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Genetic Analysis of the *claret* Locus of *Drosophila melanogaster*

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ABSTRACT

The *claret* (*ca*) locus of *Drosophila melanogaster* comprises two separately mutable domains, one responsible for eye color and one responsible for proper disjunction of chromosomes in meiosis and early cleavage divisions. Previously isolated alleles are of three types: (1) alleles of the *claret* (*ca*) type that affect eye color only, (2) alleles of the *claret-nondisjunctional* (ca^{nd}) type that affect eye color and chromosome behavior, and (3) a meiotic mutation, *non-claret disjunctional* (*ncd*), that affects chromosome behavior only. In order to investigate the genetic structure of the *claret* locus, we have isolated 19 radiation-induced alleles of *claret* on the basis of the eye color phenotype. Two of these 19 new alleles are of the ca^{nd} type, while 17 are of the *ca* type, demonstrating that the two domains do not often act as a single target for mutagenesis. This suggests that the two separately mutable functions are likely to be encoded by separate or overlapping genes rather than by a single gene. One of the new alleles of the ca^{nd} type is a chromosome rearrangement with a breakpoint at the position of the *claret* locus. If this breakpoint is the cause of the mutant phenotype and there are no other mutations associated with the rearrangement, the two functions must be encoded by overlapping genes.

MUTATIONS at the *claret* locus of Drosophila are pleiotropic, producing several distinct phenotypic effects. The *claret* locus of *Drosophila melanogaster* was originally identified on the basis of eye color mutants with reduced levels of both pteridines and ommochromes; several alleles of this type are known. The allelic mutation in *Drosophila simulans* (STURTEVANT and PLUNKETT 1926) has similar effects on eye color and also displays a greatly elevated frequency of meiotic nondisjunction and chromosome loss and mitotic chromosome loss in the early cleavage divisions (STURTEVANT 1929). This abnormal chromosome behavior is a consequence of disorganized spindles that appear in meiosis and early cleavage divisions (WALD 1936). A radiation-induced *claret* allele of *D. melanogaster*, *claret-nondisjunctional* (ca^{nd}), is phenotypically similar to *ca-simulans* (LEWIS and GENCARELLA 1952; DAVIS 1969; CHAN and DAVIS 1970; PORTIN 1978; KIMBLE and CHURCH 1983). A third type of mutation, *non-claret disjunctional* (*ncd*), displays the meiotic and mitotic phenotypes but has no effect on eye color (O'TOUSA and SZAUTER 1980). The phenotypic effects of these different alleles show that there are two separately mutable functions associated with the *claret* locus: one function required for normal eye color and one function required for normal chromosome behavior.

The two functions associated with the *claret* locus may be encoded by one or two genes. If they are encoded by a single gene, the separately mutable domains may be either distinct domains of a multifunctional gene product or entirely separate products produced by alternative modes of expressing a complex gene. If the two functions are encoded by two genes, mutations that affect both functions are either simple lesions in a region of overlap between the genes or small rearrangements that affect two separate but closely linked genes.

In order to facilitate physical analysis of the *claret* locus, we have isolated a number of new radiation-induced *claret* alleles on the basis of the eye color phenotype. Analysis of the phenotypic effects of these alleles addresses the organization of the *claret* locus, because the frequency of alleles of the ca^{nd} type among new alleles isolated solely on the basis of the eye color phenotype is related to the proximity of the two separately mutable domains. If the two functions are encoded by the same gene, we expect a high frequency of alleles of the ca^{nd} type among radiation-induced *ca* alleles. On the other hand, if the two functions are encoded by separate genes, we expect a low frequency of alleles of the ca^{nd} type among radiation-induced *ca* alleles. In the results reported here, we have found a low frequency of alleles of the ca^{nd} type among radiation-induced *ca* alleles, suggesting that the eye color

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and chromosome behavior functions are encoded by separate or slightly overlapping genes.

We have also examined the phenotypes of the new alleles with respect to mitotic loss of chromosomes during the early cleavage divisions, and find that alleles producing meiotic nondisjunction produce mitotic instability as well. This suggests that the mitotic instability of chromosomes produced by alleles of the ca^{nd} type is a consequence of the same biochemical lesion that produces the meiotic phenotype, an example of pleiotropy similar to that produced by other meiotic mutations (BAKER, CARPENTER and RIPOLL 1978).

MATERIALS AND METHODS

Culture conditions: All Drosophila cultures were grown on standard cornmeal, molasses, yeast and agar medium preserved with propionic acid and methyl *p*-hydroxybenzoate (WIRTZ and SEMEY 1982). Except for some of the crosses in large mutagenesis experiments, which were conducted at room temperature, all crosses were carried out at 25°.

Stocks: All chromosomes and markers used in this study are described in LINDSLEY and GRELL (1968), with the following exceptions: The *claret* allele denoted as ca^3 in this work was originally designated as $ca^{572jlIIa3}$. *Tubby* (*Tb*) is a larval marker described by CRAYMER (1980). *Outspread wing of Kreber* (Osw^K) causes held out wings (B. GANETZKY, personal communication). *TM6B*, D^3 *e ca* and *TM6C, e ca* are new versions of the third chromosome balancer *TM6* (CRAYMER 1984), while *TM6B, e Tb* Osw^K *ca* was derived from *TM6B, e Tb ca* by irradiation, and carries an inversion associated with the newly induced Osw^K mutation (B. Ganetzky, personal communication). Stocks were obtained from the Bowling Green and Cal Tech Stock Centers and the laboratories of L. SANDER and B. GANETZKY, or were constructed.

Mutagenesis: Gamma-ray induced *claret* alleles were isolated by irradiating Canton-S wild-type males with approximately 4000 rad from a ^{60}Co source (Gammacell 220, Atomic Energy of Canada, Ltd.) at a rate of about 1000 rad/min. Irradiated males were immediately mated to *TM6C, e ca* virgin females, using 5 males and 20 females per culture. The males were discarded 3 or 4 days after mating in order to sample only postmeiotically treated cells, while the females were transferred to fresh medium to collect additional broods.

The F_1 progeny were screened for claret animals. The number of F_1 progeny screened was estimated by counting all progeny from at least 5% of the cultures in each experiment. All claret individuals in the F_1 were crossed individually to *TM6C, e ca.* Non-ebony claret F_2 males resulting from the cross of new mutants to *TM6C* were selected and crossed to *TM6B*, D^3 *e ca/Ly* virgin females in order to establish stocks balanced with *TM6B*. These stocks were subsequently balanced with *TM6B, e Tb* Osw^K *ca.*

The eye color phenotype produced by *ca* is indistinguishable from that produced by mutations at several other loci. Dominant eye color mutations at these loci might initially be scored as *ca* F_1 progeny; these can be eliminated in the course of the crosses used to establish balanced stocks. Dominant eye color mutations on other than the third chromosome can be identified by observing independent segregation with respect to the third chromosome markers on the balancer chromosomes, and dominant eye color mutations on the third chromosome can be identified by scoring eye color in animals heterozygous for the *Ly* third chromosome.

Malpighian tubule color: The Malpighian tubules of third instar larvae were examined by dissecting larvae in Ephrussi-Beadle Ringer's solution (130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$). The color of freshly dissected Malpighian tubules from larvae carrying *claret* alleles was compared to that of larvae carrying other eye color mutations (BREHME and DEMEREC 1942).

Polytene chromosome cytology: Chromosomes carrying newly induced *ca* alleles were examined cytologically. Heterozygous larvae were produced by crossing males from balanced or homozygous stocks to Canton-S wild-type females or to females carrying a marked third chromosome known to be structurally normal. Larvae were reared at 18° prior to dissection of salivary glands. Larvae heterozygous for the *TM6B, e Tb* Osw^K *ca* balancer were distinguished from those heterozygous for third chromosomes carrying new *ca* alleles using the *Tb* marker. Permanent slides of polytene chromosomes were prepared by the method of ATHERTON and GALL (1972), modified as follows: Third instar larvae were washed in distilled water and dissected in Ephrussi-Beadle Ringer's solution. Salivary glands were freed of adhering fat body and transferred to freshly prepared 3:1 ethanol:acetic acid. After 2–5 min of fixation, salivary glands were transferred to a drop of 45% acetic acid on a siliconized coverslip. Coverslips were picked up using slides coated with gelatin. Salivary gland tissue was dissociated by stroking the coverslip with a dissecting needle. The chromosomes were then flattened by heating the slide for several minutes on a slide warming tray set to 45°. After freezing the slides on dry ice, coverslips were removed with a razor blade, and the slides were dehydrated in 95% ethanol. The slides were air dried prior to staining with Giemsa in 20 mM sodium phosphate buffer (pH 7.5). Stained slides were dried in air, and coverslips were permanently mounted using Preservaslide (EM Science). Slides were examined with a Zeiss Axioplan microscope and photographed using Kodak Technical Pan Film. The film was processed with Kodak HC-110 developer using the procedure recommended for moderate contrast.

Analysis of mitotic chromosome loss: Females carrying wild-type *X* and fourth chromosomes and a third chromosome combination to be tested for its effect on mitotic stability were crossed to *y w* sn^3 f^{36a} or *y w* sn^3 f^{36a}; *C(4)RM, ci* ey^R/O males, using a single female and three to five males per vial. The progeny were examined for mosaicism with respect to sex or cuticle markers. Both marked gynandromorphs, in which the male tissue is marked with *y w* sn^3 f^{36a}, and unmarked gynandromorphs, in which the male tissue is phenotypically wild type, can be scored among the progeny of these crosses. Marked gynandromorphs originate as regular (mono-*X*) ova fertilized by *X*-bearing sperm, with subsequent mitotic loss of the maternally derived *X* chromosome. Unmarked gynandromorphs may result from either of two mechanisms: (1) the mitotic loss of the paternally derived *X* chromosome from zygotes originating as mono-*X* ova fertilized by *X*-bearing sperm, or (2) the mitotic loss of a maternally derived *X* chromosome from zygotes originating as diplo-*X* ova fertilized by *Y*-bearing sperm.

Analysis of meiotic and mitotic chromosome behavior: The *X* and fourth chromosomes of stocks carrying *ca* alleles were replaced with marked chromosomes carrying *y* and spa^{pol}, respectively. Marked females were crossed to males carrying a reversed metacentric compound fourth chromo-

some ($C(4)RM$, *ci* ey^R/O) and a Canton-S wild-type *X* chromosome, using a single female and three to five males per vial.

All possible regular and exceptional segregational classes of the *X* and fourth chromosomes can be scored in these crosses. With respect to the *X* chromosome, all regular (mono-*X*) ova are recovered as wild-type females if fertilized by *X*-bearing sperm, or as yellow males if fertilized by *Y*-bearing sperm. Half of the exceptional (diplo-*X* or nullo-*X*) ova are recovered. Half of the diplo-*X* ova are recovered as matroclinous females (phenotypically yellow) if fertilized by *Y*-bearing sperm, while half of the diplo-*X* ova are lost as inviable metafemales if fertilized by *X*-bearing sperm. Half of the nullo-*X* ova are recovered as patroclinous males (phenotypically wild type) if fertilized by *X*-bearing sperm, while half of the nullo-*X* ova are lost as inviable *YO* zygotes if fertilized by *Y*-bearing sperm.

With respect to the fourth chromosome, half of all regular (mono-*4*) or exceptional (diplo-*4* or nullo-*4*) ova are recovered in these crosses. Males carrying the compound fourth chromosome and no homologous fourth chromosome ($C(4)RM$, *ci* ey^R/O) produce sperm that are diplo-*4* and nullo-*4* in equal proportions. Half of the regular (mono-*4*) ova are recovered as zygotes trisomic for the fourth chromosome (phenotypically wild type), while half are recovered as zygotes monosomic for the fourth chromosome (phenotypically sparkling-poliert and Minute). Because Minute animals exhibit erratic viability, they are excluded from all tabulations, so half of the mono-*4* ova may be considered lost. Half of the exceptional (diplo-*4* or nullo-*4*) ova are recovered. Half of the diplo-*4* ova are recovered as zygotes disomic for the fourth chromosome (phenotypically sparkling-poliert) if fertilized by nullo-*4* sperm, while half of the diplo-*4* ova are lost as inviable zygotes tetrasomic for the fourth chromosome if fertilized by diplo-*4* sperm. Half of the nullo-*4* ova are recovered as zygotes disomic for the fourth chromosome (phenotypically cubitus interruptus and eyeless) if fertilized by diplo-*4* sperm, while half of the nullo-*4* ova are lost as inviable zygotes nullosomic for the fourth chromosome if fertilized by nullo-*4* sperm.

Mitotic loss of the *X* or fourth chromosomes can be detected in the progeny of these crosses. Loss of an *X* chromosome during cleavage divisions in an *XX* zygote produces an *XX*-*XO* gynandromorph. If the maternally derived *X* chromosome (marked with *y*) is lost, the gynandromorph consists of unmarked male and female tissue and can only be detected if the animal is mosaic with respect to sexually dimorphic parts (sex combs, genitalia or terminal abdominal segments). If the paternally derived *X* chromosome is lost, the gynandromorph consists of unmarked female and yellow male tissue, and can be detected if any part of the adult cuticle is mosaic. In these crosses, *XXY* matroclinous exceptions are recovered as yellow females; loss of one of the maternally derived *X* chromosomes produces an *XXY*-*XY* gynandromorph consisting of yellow male and female tissue, which can be detected if two sexually dimorphic structures in a mosaic individual are of different sex.

Mitotic loss of the fourth chromosome is less readily detected in these crosses. Loss of the maternally derived spa^{pol} fourth chromosome during cleavage divisions in a zygote trisomic for the fourth chromosome ($spa^{pol}/C(4)RM$, *ci* ey^R) produces a trisomic-disomic mosaic animal in which the disomic parts are phenotypically cubitus interruptus and eyeless. Because these two markers affect only small parts of the adult (part of the wing and the entire eye, respectively) and because both markers show variable expressivity, it is difficult to distinguish these mosaics from nonmosaic animals that are wholly cubitus interruptus and eyeless. Loss of the paternally derived compound fourth chromosome during cleavage divisions in a trisomic zygote produces a trisomic-monosomic mosaic animal in which the monosomic parts are Minute and marked with spa^{pol}. This class is only detected if at least part of the eyes are monosomic and at least part of the cuticle elsewhere is trisomic. Mitotic loss of one of the maternally derived fourth chromosomes in a zygote resulting from a disomic ovum (spa^{pol}/spa^{pol}) produces a disomic-monosomic mosaic animal (marked with spa^{pol}) in which the monosomic parts are Minute. This class is readily detected if the mosaic patches are large, but such animals may have reduced viability. Mitotic loss of the paternally derived compound fourth chromosome in a zygote resulting from a nullosomic ovum ($C(4)RM$, *ci* ey^R/O) produces an inviable disomic-nullosomic mosaic animal.

Crosses designed to measure third chromosome crossing over were carried out with one heterozygous female and two to five *ru h th e ca* males per vial.

Statistical methods: The standard errors of the frequencies of exceptions and map lengths were assumed to be binomial; that is, $(p(1 - p)/N)^{1/2}$, where p is the frequency exceptions or recombination and N is the corrected total number of progeny observed in the experiment (SZAUTER 1984).

RESULTS

Isolation of new *ca* alleles: Approximately 254,000 F_1 progeny were screened in a series of 19 experiments. Of 117 F_1 animals initially scored as having a claret-like eye color, 19 were sterile, 42 failed to breed true, 21 carried unlinked dominant eye color mutations, and 33 carried new *ca* alleles. No dominant mutations on the third chromosome were recovered as potential *ca* alleles. Included among the sterile progeny and those that failed to breed true were six mosaic animals with one claret-like eye and one wild-type eye. Thirty-one of the new *ca* alleles were established as permanent stocks; two were lost as a result of poor viability or fertility. The 31 *ca* alleles established as permanent stocks were all independently isolated except for one cluster of ten alleles (ca^{16}) and one cluster of four alleles (ca^{10}) arising from the same culture bottles. If all members of a cluster resulted from a single event, the 31 alleles established as permanent stocks represent nineteen independent mutational events.

The experimental design ensures the recovery of independent mutations within a culture unless one of the irradiated males is not removed prior to the time at which clusters of sperm derived from a mutant stem cell are produced. However, it is possible that a cluster may be the result of a spontaneous mutation that occurred in the germ line of one of the irradiated males or in the germ line of one of his wild-type parents. All mutant chromosomes from the culture producing the cluster of ten alleles (ca^{16}) are identical with respect to two unusual properties: (1) they all carry a third-chromosome recessive lethal mutation while most other irradiated third chromosomes car-

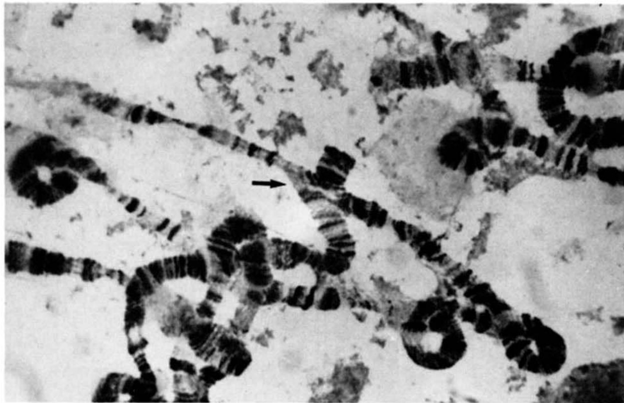

FIGURE 1.—Polytene chromosomes from a larva heterozygous for $T(2;3)ca^{nd2}$. The arrow indicates the position of the translocation breakpoint.

rying new *ca* alleles do not, and (2) they all produce light yellow Malpighian tubules in heteroallelic larvae (ca^{16}/ca), while all other *ca* alleles produce colorless Malpighian tubules. The light yellow Malpighian tubules of ca^{16} are intermediate in color between those produced by *carmine* (*cm*) and *cinnabar* (*cn*). The eye color produced by each of the new alleles, including ca^{16}, is indistinguishable from that produced by *ca* at the level of examination under a dissecting microscope.

Cytogenetic analysis: Males from balanced or homozygous stocks of all new *ca* alleles were crossed to Canton-S wild-type females to produce heterozygous larvae; the salivary gland polytene chromosomes from these larvae were examined in order to detect chromosome rearrangements.

Both new alleles of the ca^{nd} type are associated with chromosome rearrangements. The ca^{nd2} mutation is associated with a translocation between chromosome arms 2R and 3R with breakpoints at 44AB and 100B, designated $T(2;3)ca^{nd2}$; a photomicrograph is shown in Figure 1. The breakpoint at 100B in 3R is near the cytological position of the *ca* locus (99B5–9) but distinct from it. The ca^{nd3} mutation is associated with a chromosome rearrangement at 99B, the cytological position of the *ca* locus. In $ca^{nd3}/+$ heterozygotes, the chromosome banding of the 99B region is altered by what appears to be a small inversion. In well-stretched preparations, the 99B region does not synapse properly with the homologous chromosome. Four views of the ca^{nd3} rearrangement are shown in Figure 2. The ca^{5} mutation was found to be associated with a complex chromosome rearrangement having two breakpoints in chromosome arm 3R, in regions 90C and 92D, and one breakpoint in chromosome arm 2R at 33A. It therefore appears that ca^{5} is associated with an insertional translocation that is free of rearrangement breakpoints near the *claret* locus. None of the other *ca* mutations are associated with cytologically detectable chromosome rearrangements.

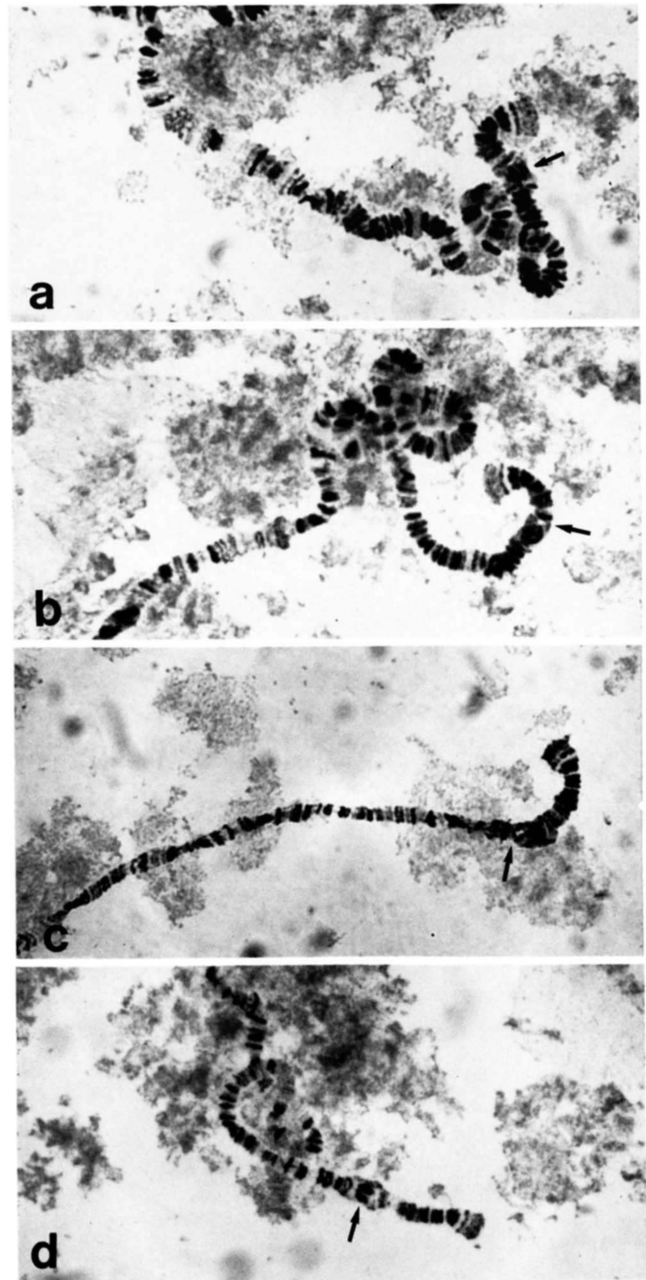

FIGURE 2.—Polytene chromosomes from larvae heterozygous for ca^{nd3}. The arrow indicates the position of the *claret* locus and the associated rearrangement: a) polytene chromosome bands turned sideways appear at 99B; b) asynapsis appears at the distal end of the rearrangement, starting just proximal to 99C; c) asynapsis appears at the proximal end of the rearrangement, starting just distal to 99A; and d) asynapsis appears throughout the 99B region, with the heavy bands of 99B flanked by asynapsis on both sides.

We have confirmed the cytological analysis of several *ca* alleles with genetic experiments. Because inversions and translocations act as dominant chromosome-specific suppressors of crossing over [see ROBERTS (1976) for a review], we measured crossing over in females heterozygous for wild-type or *ca* mutant third chromosomes. The results are presented in Table 1. The wild-type control chromosome used in

TABLE 1

Results of crossing females heterozygous for multiply marked third chromosomes and a wild-type or *ca* third chromosome to *ru h th e ca* males

	Third chromosome			
Class[a]	Canton-S	ca^5	ca^{nd2}	ca^{nd3}
NCO	1396	349	259	277
1	349	244	109	105
2	328	142	102	104
3	389	47	155	218
4	513	80	76	173
1, 2	14	27	12	9
1, 3	123	14	56	57
1, 4	195	34	28	62
2, 3	142	14	64	61
2, 4	195	34	23	52
3, 4	139	13	19	52
1, 2, 3	19	3	6	6
1, 2, 4	15	7	3	6
1, 3, 4	29	6	6	13
2, 3, 4	24	4	4	7
1, 2, 3, 4	2	0	1	1
Total	3872	1018	923	1203
Map length[b]:				
Region 1	19.3 (0.6)	32.9 (1.5)	23.9 (1.4)	22.7 (1.2)
Region 2	19.1 (0.6)	22.7 (1.3)	23.3 (1.4)	21.5 (1.2)
Region 3	22.4 (0.7)	9.9 (0.9)	33.7 (1.6)	36.3 (1.4)
Region 4	28.7 (0.7)	17.5 (1.2)	17.3 (1.2)	32.0 (1.4)
Total	89.5 (2.7)	83.0 (4.9)	98.2 (5.6)	112.5 (5.3)

[a] Progeny are tabulated by crossover class, listed by the third chromosome region or regions in which crossovers were observed (*e.g.*, "1, 2" is a double crossover in regions 1 and 2). "NCO" denotes the noncrossover class. The regions are: (1) *ru-h*, (2) *h-th*, (3) *th-e* (4) *e-ca*. The experiment with the unmarked Canton-S third chromosome used heterozygotes with a third chromosome carrying all of the markers (*ru h th e ca*); in the other experiments, the third chromosome under study carries a *ca* allele, and was heterozygous with a third chromosome carrying all of the markers except *ca* (*ru h th e*).

[b] Map lengths (in cM) are presented with standard errors (see MATERIALS AND METHODS). The standard map positions for the markers used are: *ru* (0.0), *h* (26.5), *th* (43.2), *e* (70.7), *ca* (100.7). The standard map lengths derived from these positions are as follows: *ru-h* (26.5), *h-th* (16.7), *th-e* (27.5), *e-ca* (30). The observed map lengths are expected to be less in all cases as a consequence of undetected double crossovers within a region.

these experiments is derived from Canton-S, the same stock that we used as a parental stock for mutagenesis. As shown in Table 1, the ca^5 third chromosome, which is associated with an insertional translocation, reduces the frequency of crossing over in two adjacent regions, between *th* and *e* and between *e* and *ca*. The ca^{nd2} third chromosome, which is associated with a reciprocal translocation, reduces the frequency of crossing over between *e* and *ca*, the interval immediately proximal to the third chromosome breakpoint. The ca^{nd3} chromosome has no effect on crossing over, except for a possible increase in crossing over between *th* and *e*.

Recessive lethality: Four of the newly recovered *claret* alleles (ca^{nd2}, ca^{nd3}, ca^{12} and ca^{16}) behaved as recessive lethal mutations in balanced stocks. Because the chromosomes carrying new *claret* alleles were recovered and maintained as heterozygotes with multiply inverted balancer chromosomes, the recessive lethality could have been produced by mutations anywhere on the third chromosome. To determine whether the recessive lethal mutations in these stocks were allelic, balanced stocks carrying these four *claret* alleles were crossed in all possible combinations. All heteroallelic combinations were viable, demonstrating that the recessive lethality of these four chromosomes is the consequence of mutations in four different complementation groups, and therefore in all but at most one case is not associated with mutations at the *claret* locus. In addition, we were able to remove the associated recessive lethal mutations from the third chromosomes of two of the *claret* mutants (ca^{12} and ca^{16}) by allowing recombination with a Canton-S wild-type chromosome. We were unable to separate the recessive lethality of ca^{nd3} from the *ca* mutation in a small experiment (12 recombinant chromosomes tested), and did not attempt to separate the recessive lethality of ca^{nd2} from the *ca* mutation. The cytogenetic analysis of these rearrangements suggests that it is impractical to dissociate ca^{nd2} and ca^{nd3} from their associated chromosome rearrangements.

Chromosome behavior: Each new *ca* allele was tested for its effect on the mitotic stability of the *X* chromosome by crossing females homozygous for *ca* alleles or heteroallelic for a new *ca* allele and ca^{nd} to *y w* sn^3 f^{36a} or *y w* sn^3 f^{36a}; *C(4)RM, ci* ey^R/O males. This cross also permits the recognition of nullo-*X* ova, one of the classes of exceptional ova resulting from meiotic nondisjunction or loss. Results are shown in Table 2.

Three *claret* alleles (ca^{nd}, ca^{nd2} and ca^{nd3}) produce a high frequency of gynandromorphs. The original allele of this type, ca^{nd}, produced 209 gynandromorphs among a total of 1823 zygotic females, a frequency of 11.5%. Two new alleles also produce a high frequency of gynandromorphs: ca^{nd2} produced 10.7% (3/28) gynandromorphs, while ca^{nd3} produced 13.8% (48/349) gynandromorphs. The observed gynandromorphs are of two types, marked and unmarked. Marked gynandromorphs have male tissue marked with *y w* sn^3 f^{36a} and result from the mitotic loss of the maternally derived wild-type *X* chromosome in *y w* sn^3 f^{36a}/+ embryos. Unmarked gynandromorphs have wild-type male tissue and result from either the mitotic loss of the paternally derived *X* chromosome in *y w* sn^3 f^{36a}/+ embryos or the mitotic loss of one of the maternally derived *X* chromosomes in exceptional +/+/*Y* females. Unmarked gynandromorphs are more difficult to identify because they can only be detected when two sexually dimorphic structures in one individual are of different sex. In order to estimate the frequency of unmarked gynandromorphs that were

TABLE 2

Results of crossing females carrying wild-type *X* and fourth chromosomes and the indicated third chromosomes to $y\ w\ sn^3\ f^{36a}$ or $y\ w\ sn^3\ f^{36a}$; *C(4)RM, ci* ey^R/O males

Third chromosomes[a]	+ Female	Gynandromorphs Marked[b]	Gynandromorphs Unmarked[c]	+ Male	$y\ w\ sn^3\ f^{36a}$ Male[d]	Exceptional mosaic[e]	Total
Canton-S	2139	0	0	2032	3	0	4174
ca^{nd}	1614	185	24	1547	191	0	3561
ca^{nd2}/ca^{nd}	25	2	1	25	3	0	56
ca^{nd3}/ca^{nd}	301	43	5	262	41	0	652
ca	263	0	0	310	1	0	574
ca^3	269	0	0	300	0	0	569
ca^4	667	0	0	660	0	0	1327
ca^5	820	0	0	817	1	1	1639
ca^6	382	0	0	443	0	0	825
ca^7	1033	0	0	1011	0	0	2044
ca^8	408	0	0	448	1	0	857
ca^9	1104	0	0	1121	0	0	2225
ca^{10}	946	1	0	896	3	0	1846
ca^{11}	496	0	1	540	0	0	1037
ca^{12}/ca^{nd}	275	0	0	294	1	0	570
ca^{13}	656	0	0	604	2	2	1264
ca^{14}	338	0	0	322	1	0	661
ca^{15}	1246	0	0	1179	1	0	2426
ca^{16}/ca^{nd}	364	0	0	348	0	0	712
ca^{17}	307	0	0	237	0	0	544
ca^{18}	801	0	0	807	0	0	1608
ca^{19}	525	0	0	498	0	0	1023
ca^{20}	347	0	0	343	0	0	690

[a] Third chromosomes carrying recessive lethal mutations were tested as heterozygotes with ca^{nd}.
[b] Marked gynandromorphs are those in which the female tissue is wild type and the male tissue is marked with $y\ w\ sn^3\ f^{36a}$.
[c] Unmarked gynandromorphs are those in which both the male and the female tissue is phenotypically wild type.
[d] Males marked with $y\ w\ sn^3\ f^{36a}$ are patroclinous exceptions, resulting from the fertilization of ova nullosomic for the *X* chromosome by *X*-bearing sperm.
[e] Exceptional mosaics are animals containing both wild type and $y\ w\ sn^3\ f^{36a}$ tissue with sexual differentiation unlike that expected in marked gynandromorphs (see text for explanation).

not detected, we analyzed all marked gynandromorphs recovered from ca^{nd}/c^{nd} females, recording the phenotypes of each of 93 cuticular landmarks for each gynandromorph. Analysis of this data showed that approximately 70% of our marked gynandromorphs could have been identified as gynandromorphs if the male tissue had been unmarked. Therefore, if the fraction of the adult cuticle that is male is similar between the marked and unmarked gynandromorphs, we expect to be able to detect the majority of unmarked gynandromorphs. Despite the difficulty of identifying unmarked gynandromorphs, the excess of marked over unmarked gynandromorphs in these experiments indicates that maternally derived *X* chromosomes are more likely to be lost mitotically than are paternally derived *X* chromosomes.

It is possible to estimate the relative frequencies of maternal-loss and paternal-loss gynandromorphs from the data in Table 2. Of the 209 gynandromorphs recovered from ca^{nd}/ca^{nd} mothers, 185 were marked and 24 were unmarked. If all of the unmarked gynandromorphs result from the mitotic loss of the paternally derived *X* chromosomes in $y\ w\ sn^3\ f^{36a}/+$ females, and the frequency of unmarked gynandromorphs that can be detected is 70% (as estimated above), then the relative frequencies of maternal and paternal *X* chromosome mitotic loss are 84% (185/219) and 16% (34/219). For the 48 gynandromorphs recovered from ca^{nd3}/ca^{nd} mothers, similar calculations produce frequencies of 86% (43/50) maternal-*X* loss and 14% (7/50) paternal-*X* loss. Because of the poor fertility of ca^{nd2}/ca^{nd} mothers, too few gynandromorphs were recovered to permit meaningful calculations.

In the experiments presented in Table 2, we also observed "exceptional mosaics." These are mosaic animals containing both marked and unmarked tissue with sexual differentiation unlike that expected in marked gynandromorphs. The exceptional mosaic recovered from ca^5 had both unmarked and $y\ w\ sn^3\ f^{36a}$ tissue, but all sexually dimorphic structures in both tissue types were male. One of the exceptional mosaics recovered from ca^{13} was a wild-type male with a portion of the head marked with $y\ w\ sn^3\ f^{36a}$ (this tissue did not include any sexually dimorphic structures). These exceptional mosaics may have resulted from double mitotic loss of the paternally derived *X* chromosome in one lineage and of the maternally derived *X* chromosome in another lineage during the devel-

opment of a regular female, or some other unusual occurrence. The other exceptional mosaic recovered from ca^{13} was also a wild-type male with a portion of the head marked with $y\ w\ sn^3\ f^{36a}$; this mosaic was also missing a sex comb on one wild-type foreleg. The absence of a sex comb on one wild-type foreleg of this exceptional mosaic is characteristic of female development, and suggests that the wild-type tissue was itself chromosomally mosaic, which is also consistent with the double mitotic loss of the maternally derived and paternally derived *X* chromosomes in different lineages, with the loss of the paternally derived chromosome delayed in the wild-type lineage. The *ca* alleles carried by the mothers of these exceptional mosaics (ca^5 and ca^{13}) are not alleles of the ca^{nd} type, and did not otherwise affect chromosome behavior. Exceptional mosaics were not found among the progeny of females carrying alleles of the ca^{nd} type. Therefore, whatever the mechanism giving rise to exceptional mosaics, it does not appear to be associated with the effects of alleles of the ca^{nd} type on mitotic chromosome loss.

The results presented in Table 2 also show that ca^{nd}, ca^{nd2} and ca^{nd3} all produce meiotic nondisjunction or chromosome loss. Exceptional ova nullosomic for the *X* chromosome are detectable in these crosses, resulting in patroclinous males that are genotypically $y\ w\ sn^3\ f^{36a}/O$. Because half of the nullo-*X* ova are fertilized by *Y*-bearing sperm to produce inviable *YO* zygotes, the actual frequency of nullo-*X* ova produced by the three alleles may be calculated by doubling the number of patroclinous males. These calculations show that all three alleles produce comparable frequencies of ova nullosomic for the *X* chromosome. The frequencies of nullo-*X* ova are: for ca^{nd}, 10.2% (382/3762), for ca^{nd2}, 10.2% (6/59), and for ca^{nd3}, 11.8% (82/693). All of the other genotypes tested produce patroclinous males at spontaneous frequencies; the highest frequencies of nullo-*X* ova observed among alleles of the *ca* type were from ca^{12} and ca^{13} mothers, which both produced 0.7% nullo-*X* ova.

In summary, our new *claret* alleles are of two types: those of the *ca* type, which produce neither meiotic nor mitotic chromosome loss, and those of the ca^{nd} type, which produce both meiotic and mitotic chromosome loss. We have not recovered any *ca* alleles that affect one type of chromosome behavior but not the other, although there is no selection against such alleles in our screen.

The experiments presented in Table 2 do not fully document the meiotic effects of each *ca* allele; only a single exceptional segregational class (nullo-*X* ova) can be detected. In order to compare the meiotic effects of ca^{nd} and ca^{nd3}, stocks were constructed in which the *X* and fourth chromosomes were marked with *y* and spa^{pol}, respectively. (The ca^{nd2} mutation is associated with a reciprocal translocation; heterozygosity for the translocation in combination with the ca^{nd} phenotype results in extremely poor fertility, making the further analysis of this mutation impractical.) Crosses were performed that permit the identification of all regular and exceptional segregational classes for the *X* and fourth chromosome. These crosses also permit the identification of mosaic progeny resulting from the mitotic loss of the *X* or fourth chromosome (see MATERIALS AND METHODS). The results are presented in Table 3.

The data in Table 3 show the number of observed progeny of each phenotypic class. Among the progeny in these experiments are gynandromorphs resulting from the mitotic loss of an *X* chromosome in an *XX* zygote. Because the maternally derived *X* chromosome is marked with *y* and the paternally derived *X* chromosome is wild type, mitotic loss of the maternally derived *X* chromosome will lead to an unmarked gynandromorph, while mitotic loss of the paternally derived *X* chromosome will lead to a marked gynandromorph, the opposite of the results presented in Table 2. Because matroclinous exceptions resulting from diplo-*X* ova are wholly marked with *y*, the occurrence of marked gynandromorphs unequivocally demonstrates that ca^{nd} and ca^{nd3} cause the mitotic loss of paternally derived *X* chromosomes. Unmarked gynandromorphs can only result from the mitotic loss of maternally derived *X* chromosomes. The results confirm that there is a preferential mitotic loss of the maternally derived *X* chromosome in zygotes produced by ca^{nd} and ca^{nd3}. Correcting for the detection of approximately 70% of the unmarked gynandromorphs (as before), 93% (63/68) of the gynandromorphs produced by ca^{nd} and 90% (43/48) of those produced by ca^{nd3} result from the loss of the maternally derived *X* chromosome. These results are comparable to those obtained in the experiments presented in Table 2, even though it was not possible to distinguish between loss of paternally derived *X* chromosomes in regular females and loss of maternally derived *X* chromosomes in matroclinous exceptional females as the source of unmarked gynandromorphs in those experiments. The results presented in Table 3 show that matroclinous exceptions are rare, supporting the conclusion that virtually all of the unmarked gynandromorphs in the previous experiments must result from loss of paternally derived *X* chromosomes in regular females.

We also observed mitotic loss of the fourth chromosome in these experiments. Because the fourth chromosome markers do not permit the detection of many of the mosaic progeny (see MATERIALS AND METHODS), mosaics of this type are not tabulated separately, but rather are tabulated as the zygotic genotype. Mitotic loss of both the maternally derived

TABLE 3

Results of crossing females carrying the indicated third chromosomes and *X* and fourth chromosomes marked with *y* and spa^{pol} to +/*Y*; *C(4)RM, ci ey*^{R}/O males

	y/+ Females			*y*/*Y* Males			*y*/*y* Females			+/O Male			Gynandromorphs[a]				
													Unmarked[b]		Marked[c]		
	ci ey/ *ci ey*/ *spa*	*ci ey*/ *ci ey*	*spa*/ *spa*	*ci ey*/ *ci ey*/ *spa*	*ci ey*/ *ci ey*/	*spa*/ *spa*	*ci ey*/ *spa*	*ci ey*/ *ci ey*	*spa*/ *spa*	*ci ey*/ *spa*	*ci ey*/ *ci ey*	*spa*/ *spa*	*ci ey*/ *ci ey*/ *spa*	*ci ey*/ *ci ey*	*ci ey*/ *ci ey*/ *spa*	*ci ey*/ *ci ey*	Total
+/+	1550	1	0	1222	2	0	0	0	0	2	0	0	0	0	0	0	2777
ca^{nd}/ca^{nd}	93	124	3	67	84	1	8	7	0	27	80	0	12	32	1	4	543
ca^{nd3}/ca^{nd}	122	105	9	102	78	2	0	3	0	23	32	4	9	21	2	3	515

[a] No gynandromorphs were observed among the small number of diplo-*4* female progeny homozygous for spa^{pol}.
[b] Unmarked gynandromorphs are those in which both male and female tissue is wild type.
[c] Marked gynandromorphs are those in which the female tissue is wild type and the male tissue is marked with *y*.

TABLE 4

Ova classified by *X* and fourth chromosome segregational type from the data in Table 2

Third chromosome	X4	XO	X44	XX4	XXO	XX44	O4	OO	O44	Total
+/+	2772	3	0	0	0	0	4	0	0	2779
ca^{nd}/ca^{nd}	173	244	4	16	14	0	54	160	0	665
ca^{nd3}/ca^{nd}	235	207	11	0	6	0	46	64	8	577

Data are corrected to reflect the loss of half of the *X*-exceptional zygotes (see MATERIALS AND METHODS).

fourth chromosome and the paternally derived compound fourth chromosome was observed. Because mitotic loss of the maternally derived fourth chromosome results in mosaics marked with *cubitus interruptus* or *eyeless*, markers that have variable expressivity, the occurrence of these types was verified by progeny testing two of these mosaics. Animals thought to be mosaics of this type were crossed to spa^{pol}/spa^{pol}; both wild-type and non-Minute sparkling-poliert progeny were recovered, demonstrating that at least some cells in the germ line carried a spa^{pol} fourth chromosome in addition to the paternally derived compound fourth chromosome. Mitotic loss of the paternally derived compound fourth chromosome can be reliably scored because the sparkling-poliert and Minute phenotypes have uniform expressivity and complete penetrance.

The frequency of each segregational class of ova can be calculated from the data in Table 3 by combining different phenotypic classes that originate from genetically identical ova and correcting for the loss of half of the *X*-exceptional ova (see MATERIALS AND METHODS). For example, wild-type females, yellow males, and both marked and unmarked gynandromorphs originate as regular (mono-*X*) ova; the different phenotypic classes are the consequence of fertilization by different sperm types or the mitotic loss of an *X* chromosome in *XX* zygotes. The corrected frequencies of each segregational class of ova, calculated from the data in Table 3, are presented in Table 4.

The data in Table 4 show that females carrying ca^{nd} or ca^{nd3} produce a high frequency of gametes exceptional for the *X* and fourth chromosome. For both the *X* and fourth chromosome, nullosomic gametes are more frequent than are disomic gametes. Because meiotic nondisjunction is expected to produce equal frequencies of nullosomic and disomic gametes, this demonstrates that meiotic chromosome loss rather than nondisjunction is the major cause of exceptional gametes. The frequencies of gametes exceptional for the *X* chromosome, the fourth chromosome, and simultaneously exceptional for both are presented in Table 5.

The results in Table 5 show that the frequency of exceptions produced by ca^{nd}/ca^{nd} and ca^{nd}/ca^{nd3} females is comparable, although somewhat lower for ca^{nd}/ca^{nd3} females. In both cases, high frequencies of exceptions for the *X* and fourth chromosomes are found. The simultaneous nondisjunction or loss of both the *X* and fourth chromosomes is observed more frequently than expected on the basis of independence.

DISCUSSION

Comparison of results to previous studies: We have recovered two new radiation-induced *claret* alleles of the ca^{nd} type and compared their effects on mitotic and meiotic chromosome behavior to the original ca^{nd} allele. We find that the mitotic effects of ca^{nd2} and ca^{nd3} are similar to the mitotic effects of ca^{nd}: there is a high frequency of mitotic loss of *X* chromosomes during the development of *XX* zygotes, leading to the production of gynandromorphs. There is a strong preference for the loss of maternally derived *X* chromosomes rather than paternally derived chromo-

TABLE 5

Frequencies of *X* and fourth chromosome exceptions from the data in Table 3

Allele	*X* Exceptions	*4* Exceptions	Observed *X-4* exceptions	Expected *X-4* exceptions[a]	N
+	0.0014 (0.0007)	0.0011 (0.0006)	0.00	10^{-6}	2779
ca^{nd}	0.367 (0.019)	0.635 (0.019)	0.26 (0.017)	0.233	665
ca^{nd3}	0.201 (0.017)	0.513 (0.021)	0.14 (0.021)	0.103	577

The frequency of ova exceptional for the X chromosome is calculated, using the corrected frequencies from Table 3, as (*XX4* + *XXO* + *XX44* + *O4* + OO + *O44*)/Total. The frequency of ova exceptional for the fourth chromosome is similarly calculated as (*XO* + *X44* + *XXO* + *XX44* + *OO* + *O44*)/Total. The frequency of ova exceptional for both the X and the fourth chromosome is calculated as (*XXO* + *XX44* + OO + *O44*)/Total. The frequencies are presented with standard errors (see MATERIALS AND METHODS).

[a] The expected frequency of ova exceptional for both the *X* and fourth chromosome is calculated under the assumption of independence, that is, as the product of the frequencies of *X* chromosome exceptions and fourth chromosome exceptions.

somes, although it is clear that paternally derived *X* chromosomes can be lost as well. Alleles of the ca^{nd} type that produce mitotic loss also produce meiotic nondisjunction and chromosome loss; the frequency of nondisjunction or loss of the *X* chromosome is similar among the three alleles. There is no evidence that the meiotic and mitotic effects of mutations of the ca^{nd} type are separately mutable; all alleles either exhibit both phenotypes or neither.

Our results may be compared to those from previous studies. STURTEVANT (1929) performed crosses of *Drosophila simulans* females homozygous for *ca* (an allele resembling ca^{nd} of *Drosophila melanogaster*) to males carrying marked *X* chromosomes, a cross resembling the cross presented in Table 2. He observed gynandromorphs at a frequency of 16.2%; these consisted of 88% unmarked and 12% marked gynandromorphs. The data obtained by Sturtevant for *ca-simulans* therefore show a slightly higher frequency of gynandromorphs, but a similar disparity in the frequency of marked and unmarked gynandromorphs, reflecting a greater tendency for maternal *X* chromosome loss. The frequency of nullo-*X* ova, estimated from the frequency of patroclinous males, was 29.1%, nearly three times the frequency of nullo-*X* ova observed in our experiments. This may be a consequence of the decreased viability or delayed hatching of *XO* males carrying the *y w* sn^3 f^{36a} chromosome, because we observed a higher frequency of nullo-*X* ova in experiments in which the patroclinous exceptions were marked with *y* alone.

We were also able to detect the mitotic loss of chromosomes in experiments primarily conducted to document meiotic chromosome behavior. These results may be compared to previous studies as well. STURTEVANT (1929) crossed *ca-simulans* females homozygous for *X*-linked markers to males not carrying the same *X*-linked marker. As in the cross presented in our Table 3, three types of gynandromorphs are distinguishable among the progeny of this cross: (1) gynandromorphs resulting from the mitotic loss of the maternally derived *X* chromosome in regular female zygotes, (2) gynandromorphs resulting from the mitotic loss of the paternally derived *X* chromosome in regular female zygotes and (3) gynandromorphs resulting from the mitotic loss of one of the maternally derived *X* chromosomes in exceptional female zygotes. STURTEVANT found that 64 of the 66 gynandromorphs in his experiment were of the first type, two of the 66 gynandromorphs were of the third type, and none was of the second type. On the basis of these results, STURTEVANT concluded that the *ca-simulans* mutation did not cause the loss of paternally derived *X* chromosomes, and attributed the appearance of unmarked gynandromorphs in his first experiment to loss of one of the maternally derived *X* chromosomes in exceptional females, rather than to the loss of the paternally derived *X* chromosome in regular females. In contrast, in a similar experiment presented in Table 3, we have detected gynandromorphs of the first and second type, but none of the third type. On the basis of our results, we conclude that ca^{nd} can cause the loss of paternally derived chromosomes, and interpret the results presented in Table 2 on this basis.

HINTON and MCEARCHERN (cited in DAVIS 1969) crossed *y/y*; ca^{nd}/ca^{nd} females to *+/Y*; spa^{pol}/spa^{pol} males, an experiment similar to that presented in Table 3. In this experiment, all three classes of gynandromorphs are distinguishable. In an earlier report on the same experiment, HINTON and MCEARCHERN (1963) report that gynandromorphs attributable to paternal chromosome loss were found. Combining the data presented in the two reports, the frequency of regular gynandromorphs was 12.6%, of which 91.1% resulted from maternal *X* chromosome loss and 8.9% resulted from paternal *X* chromosome loss. These results are similar to ours.

Our results on the meiotic behavior of chromosomes in ca^{nd} mothers are comparable to those of DAVIS (1969), who crossed *y/y*; ca^{nd}/ca^{nd}; ci^D/spa^{Cat} females to *+/Y*; *C(4)RM*, +/O males. Using the same methods of calculation as for the data presented in Table 5, the frequencies of exceptional ova with standard errors are as follows: for the *X* chromosome, 0.317 (0.006), for the fourth chromosome, 0.691 (0.006) and for both the *X* and fourth chromosome, 0.267

(0.006) (see Table 2 of DAVIS 1969). These results are similar to ours despite the differences in experimental design.

The nature of the eye color and chromosome behavior functions: The *claret* mutation causes a reduction in the levels of both pteridines (red pigments) and ommochromes (brown pigments). The likely biochemical basis of the Malpighian tubule and eye color phenotypes produced by *claret* mutations is a reduced ability to transport metabolic intermediates into Malpighian tubules. Pteridines are synthesized from GTP, while ommochromes are synthesized from tryptophan [see PHILLIPS and FORREST (1980) and SUMMERS, HOWELLS and PYLIOTIS (1982) for reviews]. Because these two classes of pigments have distinct biosynthetic pathways, the biochemical basis of the eye color phenotype produced by *claret* cannot be a substrate or enzymatic activity shared by the two pathways. The synthesis of ommochromes in the developing eye disc requires not only the enzymes that carry out the reactions, but also the proper transport of metabolic intermediates between different tissues in which particular biosynthetic steps take place. The Malpighian tubules of larvae carrying *claret* and other mutations that affect both pigment pathways have been shown to be defective in the transport of kynurenine, an intermediate in ommochrome biosynthesis (SULLIVAN and SULLIVAN 1975; HOWELS, SUMMERS and RYALL 1977). Some eye color mutations that interfere with kynurenine transport have also been shown to block the transport of guanine, and hence would be expected to be deficient in pteridine biosynthesis (SULLIVAN *et al.* 1979). There is evidence on the molecular basis of the transport defect for two eye color loci. Both the *white* locus, which is required for both pteridine and ommochrome biosynthesis, and the *brown* locus, which is required for pteridine biosynthesis, have sequence similarity to each other and to components of bacterial active transport systems (MOUNT 1987; DREESEN, JOHNSON and HENIKOFF 1988).

Several lines of evidence show that the effects on chromosome behavior produced by the pleiotropic mutations *ca-simulans* and *claret-nondisjunctional* must have a biochemical basis distinct from the eye color defect. First, the eye color phenotype produced by the ca^{nd} allele is indistinguishable from that produced by the *ca* allele, and there is no difference between the two alleles with respect to the pteridines present (CHAN and DAVIS 1970). Second, mutations that are epistatic to the eye color effects of ca^{nd} have no effect on chromosome behavior, showing that the chromosome behavior phenotype does not result from the accumulation of some toxic intermediate in pteridine or ommochrome biosynthesis (STURTEVANT 1956). Third, the chromosome behavior phenotype produced by ca^{nd} is autonomous when mutant ovaries are transplanted to wild-type hosts, showing that abnormal chromosome behavior can occur in animals that have normal pteridine and ommochrome biosynthesis in most of their somatic tissue (ROBERTS 1962). Finally, the phenotype of the *non-claret disjunctional* (*ncd*) mutation, which fails to complement the chromosome behavior defect of ca^{nd} but complements the eye color defect, also demonstrates that a defect in pteridine and ommochrome biosynthesis is not required for abnormal chromosome behavior (O'TOUSA and SZAUTER 1980).

Several different hypotheses have been proposed to explain the chromosome behavior phenotype produced by *claret-simulans* and *claret-nondisjunctional.* Any hypothesis must explain the basic observations, which are summarized here. First, meiotic chromosome behavior in ca^{nd}/ca^{nd} females is abnormal, with high frequencies of nondisjunction and chromosome loss of all chromosome pairs, despite normal pairing and recombination (STURTEVANT 1929; DAVIS 1969). Second, mitotic chromosome behavior in zygotes from *ca-simulans* or ca^{nd} mothers is abnormal during the first few cleavage divisions, leading to the production of genetic mosaics through chromosome loss (STURTEVANT 1929; DAVIS 1969; PORTIN 1978). Third, there is no generalized effect on mitosis in animals homozygous for *ca-simulans* or ca^{nd}; that is, homozygous progeny from heterozygous mothers grow normally with no evidence of the degree of mitotic nondisjunction or chromosome loss observed among the progeny of homozygous mothers. Finally, both ca^{nd} and *ca-simulans* females produce zygotes that exhibit abnormalities during the earliest stages of development. These include disorganized meiotic and early cleavage spindles with abnormal numbers of poles (both unipolar and multipolar spindles are seen), abnormalities in the number or shape of nuclei formed following a division, and failure of the cleavage nuclei to disperse (WALD 1936; KIMBLE and CHURCH 1983).

It is evident from these observations that the basis of meiotic and mitotic chromosome loss caused by ca^{nd} is the faulty segregation of chromosomes in morphologically abnormal spindles. The mitotic phenotype of ca^{nd} is observed only as a maternal effect, demonstrating that defective mitotic spindles result from the persistence of the meiotic spindle defect into the early cleavage divisions. Either the spindle component that is defective in ca^{nd} is dispensable in later cleavage divisions or the egg cytoplasm contains an analogous maternally supplied spindle component, used for the later divisions, which gradually displaces the faulty spindle component as new spindles are assembled. The spindle phenotype produced by the ca^{nd} mutation, which is confined to meiotic and early cleavage spindles, is distinct from that produced by another class of mutations that affect mitotic spindle organi-

zation throughout development. These mutations include the recessive lethal mutations *polo* (SUNKEL and GLOVER 1988) and *mgr* (GONZALEZ, CASAL and RIPOLL 1988), both of which produce both unipolar and multipolar spindles in larval tissues similar to the abnormal spindles produced by ca^{nd} in meiosis and early cleavage. In contrast to ca^{nd}, the spindle defects produced by *polo* and *mgr* are observed in homozygous larvae that are the progeny of heterozygous mothers. The delay in the onset of the spindle phenotype in *polo* and *mgr* suggests that maternally supplied wild-type gene product is capable of supporting the early mitotic divisions. It is possible that mutations like *polo* and *mgr* identify genes that encode, regulate or modify components of the spindle that displace the spindle component affected by the ca^{nd} mutation during the early cleavage divisions.

DAVIS (1969) proposed that the defective spindle component produced by ca^{nd} is the spindle fiber. BAKER and HALL (1976) proposed that the defective spindle component produced by ca^{nd} is the centromere region or kinetochore. KIMBLE and CHURCH (1983) proposed that the defective spindle component produced by ca^{nd} is the microtubule organizing center of the spindle pole. If the centrosome can act to organize the cytoskeleton, the hypothesis of KIMBLE and CHURCH explains not only the occurrence of unipolar and multipolar spindles but also the failure of cleavage nuclei to disperse. The evidence for a role of the centrosome in organizing the cytoskeleton was indirect at the time that KIMBLE and CHURCH proposed their model. For example, ZALOKAR and ERK (1976) found that both colchicine and cytochalasin B interfered with the dispersal of the cleavage nuclei, an effect similar to the phenotype of ca^{nd}; both the spindle abnormalities and the abnormal dispersion of the cleavage nuclei could be explained by proposing that defective centrosomes fail to properly organize both spindles and the cytoskeleton.

Since the publication of the work of KIMBLE and CHURCH, there have been a number of studies on the cytoskeleton of Drosophila embryos and the involvement of centrosomes in the organization of the cytoskeleton at the syncytial blastoderm stage [see WARN (1986) for an overall description]. KARR and ALBERTS (1986) studied the organization of the cytoskeleton in Drosophila embryos at the syncytial blastoderm stage using indirect immunofluorescence, and found that during nuclear cycle 10, the division of the centrosomes results in a redistribution of not only the microtubules of the cytoskeleton, as expected, but also cortical actin filaments. This suggests that the centrosome can act as an organizing center for actin filaments. Spindle poles at the syncytial blastoderm stage also stain with rhodamine-labeled phalloidin, demonstrating an association of cortical actin filaments with centrosomes (WARN, MAGRATH and WEBB 1984). Microinjection of antitubulin antibodies at a concentration sufficient to inactivate microtubules results in a loss of surface cap structure, further supporting a role of the centrosomes in organizing cortical actin filaments at the syncytial blastoderm stage (WARN, FLEGG and WARN 1987). Visualization of microtubules and microfilaments by the microinjection of fluorescent tubulin or actin supports the description derived from fixed material (KELLOGG, MITCHISON and ALBERTS 1988). However, although these studies indicate that centrosomes may play a role in organizing the cytoskeleton at the syncytial blastoderm stage, there is at present no direct evidence that they do so at earlier cleavage stages, or that actin microfilaments are involved in the dispersal of cleavage nuclei during the earliest nuclear cycles.

The preferential mitotic loss of maternally derived chromosomes was emphasized by BAKER and HALL (1976) as evidence that the defective spindle component produced by ca^{nd} is the centromere region rather than a spindle component such as spindle fibers or the centrosome. Under this view, ca^{nd} is a maternal effect mutation resembling the paternal effect mutation *paternal loss* (*pal*), which causes the mitotic loss of paternally derived chromosomes transmitted by homozygous males. BAKER (1975) has argued that *pal* causes defective centromere regions or kinetochores in paternally derived chromosomes because: (1) only paternally derived chromosomes are lost in zygotes from *pal* fathers, (2) paternally derived chromosomes are only lost during the first few cleavage divisions, suggesting that the defect is repaired by chromosomal replication in a wild-type cytoplasm, and (3) *X* chromosomes differ in their sensitivity to *pal*, and this polymorphism maps to the proximal region of the *X* chromosome.

We have shown that ca^{nd} causes the loss of paternally derived chromosomes as well as maternally derived chromosomes. If defective centromere regions or kinetochores are the basis of the ca^{nd} phenotype, this defect must somehow be able to affect paternally derived chromosomes. There are two possible explanations for this. First, defective maternally derived chromosomes might disrupt spindle organization, causing the loss of paternally derived chromosomes. In this case, the chromosomal defects produced by ca^{nd} and *pal* must be different, with the *pal* defect being incapable of disrupting spindle organization. KIMBLE and CHURCH (1983) have argued that it is unlikely that defective centromere regions or kinetochores would be capable of disrupting spindle organization, because kinetochores are thought to capture microtubules originating from the poles, rather than playing an active role in organizing the spindle. A second explanation that reconciles a chromosomal

defect produced by ca^{nd} with the observed loss of paternally derived chromosomes is that components of maternally derived kinetochores might "contaminate" the paternally derived kinetochores by diffusion. This seems unlikely, because the restoration of normal mitosis after a few divisions demonstrates that the zygotic cytoplasm contains all components necessary for the assembly of normal kinetochores. Furthermore, because the restoration of normal mitosis occurs well before the onset of zygotic transcription at the syncytial blastoderm stage, the components necessary for normal division must be maternally supplied and present in the zygotic cytoplasm at the early divisions.

It is possible to account for the preferential loss of maternally derived chromosomes if the spindle component affected by ca^{nd} is the centrosome rather than the kinetochore. The first cleavage division in Drosophila is gonomeric; that is, it occurs prior to karyogamy (HUETTNER 1924, 1933). The maternally derived and paternally derived chromosomes undergo the first cleavage division on closely apposed but distinct spindles. If ca^{nd} causes a defect in the maternally derived centrosome, and the sperm carries a normal centrosome in addition to the paternal pronucleus, then paternally derived chromosomes should be immune to the effects of ca^{nd} prior to karyogamy. PORTIN (1978) has estimated that two-thirds of the loss of the maternally derived *X* chromosome occurs at the first division, so if loss of the paternally derived *X* chromosome can occur only after the first division, the expected phenotype of ca^{nd} would be the preferential loss of maternally derived chromosomes.

Organization of the *claret* locus: There are three general models of the genetic organization of the *claret* locus that explain the pleiotropic effects of mutations of the ca^{nd} type. First, the *claret* locus may be a single genetic unit that encodes a complex multifunctional protein or that is capable of producing distinct protein products. Second, the *claret* locus may consist of a pair of distinct but overlapping genes. Third, the *claret* locus may consist of two separate but closely linked genes. The results of our search for new radiation-induced alleles of the *claret* locus help to distinguish among these three possible models, suggesting that the the two functions are unlikely to be encoded by a single gene.

If the *claret* locus is a single complex gene, we expect that amorphic alleles that produce no active gene product would lack both the eye color and chromosome behavior functions. The complex loci of Drosophila include several well-characterized genes that are known to have two or more distinct functional domains [see Scott (1987) for a review]. For example, the *rudimentary* (*r*) locus encodes a multifunctional protein that catalyzes three different steps of pyrimidine biosynthesis. Mutations at the *rudimentary* locus can affect one or more of these activities. If the *claret* locus is a gene of this type, we would expect that a collection of mutations that includes amorphic alleles would include mutations of the ca^{nd} type.

If the *claret* locus consists of two distinct but overlapping genes (*ca* and *ncd*), alleles of the ca^{nd} type could be point mutations or rearrangement breakpoints in the region of overlap or rearrangements involving the nonoverlapping parts of both genes. There are examples of overlapping genes in Drosophila; the best characterized example is the *Gart* locus, which contains a pupal cuticle protein gene in one of its introns (HENIKOFF *et al.* 1986).

If the *claret* locus consists of two entirely separate genes (*ca* and *ncd*), alleles of the ca^{nd} type could be rearrangements involving both genes. The two genes must be in close proximity, because (1) both functions have mutated together in at least four cases (*ca-simulans*, ca^{nd}, ca^{nd2} and ca^{nd3}, and (2) the ca^{nd} allele cannot be separated into two mutations by recombination (CHAN and DAVIS 1970). The original ca^{nd} allele is cytologically normal, but it could be a rearrangement too small to be cytologically detected. If there is no vital locus between the genes, ca^{nd} could be a cytologically undetectable deficiency. It is also possible that ca^{nd} is a cytologically undetectable inversion with a breakpoint in adjacent genes.

We have isolated nineteen new radiation-induced alleles of the *claret* locus on the basis of the eye color phenotype. Of these, two are of the ca^{nd} type, producing effects both on eye color and on chromosome behavior, while seventeen are of the *ca* type, producing only the eye color phenotype. This result demonstrates that alleles of the ca^{nd} type are not the result of a very rare mutational event, such as the simultaneous mutation of two widely separated genes. On the other hand, alleles of the ca^{nd} type are not frequent among *ca* alleles. The low frequency of alleles of the ca^{nd} type among radiation-induced alleles argues against the hypothesis that the *claret* locus is a single complex gene, as long as amorphic alleles are not selected against in our experiments. In addition, the ca^{nd3} mutation appears to be a small inversion with a breakpoint at the position of the *claret* locus. If this mutation has a single breakpoint as the only lesion at the *claret* locus, the two functions must be encoded by overlapping genes, with the ca^{nd3} breakpoint in the region of overlap.

It is important to consider whether amorphic alleles of either function would be selected against in our mutagenesis experiment. The mutant alleles were isolated using a scheme in which the new alleles were isolated and placed into balanced stocks with no selection against recessive lethal alleles or alleles of the ca^{nd} type. There is no reason to believe that amorphic

mutations in either the *ca* or *ncd* domain would be selected against in our mutagenesis scheme. Because our scheme does not select against recessive lethal mutations, amorphic alleles could be recovered even if either function is vital. Four of the alleles, including both alleles of the ca^{nd} type, behave as recessive lethal mutations, but because all of these alleles complement for viability, in all but at most one case the recessive lethality is not the result of mutation at the *claret* locus. Furthermore, in the case of two of the recessive lethal alleles not associated with chromosome rearrangements, we were able to separate the *ca* mutation from the recessive lethal mutation by recombination. These results suggest that the *claret* locus is incapable of mutating to a recessive lethal phenotype, and therefore the function of the *claret* locus is probably dispensable. It is possible that our collection of *claret* alleles includes truly amorphic alleles of the *ca* domain that nevertheless have no effect on chromosome behavior. Amorphic mutations of the ca^{nd} type might produce a meiotic phenotype so strong as to cause female sterility. We have only been able to test the two new alleles of the ca^{nd} type, ca^{nd2} and ca^{nd3}, as heterozygotes with ca^{nd}. The effects on chromosome behavior are comparable to those observed in ca^{nd} homozygotes. Because the effects of ca^{nd}, ca^{nd2} and ca^{nd3} are similar, it cannot be that the homozygous viable allele (ca^{nd}) is hypomorphic, while the alleles associated with recessive lethality (ca^{nd2} and ca^{nd3}) are amorphic. Unless amorphic alleles of the ca^{nd} type act as dominant female sterile mutations that would be selected against in our screen, it may be that the phenotype produced by ca^{nd}, ca^{nd2} and ca^{nd3} is the consequence of a complete absence of *ncd* function.

There are no deficiencies among the new *claret* alleles that we have obtained. Previous studies of the genetic organization of the *claret* region explain these results: there is a strong selection against deficiencies that remove a very closely linked Minute locus, *M(3)1*. FRISARDI and MACINTYRE (1984) used *X* rays to induce *claret* alleles on *Dp(3:1)B152*, a duplication of the tip of 3R (98F14 to the telomere) on the right arm of the *X* chromosome. The *claret* alleles in their study were isolated in *Dp(3:1)B152/Y; ca/ca* males that have at least two copies of all material from 98F14 to the tip in addition to any material remaining on the mutagenized duplication. Of 37 *ca* alleles characterized as deficiencies, all lacked the *M(3)1* locus, which was previously known to be closely linked to *ca*. The *M(3)1* locus is a haplo-insufficient Minute locus that causes slow development and reduced body size. KONGSUWAN, DELLAVALLE and MERRIAM (1986) constructed synthetic deficiencies for the 99BC interval and found that deficiencies for regions including 99B5–9 produced a strong Minute phenotype and were sterile. This led them to conclude that either this region includes *M(3)1* and another more extreme Minute or that the mutation *M(3)1* is hypomorphic. Because the design of our mutagenesis experiments does not permit the recovery of deficiencies for the Minute locus or loci in the *ca* region, we do not expect to find cytologically visible deficiencies among our new *ca* alleles. If the Minute locus is very closely linked to the *ca* locus, as the results of FRISARDI and MACLNTYRE (1984) suggest, we expect that inversions or translocations with breakpoints in the *ca* locus might be difficult to recover as well. Indeed, several of the original mutant progeny from our mutagenesis experiments identified as having a claret-like eye color were phenotypically Minute and were sterile. The extremely close linkage of *M(3)1* to *ca* means that a definitive test of whether a particular *ca* allele is amorphic is not possible, because the lack of deficiencies means that it is impossible to determine whether the compound heterozygote for a particular *ca* allele and a deficiency is phenotypically identical to an animal homozygous for the same *ca* allele.

In summary, our results address the question of the organization of the *claret* locus in two ways. First, the low frequency of alleles of the ca^{nd} type among radiation-induced alleles of *claret* demonstrates that the *ncd* domain is a distinct target for mutagenesis, and is unlikely to be part of the same gene as the *ca* function. Second, one of our alleles of the $caca^{nd}$ type appears to be a small inversion with a breakpoint at the position of the *claret* locus. If this breakpoint is the cause of the mutant effect and there are no other mutations associated with the rearrangement, the two functions must be encoded by overlapping genes, with the rearrangement breakpoint identifying the region of overlap. Although we believe that our results support the model that the separately mutable *ca* and *ncd* domains are encoded by overlapping genes, a molecular analysis of the *claret* locus and its mutant alleles is required to resolve this question.

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