod-X females from unstable w^{vc} mothers and from rod-X stock matings; the females from the former source produced a significantly higher %G than those from the second source. Although the possibility of autosomal modifiers was not strictly excluded in this experiment, other experiments failed to reveal any genetic modifiers of w^{vc} instability. It is speculated that the maternal

age and cytoplasmic effects may be different aspects of a system in which the unstable w^{vc} chromosome elicits in the cytoplasm a condition requisite for w^{vc} elimination, and that this condition persists for at least one generation in the absence of the w^{vc} chromosome.

8. Consideration of the possible mechanisms of ring chromosome elimination shows that either of two causes will explain the results; these are simple anaphase lagging (L. V. Morgan) and anaphase bridges of either the continuous or interlocking ring types (McClintock). The occurrence of certain w^{vc} derivatives, including the small duplications and w^{vc} chromosomes presumably deficient for a small section, suggests that dicentric continuous rings may be formed; however, such bridges may not constitute the exclusive means of w^{vc} elimination.

References

Battacharya, P., 1950 Behaviour of the ring-chromosome in *Drosophila melanogaster*. Proc. Roy. Soc. Edin., B, 64:199-215.

Bonnier, G., and K. G. Lüning, 1952 Interaction of centromere and cytoplasm in chromosome elimination in *Drosophila melanogaster*. Hereditas 38:339-344.

Braver, G., and J. L. Blount, 1949 Somatic eliminations of ring chromosomes in *Drosophila melanogaster*. Rec. Genet. Soc. Amer. 18:78.

Bridges, C. B., and K. S. Brehme, 1944 The mutants of *Drosophila melanogaster*. Pub. Carnegie Inst. No. 552:1-257.

Brown, Spencer W., and Alohah Hannah, 1952 An induced maternal effect on the stability of the ring-X-chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 38:687-693.

Catcheside, D. G., and D. E. Lea, 1945 Dominant lethals and chromosome breaks in ring X-chromosomes of *Drosophila melanogaster*. J. Genet. 47:25-40.

Crouse, Helen V., 1943 Translocations in *Sciara*; their bearing on chromosome behavior and sex determination. Univ. Mo. Res. Bull. 379:1-75.

Griffen, A. B., and D. L. Lindsley, Jr., 1946 The production of gynandromorphs through the use of unstable ring-chromosomes in *Drosophila melanogaster*. Anat. Rec. 96:59-60.

Lindsley, D. L. Jr., and E. Novitski, 1950 The synthesis of an attached XY chromosome. D.I.S. 24:84.

McClintock, Barbara, 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.

Morgan, L. V., 1926 Correlation between shape and behavior of a chromosome. Proc. Nat. Acad. Sci. 12:180-181.

1929 Composites of *Drosophila melanogaster*. Pub. Carnegie Inst. No. 399:223-296.

1938 Origin of attached-X-chromosomes in *Drosophila melanogaster* and the occurrence of non-disjunction of X's in the male. Amer. Nat. 72:434-446.

Morgan, T. H., and C. B. Bridges, 1919 Contributions to the genetics of *Drosophila melanogaster*. I. The origin of gynandromorphs. Pub. Carnegie Inst. No. 278:1-122.

Muller, H. J., 1948 The construction of several new types of Y chromosomes. D. I. S. 22:73.

Novitski, E., and G. Rush, 1948 Desemination by low temperature shocks. D. I. S. 22:75.

Parks, Hal B., 1936 Cleavage patterns in Drosophila and mosaic formation. Ann. Entomol. Soc. Amer. 29:350-392.

Patterson, J. T., and Wilson Stone, 1938 Gynandromorphs in Drosophila melanogaster. Univ. Texas Pub. No. 3825:5-65.

Roman, Herschel, 1947 Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. Genetics 32:391-409.

Schultz, Jack, and D. G. Catcheside, 1937 The nature of closed X-chromosomes in Drosophila melanogaster. J. Genet. 35:315-320.

Sturtevant, A. H., 1929 The claret mutant type of Drosophila simulans: a study of chromosome elimination and of cell-lineage. Zeitschr. f. wiss. Zool. 135:323-356.