UNSTABLE RING-*X* CHROMOSOMES DERIVED FROM A TANDEM METACENTRIC COMPOUND IN *DROSOPHILA MELANOGASTER*^{1,2}

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ALTHOUGH it is generally true that cells resulting from mitotic division are identical in genetic constitution, several exceptions to this basic tenet have been recognized. On the one hand, differences might arise from mitotic crossing over without chromosomal aberration (STERN 1936); on the other, whole chromosomes may be lost (McCLINTOCK 1938), abnormally segregated (STURTEVANT 1929), or broken (McCLINTOCK 1941). Regardless of the derivation of these genetically diverse cells, their origin during development produces a mosaic individual.

Some of the most thoroughly studied mosaics are gynandromorphs: organisms in which adjacent regions show the sexual dimorphism of male and female tissue. Extensive analyses of numerous gynandromorphs in *Drosophila* (MORGAN and BRIDGES 1919; BONNIER and LÜNING 1951) have led to the conclusion that the majority of gynandromorphs in this organism originate by *X*-chromosome elimination at an early cleavage mitosis in a female zygote.

It might be predicted that ring-shaped chromosomes would be prone to mechanical difficulties during chromosome replication and movement and would therefore suffer extensive loss or fragmentation during cell division. However the data from ring-*X* studies in *Drosophila melanogaster* provide little support for this notion. Contrary to expectation, the most noteworthy aspect of ring-chromosome behavior in this species has been its remarkable stability.

There has been some indication of mitotic ring loss with low frequencies for *R(1)1* (MORGAN 1933) and for *R(1)2* (BHATTACHARYA 1950), two ring-*X* chromosomes generally considered stable. *R(1)1* was spontaneously generated by a *C(1)RM*, γ female, whereas *R(1)2* is believed to have arisen as a detachment of a *C(1)RM*, γ^+ parent. Only one mitotically unstable ring produced by X-ray treatment of *R(1)2* has been previously identified (GRIFFEN and LINDSLEY 1946). This ring is designated *In(1)w^{vo}* and will be referred to here as *w^{vo}*. It and its rod-shaped derivatives have been extensively investigated and characterized (HINTON 1955, 1957, 1959).

While it has long been recognized that specially constructed attached-*X* chromosomes possessing one arm that is partially or completely inverted [a tandem

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metacentric (NOVITSKI 1954)] regularly generate circularly-shaped chromosomes as a result of meiotic exchange between the arms of the compound (NOVITSKI 1967), there has been little published evidence that rings produced in this way suffer any mitotic loss whatsoever. It was, then, of considerable interest when gynandromorphs which could only be explained on the basis of ring-X elimination were found with very high frequency in a tandem metacentric stock (PASZTOR 1967a).

The experiments described here were undertaken with the aim of characterizing the behavior of these chromosomes and establishing the factors controlling ring-chromosome instability.

MATERIALS AND METHODS

Origin and description of chromosomes: Females of the constitution $Y^S X \cdot Y^L$, $In(1)EN$, $K^S B f v w \gamma \cdot K^L \gamma^+ / XY^L \cdot Y^S$, $\gamma K^L \cdot K^S \gamma^+$ were irradiated with 3,000 r (LUCCHESI, MILLS and ROSENBLEETH 1965), and a tandem metacentric, $C(1)TM5$, was recovered which had the genotype $Y^S In(1)EN \cdot +$, $K^S B f v w \gamma \cdot \gamma$. In the course of experiments to determine which, if any fertility factors (BROSSEAU 1960) were present adjacent to the centromere of this compound-X chromosome, it was recognized that gynandromorphs appeared in one line. This line, presumably derived from $C(1)TM5$, was designated $C(1)TM5A$. Subsequent tests of $C(1)TM5A$ revealed that fertility factors were absent and vermilion was lost by crossing over. Taken together, these results indicate that the structure of this derived tandem metacentric, $C(1)TM5A$, was $Y^S In(1)EN \cdot +$, $K^S B f v w \gamma \cdot \gamma$ (PASZTOR 1967a). Cytological confirmation of the metacentric nature of $C(1)TM5A$ is shown in Figure 1a.

Ring chromosomes derived from $C(1)TM5A$ were designated $R(1)5A$, and this symbol together with specific code numbers denotes individual chromosomes. The two most thoroughly tested chromosomes directly isolated from $C(1)TM5A$ were $R(1)5A-3, \gamma w$ (Figure 1b) and $R(1)5A-42, \gamma w f B$ (Figure 1c). Other ring chromosomes obtained as a result of exchange with either a rod or another ring chromosome were given a CO designation. The origins and descriptions of these chromosomes are listed in Table 1. The circular morphology of each chromosome was identified cytologically in ganglion cells.

Genetic methods: $C(1)TM5A$ and $C(1)TM5$ females were mated to males bearing marked-Y chromosomes (BROSSEAU *et al.* 1961). Since their daughters possessed this Y chromosome, these could be tested in order to assess the frequency of X0 sons in the progeny of tandem metacentric/marked-Y females. These exceptional males were easily distinguishable from regular pat-

FIGURE 1.—Mitotic cytology of chromosomes. (a) $C(1)TM5A, Y^S In(1)EN \cdot +$, $K^S B f v w \gamma \cdot \gamma$. (b) $R(1)5A-3, \gamma w$. (c) $R(1)5A-42, \gamma w f B$.

TABLE 1

Origin and brief description of ring chromosomes

Brief designation	Genotype of female parent	Brief description
$R(1)5A-3, y w$	$C(1)TM5A, Y^S In(1)EN \cdot +, K^S B f w y \cdot y$	Unstable ring, requires X duplication for male survival.
$R(1)5A-42, y w f B$	$C(1)TM5A, Y^S In(1)EN \cdot +, K^S B f w y \cdot y$	Unstable ring, requires X duplication for male survival.
$R(1)5A-49, y w f B$	$C(1)TM5A, Y^S In(1)EN \cdot +, K^S B f w y \cdot y$	Unstable ring, requires X duplication for male survival.
$R(1)5A-51, y f B$	$C(1)TM5A, Y^S In(1)EN \cdot +, K^S B f w y \cdot y$	Unstable ring, requires X duplication for male survival.
CO $R(1)5A-30, y w$	$\frac{R(1)5A-3, y w^*}{y v}$	Unstable ring, does not require X duplication for male survival.
CO $R(1)5A-32, y w sn m$	$\frac{R(1)5A-3, y w}{XY^L \cdot Y^S, y^2 su(w^a) w^a sn m K^L \cdot K^S}$	Unstable ring, requires X duplication for male survival.
CO $R(1)5A-33, y w sn$	$\frac{R(1)5A-3, y w^*}{XY^L \cdot Y^S, y^2 su(w^a) w^a sn K^L \cdot K^S}$	Unstable ring, does not require X duplication for male survival.
CO $R(1)5A-40, y w$	$\frac{R(1)5A-42, y w f B}{y sc^4 sc^8}; B^S$	Unstable ring, lost f and B , requires X duplication for male survival.
CO $R(1)5A-107, y w ec f$	$\frac{R(1)5A-42, y w f B}{R(1)2, y^+ ec f}$	Unstable ring, requires X duplication for male survival, euchromatin substituted ($5.5 \rightarrow 57.0$).
CO $R(1)5A-120, y w^+$	$\frac{R(1)5A-3, y w}{R(1)2, y^+ ec f}$	Unstable ring, requires X duplication for male survival, euchromatin substituted ($1.5 \rightarrow 5.5$).
CO $R(1)2-b, y^+ w f B$	$\frac{R(1)5A-42, y w f B}{R(1)2, y^+ ec f}$	Stable ring, does not require X duplication for male survival, euchromatin substituted $w \rightarrow B$ ($1.5 \rightarrow 57.0$).
CO $R(1)2-1, y^+$	$\frac{R(1)5A-3, y w}{R(1)2, y^+ ec f}$	Stable ring, does not require X duplication for male survival, euchromatin substituted $ec \rightarrow f$ ($5.5 \rightarrow 56.7$).
CO $R(1)2-2, y^+ w f$	$\frac{R(1)5A-3, y w}{R(1)2, y^+ ec f}$	Stable ring, does not require X duplication for male survival, euchromatin substituted $w \rightarrow ec$ ($1.5 \rightarrow 5.5$).

* Parental male carried Y chromosome; in all other crosses $B^S Y$.

roclinous males in that they lacked the phenotype of the dominant markers carried on the maternal *Y* chromosome.

The constancy of *C(1)TM5A* behavior over a period of approximately two years was established by mating these compound-*X* females to males of various genotypes to determine whether the phenomenon of ring instability as it was first recognized was either a spurious abnormality or, perhaps, only apparent when males possessing the genotype of the original stock were used.

Ring-rod heterozygotes were mated to males bearing different rod-*X* chromosomes to establish a quantitative measure of ring instability. In conjunction with those experiments, ring males were also tested.

In order to localize the region of instability of *R(1)5A* chromosomes, a concerted effort was made to recover and then examine the behavior of ring chromosomes derived by crossing over with a rod or another ring chromosome.

Calculations: Loss of the ring chromosome during development was scored by the recovery of gynandromorphs whose male tissue displayed the recessive markers present on the rod-*X* homologue. The frequency of gynandromorphs may be conveniently expressed as the *G* ratio and is calculated:

$$G = \frac{\text{Gynandromorphs}}{\text{Gynandromorphs plus Ring-Rod } \varnothing \text{ } \varnothing} \quad (1)$$

Recognition of the *X0* male class was made possible by the use of a tagged-*Y* chromosome where the ring was introduced from the male parent. Regular males received this *Y* from their father, but *X0* males either received and subsequently lost the ring chromosome at the first zygotic division or arose from nondisjunction in ring males. It was considered that the latter process contributed to the formation of only a few *X0* males in view of the scarcity of ring/rod/marked-*Y* daughters in ring-male progeny.

X0 males were characterized by their patroclinous male phenotype when the ring was introduced via the female parent. These exceptional males could have originated from nullo-*X* eggs or loss of the ring from ring/rod zygotes. Two processes, primary nondisjunction and first-anaphase bridges resulting from four-strand double exchanges (STURTEVANT and BEADLE 1936) could lead to the production of nullo-*X* eggs. Mitotic ring loss rather than nullo-*X* egg production is the mechanism favored, for when ring/*In(1)dl-49* heterozygotes were tested, an extremely low frequency of exceptional daughters and no double crossover individuals were recovered. The contribution of nullo-*X* eggs to the *X0* class probably increased somewhat due to four-strand double exchanges when the ring was tested in ring/noninverted rod females, but in the calculations reported here, it is regarded that this increase was not of sufficient magnitude to warrant special consideration. For simplicity, therefore, *X0* males are treated together with gynandromorphs as a class arising from ring loss.

HINTON (1955) suggested that an estimate of the frequency of zygotes affected by ring instability could be made on the assumption that gametes bearing the ring and those bearing its homologue (either a rod-*X* or *Y*) are produced in a 1:1 ratio. If the rod/marked-*Y* or rod/rod class is regarded as free from lethality, it provides an estimate of the number of ring gametes produced. Thus, when the ring is contributed by the male parent, the ratio:

$$\text{Loss} = \frac{\text{Gynandromorphs plus } X0 \text{ males}}{\text{Rod } \delta \delta} \quad (2)$$

Dominant lethality is calculated by the formula proposed by HINTON (1955):

$$\text{D. L.} = \frac{\text{Rod } \delta \delta \text{ minus Ring-Rod } \varnothing \text{ } \varnothing \text{ minus } X0 \text{ } \delta \delta \text{ minus Gyn.}}{\text{Rod } \delta \delta} \quad (3)$$

It should be noted that (2) and (3) assume no lethality of the rod-bearing male progeny.

When ring/rod females are crossed to rod-*X* males, the rod/rod female class is substituted in equations (2) and (3) if it is in excess of the rod-bearing male class.

Although they do not necessarily derive from a common mechanism, the sum of the loss of

TABLE 2

Results from crosses with $C(1)TM5$, Y^s In(1)EN-+, K^s B f v y-y and $C(1)TM5A$,
 Y^s In(1)EN-+, K^s B f w y-y females $\times$ $XY^L.Y^s$, y^2 su(w^a) w^a sn m KL- K^s males

Cross	$C(1)TM$ parent	Homo- logue of $C(1)TM$	Homo- logue of $XY^L.Y^s$	Regular patro- clinous males	X0 males	$C(1)TM$ females	Ring- Rod females	Gynand- romorphs	Ring males	Total
I	$C(1)TM5$	None	B^sY	1066	*	283	306	6	269	1920
II	$C(1)TM5$	B^sY	None	682	25	231	242	1	206	1387
III	$C(1)TM5$	B^sY	B^sY	1068	31	316	382	7	294	2098
IV	$C(1)TM5A$	None	B^sY	1629	*	117	30	21	31	1828
V	$C(1)TM5A$	B^sY	None	5829	371	746	451	258	0	7655
VI	$C(1)TM5A$	B^sY	B^sY	2480	157	300	247	112	247	3543
VII	$C(1)TM5A$	Y	Y	1318	*	165	104	42	0	1603

* Cannot be distinguished.

the ring chromosomes (2) plus the loss of zygotes due to dominant lethality (3) estimates the total loss (T.L.) of ring-bearing female progeny.

RESULTS AND DISCUSSION

Comparison of $C(1)TM5$ and $C(1)TM5A$: Summaries of the data obtained from matings of tandem metacentric females are given in Table 2 and calculations (see MATERIALS AND METHODS) based on these figures are presented in Table 3. Although gynandromorphs comprise a proportion of the presumptive ring-bearing progeny of $C(1)TM5$ females, greater frequencies have been reported for $R(1)2$ (BHATTACHARYA 1950; BROWN and HANNAH 1952) which is usually regarded as a mitotically stable chromosome. Other than the infrequent occurrence of gynandromorphs, the data for $C(1)TM5$ are in accord with those presented for tandem metacentric compounds by other investigators.

The most striking feature of the aberrant behavior of the rings generated by $C(1)TM5A$ is the great frequency with which they are lost in mitotic division and thus the high incidence of gynandromorphs in the progenies of $C(1)TM5A$ females. Originally the sex mosaics were observed when stock males were

TABLE 3

*Comparison of the behavior of $C(1)TM5$ and $C(1)TM5A$ **

Homologue of $C(1)TM$	Homologue of $XY^L.Y^s$	G		$X0 \sigma\sigma$ Reg. pat. $\sigma\sigma$		$C(1)TM \text{ } \sigma\sigma$ Reg. pat. $\sigma\sigma$		Ring-bearing $\sigma\sigma$ Reg. pat. $\sigma\sigma$	
		$C(1)TM5$	$C(1)TM5A$	$C(1)TM5$	$C(1)TM5A$	$C(1)TM5$	$C(1)TM5A$	$C(1)TM5$	$C(1)TM5A$
None	B^sY	.019	.41	‡	‡	.27	.07	.29	.03
B^sY	B^sY	.018	.34	.03	.06	.29	.12	.39	.14
B^sY	None	.004	.36	.04	.06	.34	.13	.36	.12

* Calculations based on data presented in Table 2.

† Ring-bearing females include ring-rod heterozygotes plus gynandromorphs.

‡ Cannot be calculated.

$XY^L \cdot Y^S, \gamma^2 su(w^a)w^a K^L \cdot K^S/0$; but large G-values were also computed when the male parents possessed a variety of genotypes (Pasztor 1967b). These results favor the hypothesis that the origin of the gynandromorphism is a consistent characteristic of the rings regularly generated by $C(1)TM5A$.

As in the case of $C(1)TM5$, exceptional patroclinous males lacking the marked- Y chromosome from their mother but bearing the rod- X from their father were scored in the progenies of $C(1)TM5A$ females. What is of special interest here is that the incidence of this class (Table 3) is somewhat greater than that found in tests not only of $C(1)TM5$ but also of many other previously studied tandem metacentric chromosomes. Increased female nondisjunction may be disregarded as contributing to this excess, for in those tests where nondisjunctional $C(1)TM5A$ females could have been detected, their occurrence was rare and approximated that found in $C(1)TM5$ females. Data presented below suggest that $X0$ males are regularly produced from mitotic ring loss from ring/rod heterozygotes.

With respect to the effect of a homologue on ring recovery, the results of NOVITSKI and SANDLER (1956) and LINDSLEY and SANDLER (1965) are corroborated. Inspection of Table 3 reveals that although the exchange behavior of either $C(1)TM5$ or $C(1)TM5A$ as evidenced by the ratio of tandem metacentric females to patroclinous males remains fairly constant, ring recovery is enhanced when the tandem metacentric female parent carries a Y homologue. This greater recovery is reflected in the increased ratios of ring females to patroclinous males and appears to be characteristic of tandem metacentric chromosomes whether they generate stable or unstable ring chromosomes.

HINTON (1957) utilized an elegant method for converting w^{vc} into tandem metacentrics which retained intact the centromere and associated heterochromatic regions of the unstable ring chromosome. These compounds, designated $w^{vc} \cdot dl-49$, were divided into two groups distinguishable by their differences in gynandromorph, $X0$ male and tandem metacentric female recovery frequencies. Quantitative comparisons between $w^{vc} \cdot dl-49$, $C(1)TM5$, and $C(1)TM5A$ are inappropriate considering the heterogeneous nature of the stocks used in those and the experiments presented here. Recognizing this difficulty, we still find some interesting parallels in the data presented by HINTON for stable and unstable $w^{vc} \cdot dl-49$ and the results given in Table 2 for $C(1)TM5$ and $C(1)TM5A$. In both pairs there is a direct relationship between $X0$ male and gynandromorph frequencies in tandem metacentric female progeny. Furthermore, a deficit in metacentric chromosome bearing female progeny characterizes $w^{vc} \cdot dl-49$ stocks which produce high frequencies of $X0$ males and gynandromorphs. We estimate (Table 3) deficiencies of 17% (29% - 12%) and 21% (34% - 13%) in the frequency of tandem metacentrics based on the incidence of regular patroclinous males for $C(1)TM5A$ compared with $C(1)TM5$.

Unlike $w^{vc} \cdot dl-49$, however, rings newly generated by interarm exchange in $C(1)TM5A$ experience a high degree of dominant lethality as indicated by a depression in ring recovery. $X0$ male plus ring female and gynandromorph frequencies estimate total ring production. In the two tests for which this computa-

tion is made (Table 3) this estimate is 42% (39% + 3%) and 40% (36% + 4%) for *C(1)TM5*, whereas for *C(1)TM5A* we calculate only 20% (14% + 6%) and 18% (12% + 6%).

Behavior of *R(1)5A* chromosomes: Males bearing ring chromosomes were not found in the progenies of *C(1)TM5A* females when the male parent lacked a free-*Y* chromosome and would, therefore, have produced *R(1)5A/0* sons (cross V, Table 2). The results of cross VII in Table 2 show that whatever the origin of ring-male deficiency, it cannot be alleviated in *R(1)5A/Y* males. If a lethal were located in one arm of the tandem metacentric, all rings produced as a result of an exchange distal to the lethal would be inviable in hemizygous individuals. In the case of *C(1)TM5A*, this would indicate that the lethal must be located extremely close to or in the centric heterochromatin (see Figure 2) in view of the complete absence of ring-bearing males in the progenies of *C(1)TM5A* females in crosses V and VII in Table 2.

Rod-X/γ⁺Y and *rod-X/B^SY* males were chosen for mating to *C(1)TM5A* females because each carried *Y* chromosomes to which *X*-chromatin material is appended. Those of the former genotype yielded no ring males in their progeny (PASZTOR 1967b), while those of the latter did (crosses IV and VI, Table 2).

It has also been established that the *Y^{Su⁺-f}* chromosome (GETHMANN 1967) would permit ring-male survival (PASZTOR 1967a). These results demonstrate that the lethal factor responsible for the death of hemizygous ring-bearing individuals is localized in the heterochromatic region of the noninverted arm of *C(1)TM5A*, at or very near the *Su⁺-f* locus.

Mitotic instability of *R(1)5A* is manifested by the occurrence of gynandromorphs and *X0* males in the progenies of unstable ring-bearing individuals (Tables 4 through 6). Although some *X0* males may have arisen from other sources (see MATERIALS AND METHODS), little error is introduced if they are all considered to originate by ring elimination at the first cleavage division in a ring/rod heterozygote. It is apparent that *X0* males are found with varying frequencies, however, depending upon their genotypes. Unequal recoverability of

FIGURE 2.—The structure of *C(1)TM5A* and one out of many possible ring chromosomes generated by meiotic exchange. (Only two of the four strands of *C(1)TM5A* are shown.) The diagrams indicate euchromatin as a fine line, heterochromatin as a heavy line, and the centromere as a small open circle. The relative lengths of heterochromatin and euchromatin as well as the location of the exchange are arbitrarily designated.

TABLE 4

Results from crosses with R(1)5A introduced by the female parent

Cross	Female parent	Male parent	Rod males	Ring males	Rod-Rod females	Ring-Rod females	Gyn.	X ⁰ males	Total	G	Loss	D.L.	T.L.
I	R(1)5A-3,y w <u>y² su(w^a) K^L.K^S</u>	<u>In(1)dl-49,y v f car</u> Y	778	0	712	361	63	54	1968	.15	.15	.39	.54
II	R(1)5A-3,y w <u>In(1)dl-49,y v f car</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	1882	340	2475	439	246	262	5644	.36	.21	.62	.83
III	R(1)5A-3,y w <u>y² su(w^a) w^a sn m K^L.K^S</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	1336	331	816	476	368	96	3423	.44	.35	.30	.65
IV	R(1)5A-42,y w f B <u>In(1)dl-49,y v f car</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	874	61	951	136	118	172	2312	.46	.30	.55	.85
V	R(1)5A-42,y w f B <u>y² cv f car</u>	<u>In(1)dl-49,y v f car</u> BSY	1164	155	1323	286	91	87	3106	.24	.14	.65	.79
VI	R(1)5A-42,y w f B <u>y² cv f car</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	285	20	333	36	30	41	745	.45	.21	.68	.89
VII	R(1)5A-51,y f B <u>In(1)dl-49,y v f car</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	976	138	993	335	43	17	2502	.11	.06	.60	.66
VIII	R(1)5A-49,y w f B <u>y² su(w^a) w^a sn m K^L.K^S</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	1507	183	1811	478	226	79	4284	.32	.17	.57	.74
IX	R(1)5A-49,y w f B <u>In(1)dl-49,y v f car</u>	<u>y² su(w^a) w^a sn m K^L.K^S</u> BSY	2026	117	2118	411	160	33	4865	.28	.09	.71	.80
X	R(1)5A-49,y w f B <u>y sc⁴ sc⁸</u>	<u>y² su(w^a) w^a m f</u> Y	1273	0	2790	860	727	291	5941	.46	.37	.33	.70

X0 males of different genotypes has also been noted by HANNAH (1955) and by MITTLER and GERDY (1967).

R(1)5A chromosomes display instability whether introduced by way of the male or female parent (Tables 4 and 5). In the tests presented here no effort was made to prepare isogenic stocks and the variability in total loss (see MATERIALS AND METHODS) shown in the various crosses is probably a reflection of modifiers present in the different stocks utilized. Nevertheless, the data indicate that instability is not markedly influenced by cytoplasmic factors or maternal effects and is demonstrable regardless of parental origin of the ring. These results permit the interpretation that the control of instability is an inherent property of *R(1)5A* chromosomes.

As has been established, a lethal in or near the *Su⁺-f* region prevents the recovery of *R(1)5A/0* or *R(1)5A/Y* males. If ring instability were also associated with this factor, then those rings which do not require an *X* duplication for ring-male survival should be stabilized. That this is not the case, however, is shown by the behavior of two infrequently recovered rings, *CO R(1)5A-30, γ w* and *CO R(1)5A-33, γ w sn* which have lost the lethal either by crossing over with a rod chromosome or by spontaneous reversion. Males bearing either of these rings with a *Y* homologue are viable. The data summarized in Table 5, crosses VI, VII, IX, and X demonstrate that these rings are still eliminated during cleavage mitoses as signaled by the high incidence of gynandromorphs and *X0* males. What is also of interest here is that in crosses VI and IX where *X0* males can be scored with accuracy, dominant lethality as calculated by equation (3) has disappeared. It must be admitted, however, that the data from crosses VII and X make this conclusion somewhat less convincing. Although the *X0* male class cannot be distinguished, it seems rather unlikely that the great disparity between the rod-bearing males and ring-bearing females could be entirely attributable to *X0* males. We could also interpret the lack of dominant lethality measured in crosses VI and IX of Table 6 as originating in *B^sY* suppressing viability of regular rod males relative to the standard *Y* chromosomes in crosses VII and X. Taken together, then, the data given in Table 5 permit the conclusion that the segments of the ring chromosome in which the properties of hemizygous lethality and instability originate are independent. Furthermore, we conclude that the unstable behavior of *R(1)5A* is manifested by mitotic loss as expressed by gynandromorphs and *X0* males. Dominant lethality originating in meiotic or mitotic events also characterizes *R(1)5A*.

The locus of ring instability: In light of the fact that *R(1)5A* chromosomes are produced from *C(1)TM5A* by crossing over between the two arms of the attached *X*, there are numerous genetically distinct, circularly shaped chromosomes generated depending upon the location of the exchange. Table 4 gives data obtained from four different rings derived directly from *C(1)TM5A*. These results suggest that the feature all these rings have in common, the centromere and its adjacent heterochromatin, contains the locus of ring instability. Furthermore, *R(1)5A* may gain varying amounts of genetic material by exchange with rod chromosomes (see Table 5) and still retain its characteristic unstable behavior.

TABLE 5
Results from crosses with R(1)5A introduced by male parent

Cross	Female parent	Male parent	Rod males	X? males	Ring-Rod females	Gyn.	Total	G	Loss	D.L.	T.L.
I	$\gamma^2 su(w^a) w^a sn m Kl.K^S$	$R(1)5A-3,y w/BSY$	1995	352	554	420	3321	.43	.39	.34	.73
II	$ln(1)dl-49, y v f car$	$R(1)5A-3,y w/BSY$	1242	42	625	98	2007	.14	.11	.38	.49
III	$ln(1)dl-49, y v g f car$	$R(1)5A-3,y w/BSY$	819	97	402	106	1424	.21	.25	.26	.51
IV	$\gamma^2 su(w^a) w^a sn m Kl.K^S$	$R(1)5A-42,y w f B/BSY$	1694	317	210	399	2620	.66	.42	.45	.87
V	$ln(1)dl-49, y v f car$	$R(1)5A-42,y w f B/BSY$	1501	88	288	73	1950	.20	.11	.70	.81
VI	$\gamma^2 su(w^a) w^a sn Kl.K^S$	$CO R(1)5A-30,y w/BSY$	2705	89	2360	331	5485	.12	.16	None	.16
VII	$\gamma^2 su(w^a) w^a sn Kl.K^S$	$CO R(1)5A-30,y w/Y$	2095	+	1210	356	3661	.23	+	+	+
VIII	$\gamma^2 su(w^a) w^a sn m Kl.K^S$	$CO R(1)5A-32,y w sn m$ <i>BSY</i>	1167	60	804	181	2212	.18	.21	.10	.31
IX	$\gamma^2 su(w^a) w^a sn m Kl.K^S$	$CO R(1)5A-33,y w sn/BSY$	352	21	269	70	712	.21	.26	None	.26
X	$\gamma^2 su(w^a) w^a sn m Kl.K^S$	$CO R(1)5A-33,y w sn/Y$	3331	+	2451	441	6623	.15	+	+	+
XI	$\gamma^2 su(w^a) w^a sn m Kl.K^S$	$CO R(1)5A-40,y w/Y^{su+}$	523	+	61	84	668	.58	+	+	+

† Cannot be distinguished.

‡ Cannot be calculated.

If, as these considerations indicate, the primary locus of instability were confined to a single genetic segment of the ring chromosome, then this region could be analyzed by the derivation of rings which have gained or lost genetic material by crossing over.

Inspection of Table 6 reveals that stable rings derived from *R(1)2* (crosses I, II, and III) which possess varying amounts of euchromatin from the white through the Bar loci of an unstable ring, still display behavior consistent with that expected for *R(1)2*. Conversely, the results of test IV which uses an unstable ring chromosome possessing most of the euchromatin from the stable *R(1)2* chromosome show that ring instability is still manifest. In addition, *R(1)5A-120* (crosses V and VI) demonstrate continued instability with yet further euchromatin substitution of the unstable ring.

The data permit the interpretation that the locus of instability is outside the Bar to white region. Previous discussion of crossover ring chromosomes alluded to the finding that the hemizygous lethal present on the ring could be absent with no change in unstable ring behavior. Since the lethal is at or near the *Su⁺-f* locus (64.0) located in heterochromatin (COOPER 1959), the instability region is even more proximally located. Finally, the only regions not accounted for are the centromere (likely from a Y chromosome), the centric heterochromatin of uncertain provenance, and the euchromatin from the yellow to the white loci.

CONCLUSIONS

A comparison of the only two mitotically unstable ring-X chromosomes in *Drosophila*, *w^{vo}* and *R(1)5A*, reveals striking similarities and few departures in their behavior. Gynandromorphs, *X0* males, and dominant lethals among the prospective ring-bearing offspring of ring males or females characterize the unstable nature of both chromosomes. However the failure to observe increased nondisjunction, breakage products from *R(1)5A*, or its eventual stabilization constitute significant differences between the two.

On the basis of crossover ring-chromosome behavior and other considerations discussed previously, it is concluded that the control of *R(1)5A* instability resides in the chromosome itself, specifically in the centrometric, heterochromatic or yellow-white portion of the ring. HINTON's results for duplications (1955) and rod chromosomes (1957) produced from *w^{vo}* led him to restrict its instability locus to the very same region.

Analogous also are many characteristics of stable and unstable tandem metacentric chromosomes derived from *w^{vo}* compared with *C(1)TM5* and *C(1)TM5A*. Like unstable *w^{vo}·dl-49* tandem metacentric chromosomes, *C(1)TM5A* manufactures unstable ring chromosomes by the criteria stated above. Although *C(1)TM5A* suffers no mitotic loss as evidenced by the lack of superfemale/*X0* male mosaics, it is unstable by the criterion of dominant lethality. Unlike the rings newly generated by *w^{vo}·dl-49*, the data indicate dominant lethality for those produced by *C(1)TM5A*.

Anaphase bridges formed as a consequence of sister-strand union provided a

TABLE 6
*Results from crosses of unstable and stable rings recovered as products of crossing over with ring chromosomes**

Cross	Ring in male parent†	Stability	Female parent	Rod males	X0 males	Ring-Rod females	Gyn.	Total	G	Loss	D.L.	T.L.
I	CO R(1)2-b,y+ w f B	Stable	In(1)dl-49,y v f car	2425	7	2737	15	5185	.005	.008	None	.008
II	CO R(1)2-1,y+	Stable	In(1)dl-49,y v f car	571	2	649	6	1228	.009	.014	None	.014
III	CO R(1)2-2,y+ w f	Stable	In(1)dl-49,y v f car	2076	0	2642	11	4729	.004	.005	None	.004
IV	CO R(1)5A-107,y w ec f	Unstable	In(1)dl-49,y v f car	438	7	304	26	775	.08	.08	None	.08
V	CO R(1)5A-120,y w+	Unstable	In(1)dl-49,y v f car	412	8	327	26	773	.07	.08	.12	.20
VI	CO R(1)5A-120,y w+	Unstable	y ^a su(w ^a) w ^a sn m K ^L .K ^s	531	35	316	89	971	.22	.23	.17	.40

* For origin and description see Table 1.
† All males carried B^SY.

satisfactory mechanism to account for w^{vG} instability (HINTON 1955, 1957). Briefly stated, this hypothesis proposes that sister-strand fusion rather than sister-strand exchange occurs in unstable rings and their rod derivatives. In the former, the resultant dichromatid bridge is either (1) lost, converting a prospective female zygote into an XO male or gynandromorph or (2) broken, leading to a dominant lethal or duplication. In the latter, it is assumed that the anaphase bridge formed by a rod always breaks, producing dominant lethals. Our results are in agreement with HINTON's and suggest as did his that sister-strand exchange be discarded as the process responsible for anaphase bridge formation.

The unstable behavior of $C(1)TM5A$ and $R(1)5A$ can be also adequately explained on the basis of the anaphase bridge model cited above. This does not preclude the possibility that the locus of instability or the ultimate basis for sister-strand union is different for w^{vG} and $R(1)5A$.

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SUMMARY

Ring chromosomes regularly generated by meiotic exchange within the arms of a tandem metacentric display genetic behavior inconsistent with those previously reported for these types of chromosomes. Gynandromorphs which can only be explained by the elimination of ring chromosomes during development comprise from 34% to 41% of the sum of ring-rod plus gynandromorph classes in $C(1)TM5A$, $Y^S In(1)EN \cdot +$, $K^S B f v w \gamma \cdot \gamma$ progeny. A lethal in the extreme proximal region of the noninverted arm of the tandem metacentric results in the formation of viable ring-bearing sons only if they carry a Y chromosome to which this portion of the X has been appended.—Mitotic instability of rings produced by $C(1)TM5A$ is manifested by the high incidence of gynandromorphs as well as XO males in the progeny of ring-bearing individuals. Although subject to variability, ring instability resides in the circular chromosome *per se* and is demonstrable regardless of parental origin. The aberrant mitotic behavior of these circular chromosomes is localized to a segment restricted to the centromere, its centric heterochromatin, or a region from the yellow to the white loci. These conclusions regarding unstable $R(1)5A$ behavior are considered within a framework of those previously made for w^{vG} chromosomes.

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