THE GENETICS OF A MUTABLE GENE AT THE WHITE LOCUS OF *DROSOPHILA MELANOGASTER*[1]

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ALMOST coincidental with the rediscovery of Mendelism, genes of high spontaneous mutation rate, often referred to as unstable, labile or mutable genes, were found. Such mutable genes, although not described as such, were identified in association with variegated phenotypes especially noticeable in plants as flower color variegation. R. A. EMERSON (1914, 1917) from his studies of variegated pericarp color in maize was the first to suggest that the variegation was a consequence of frequent mutation of the gene for colorless pericarp, in short associated with a mutable gene. Subsequent studies all pointed to the conclusion that the high mutation rate of mutable genes was an inherent, autonomous property of the gene itself (see reviews by DEMEREC 1935 and STUBBE 1933). Especially noteworthy in this connection are the studies of DEMEREC who over a period of 15 years described and characterized several mutable genes in *Drosophila virilis* (summarized in DEMEREC 1941). His detailed studies, particularly of the mutable miniature wing genes, led him to conclude that mutability, although influenced by specific genetic and environmental factors, was fundamentally a property of the mutable gene itself. More or less concurrently RHOADES (1938) described the then unique situation in which mutational instability was conferred upon the normally stable *a* gene in maize in the presence of the gene Dotted.

We are indebted to MCCLINTOCK for the most extensive and detailed cytogenetic analysis of mutable genes in maize. Beginning with a report in 1950 (MCCLINTOCK 1950, 1951), she was able to show in a brilliant series of investigations that high mutability of a number of genes in maize is controlled by genetic elements independent of the mutating gene itself. The diversity of interaction and control of the elements involved have been recently summarized and no further details need be elaborated here except to note that correctness of MCCLINTOCK's conclusion has been amply verified by maize studies of BRINK and associates on P^{vv}, by PETERSON on *pg*, a_1 and a_2 and by NUFFER on bz_2 (MCCLINTOCK 1965).

In contrast to the apparent widespread occurrence of mutable genes in maize, the mutable genes described by DEMEREC in *D. virilis* represent the only other adequately investigated examples in multicellular organisms. Significantly, despite an intensive search for mutable genes by a number of investigators, especially in *D. melanogaster*, no good example has been described. Since DEMEREC's mutable genes have been lost for some time, it has not been possible to determine

[1] Dedicated with esteem and affection to my friend and colleague PROFESSOR CURT STERN on the occasion of his sixty-fifth birthday.

TABLE 1

Synopsis of gene symbols used in text

Symbol	Phenotype	Chromosome and location
y	yellow body color	X-0.0
y^2	allele of *y*	
sc	scute bristles	X-0.0
kz	kurz bristles	X-0.7
w	white eye color	X-1.5
w^a	white-apricot, allele of *w*	
w^{a2}	white-apricot-2, allele of *w*	
w^{ch}	white-cherry, allele of *w*	
w^i	white-ivory, allele of *w*	
sp-w	spotted-white, allele of *w*	
spl	split bristles	X-3.0
dm	diminutive bristles	X-4.6
cho	chocolate eye color	X-5.4
ec	echinus eyes	X-5.5
f	forked bristles	X-56.7
B	Bar eye	X-57.0
w^{m4}	white-mottled-4	Inversion of X
rst^3	roughest-3	Inversion of X
Cy	Curly wing	Inversion of 2
Ubx	Ultra bithorax-130	Inversion of 3

whether the situation in maize is unique or general. The subject of this report will be a description of a newly discovered mutable gene at the white (*w*) locus in *D. melanogaster* together with an analysis of its properties and those of some of its derivatives.

MATERIALS AND METHODS

Table 1 lists the mutants used. Details of specific crosses will be given where appropriate. Standard Drosophila culture methods were employed throughout.

EXPERIMENTAL

Origin of the mutable gene white-crimson (w^c)*:* In a series of experiments designed to measure the frequency of reversion of w^i following X-ray treatment, attached-X females homozygous $w^i spl$ were treated with about 4000R X rays and crossed to y^2 *sc* w^i *spl* males. In one such experiment a single female was found which on basis of her eye color phenotype was judged to be heterozygous for w^i and a partial reversion of w^i. Since such partial reversions are rare as compared to reversions of w^i to w^+ (BOWMAN and GREEN 1966), the female was bred and her progeny were scored. Based on her female progeny, it was clear that the exceptional female was heterozygous for w^i and an allelic eye color mutant roughly equivalent to w^{a2} in eye color phenotype. This new mutant was designated w^c. A stock was established in which the attached-X females were judged, on the basis of their phenotype, to be homozygous w^c *spl*. Inspection of this stock

after a few generations disclosed the unexpected fact that, in addition to w^c *spl* females, w^+ *spl* females were also present. Since the two partial reversions of w^i previously analyzed were found to be refractory to both spontaneous and X-ray induced reversion (BOWMAN and GREEN 1966), a detailed study of w^c was undertaken.

As a first experiment, 50 homozygous w^c *spl* attached-X females were crossed individually to *B* males and their female progeny were scored. Among those tested, 43 produced a minimum of 50 female progeny and were, therefore, included in the summarized results of the experiment. These 43 females produced a total of 5,866 female offspring. Among the 43, 15 females produced one or more exceptional progeny not homozygous w^c in eye color which were tentatively designated as w^+, dilute w^c, w^i, w and dark w^c. These eye color phenotypes mean: w^+, inseparable from wild type; dilute w^c, lighter than homozygous w^c but darker than w^i; w^i, inseparable from standard w^i; w, inseparable from standard w; and dark w^c, darker than homozygous w^c but clearly lighter than w^+. The distribution of the exceptional progeny recoverd from each female is summarized in Table 2. These data confirm the occurrence of w^+ exceptions among the progeny of w^c females and establish the occurrence of at least two additional exceptional types, viz. w^i and w. Progeny tests of the females designated dark w^c proved them to be heterozygous for w^c and a third mutant with a deep maroon eye color designated w^{dc}. Progeny tests of the dilute w^c females established each to be heterozygous for w^c and either w^i or w. Where a female produced both dilute w^c and w^i exceptions, e.g. female 8 of Table 2, progeny tests of the dilute w^c females invariably established each to be heterozygous for w^c and w^i. Similarly, where a female produced both dilute w^c and w exceptions, e.g. female 37 of Table 2, prog-

TABLE 2

Number and phenotype of female exceptions derived from single female attached-X w^{c}spl *homozygotes*

Female No.	Number and phenotype of exceptions				
	w^+	dilute w^c	w	w^i	dark w^c
3	.	1	.	.	.
5	3	.	.	.	.
7	4	.	.	.	.
8	.	6	.	3	.
10	4	3	.	.	.
11	1	.	.	.	.
15	.	.	.	1	.
16	.	3	.	1	.
18	.	1	.	.	.
21	.	1	2	.	.
23	1	.	.	.	.
25	.	.	.	.	2
28	2	.	.	.	.
37	2	4	3	.	.
41	2	5	.	.	.

eny tests of the dilute w^c females showed that they were heterozygous for w^c and w. Where the exceptions consisted solely of dilute w^c females, they proved to be heterozygous either for w^c and w^i or for w^c and w. One exception was found: Female 47 produced five dilute w^c exceptions which on progeny testing proved to include four w^c/w^i heterozygotes and one w^c/w heterozygote. Equivalent results were obtained on progeny-testing the w^+ exceptions; some proved to be heterozygotes of the genotype w^+/w^c, other proved to be homozygous w^+.

Considering these data together, the following tentative conclusions appear to be in order. In females, w^c mutates to at least four different states as judged by the resultant phenotypes. The occurrence of exceptions in clusters strongly favors the notion that the exceptions occur primarily as a premeiotic event rather than as a meiotic or postmeiotic one. The reciprocal of each exception appears in all cases to be w^c, i.e. neither w nor w^i occurred as reciprocals of w^+. As a first approximation this suggests that a mutational rather than a recombinational event is involved, although mitotic recombination is not entirely excluded. The origin of the several exceptions as a premeiotic event provides a ready explanation for the occurrence of homozygous w^+, w^i and w individuals. Where w^c changed to w^+, w^i or w several cell divisions prior to meiosis, it is expected that a number of heterozygous nuclei will be available at meiosis. Since the w locus is more than 50 map units distal to the centromere, it is expected that in these heterozygotes half the nonreciprocal crossovers occurring between the w locus and the centromere will result in females homozygous for w^+, w^i or w as the case may be. Thus, homozygotes should be comparatively frequent in occurrence.

It follows that if each cluster is scored as a single event, among the 43 homozygous w^c *spl* females tested, equal to 86 w^c genes, the following frequencies of changes were found: $w^+ = 8$, $w = 4$, $w^i = 6$, $w^{dc} = 1$.

Since 28 of the 43 attached-X females tested produced no exceptions, the question was asked wether their w^c genes are refractory to change. A test of this possibility was made by selecting 6 to 10 F_1 w^c *spl* daughters of each of the 28 females and testing each group *en bloc* to see whether they produce exceptions. A detailed presentation of these results is unnecessary here; it will suffice to note that each group of w^c *spl* daughters produced exceptions. Thus, the failure to recover exceptions among the 28 parental females is most likely a matter of chance.

While the occurrence of w^+, w^i and w exceptions in clusters strongly argues for their premeiotic origin, there is nothing inherent in the experimental results of Table 2 which excludes the possibility that whatever the process, it involves a premeiotic event followed by meiotic crossing over. A test of the latter contingency, viz. that crossing over is in some way involved, was carried out after first synthesizing attached-X females of the genotype w^c *spl*/y^2 *sc* w^a *ec*. Among 41 such females, 14 changes in w^c were detected including 7 w^+, 2 w^i and 5 w. Progeny tests established that each carried the parental *spl* marker and none carried markers of the homologous w^a X chromosome. Thus, conventional crossing over is not involved. It should be added that following detachment of the attached-Xs, mutation of w^c was tested in a variety of heterozygotes in which one X chromosome carried w^c *spl* and the homologous X carried either a deficiency of the w

gene, or w mutants, or a complex inversion. It will suffice to note here that in each heterozygote, w^c mutated as in the attached-Xs suggesting that neither the genetic constitution of the homologous X chromosome nor the residual genetic background have any effect on the mutability of w^c.

A test of the possible relationship of mitotic crossing over to mutability of w^c was carried out by determining the frequency, if any, of mutations in w^c *spl* males. While mitotic crossing over occurs in Drosophila males, it is not possible in males which have but a single X chromosome. Should w^c mutate with an appreciable frequency in males, mitotic crossing can be effectively excluded as a factor in the process. Tests of 183 individual w^c *spl* males were obtained. Each was crossed to 5–10 attached-X females and from 300 to 600 F_1 males per parental male were scored. In Table 3 the results of this experiment are tabulated. It will be noted that 19 changes of w^c were obtained with the cluster size ranging from 1 to 116. This is a minimum estimate of the mutation frequency. By using the attached-X method only those X chromosome changes transmitted to male progeny will be recovered. Those transmitted to super females, i.e. to zygotes with the attached-Xs plus the free X from the male, will be lost owing to lethality. These results mean that mitotic crossing over is in all probability not involved in the mutation of w^c.

In a parallel experiment, the mutation of w^c was compared in females carrying a sex-linked Minute gene, known to increase mitotic crossing over (STERN 1936), and with females lacking the Minute.

The presence of the Minute was without effect on the frequency of mutation of w^c.

TABLE 3

Frequency of male exceptions derived from w^{c}spl *males (type cross* w^{c}spl *male* × *attached-X females; total males tested = 183)*

Male No.	Number and phenotype of exceptions per male			
	w^+	w^i	w	w^{dc}
1	25	.	.	.
2	1	.	.	.
3	116	.	.	.
4	.	.	1	7
5	.	22	.	.
6	.	9	.	.
7	.	5	.	.
8	2	.	.	.
9	1	2	.	.
10	5	.	2	.
11	.	3	.	.
12	.	1	.	.
13	3	.	.	.
14	.	1	.	.
15	.	1	.	.
16	2	.	.	.

One additional recombinational event could conceivably be involved in the mutation of w^c. This is the association of mutations of w^c with some kind of exchange between sister chromatids. Presumably, such an exchange would be comparable to crossing over between nonsister chromatids and could occur in both females and males. In principle, an experimental test of such a possibility can be made by comparing the mutation frequency of w^c located in ring X and in rod X chromosomes. The rationale of this experiment derives from the observation that conventional crossing over between ring X and rod X or between homologous ring X chromosomes is limited to double crossovers (L. V. MORGAN 1933).

Single exchanges apparently occur but lead to inviable products, ostensibly interlocking rings or products derived from broken rings. From these facts one can argue that if mutation of w^c is associated primarily with a sister chromatid exchange event comparable to single crossing over, such mutants would not be recovered in ring X chromosomes for the same reason that regular single crossovers between ring chromosomes are not recovered. If a part of the sister-strand events occurred as double sister-strand crossovers, they and, therefore, mutants, would be recovered but at a reduced frequency. An experimental test of this possibility was carried out in the following manner. By crossing over, w^c together with the markers *spl* and *f* were inserted in the ring X chromosome, X^{c2}. Subsequently, heterozygous females $y\ w^c$ (rod X)/w^c *spl f* (ring X) were obtained and crossed individually to $y^2\ w$ *spl* males. Thus, mutations of w^c in ring and rod X could be determined simultaneously among their progeny. Where a mutation occurred in a y marked individual it was scored as a rod X event and where it occurred in a *spl* marked individual it was scored as a ring X event. Among a total of 90 heterozygous females producing on the average 388 progeny each (range 219 to 576), 25 mutants of w^c were found. Of these 15 (6 w^+, 3 w^i, 6 w) occurred in the ring X chromosome, 10 (5 w^+, 2 w^i, 3 w) in the rod X chromosome. In Table 4, a distribution of the mutant cluster size occurring in ring and rod X chromosomes is presented. In general the cluster sizes are equivalent. The overall conclusion from these results is that the localization of w^c in a ring X chromosome does not prejudice its mutation either as to frequency or as to cluster size. This means that very likely a sister-strand exchange event is not involved.

The conclusion to be drawn from the foregoing data is that as a first approximation the mutational behavior of w^c is best explained as an autonomous property of the w^c gene.

TABLE 4

Distribution of mutant clusters derived from w^c *in ring and rod X chromosomes*

	Number of independent mutants of cluster size										
	1	2	3	4	5	6	7	8	9	. . .	20
rod X	1	2	4	.	1	.	.	.	.		1
ring X	6	2	1	1	.	2	.	.	2		.

Genetic analysis of the w^i, w *and* w$^+$ *derivatives of* w^c: The next step in the study of w^c entailed an analysis of the w^i, w and w^+ mutants derived from w^c. For the analysis of w^i mutants, eight independent such mutants were individually tested for intragenic recombination and for spontaneous reversion to w^+. Each w^i was crossed to a w^{ch} tester stock such that F_1 females $y\ w^{ch}\ spl/w^i$ and also heterozygous for the autosomal inversions *Cy* and Ubx^{130} were obtained. These females were crossed to $y^2w\ spl$ males and their progeny scored. The results of the several experiments are given in Table 5. It will be recalled that the frequency of reversions to w^+ associated with intragenic recombination between w^i and w^{ch}, about 1/50,000, is much reduced when compared to comparable reversions derived from recombination between w^a and w^{ch}, about 1/10,000 (LEWIS 1959; BOWMAN and GREEN 1966). Reversion of w^i to w^+ without recombination occurs in females at a frequency of 1 in 15 to 20,000 chromosomes (LEWIS 1959; BOWMAN 1965). As will be seen from the data in Table 5, the reversion frequencies of individual w^i mutants associated with intragenic crossing over are inadequate for a valid comparison with equivalent reversion rates of the original w^i mutant. However, by considering all eight mutants together it will be noted that the overall frequency of reversion associated with recombination was 13 in 515,793 or about 1/40,000—not dissimilar from that described for the original w^i mutant. Similarly, a spontaneous reversion frequency of 19/257,896 w^i chromosomes tested (half the progeny scored) or about 1/14,000 appears to be equivalent to the spontaneous reversion rate of the original w^i. Subsequent to these experiments, yet another w^i derived from w^c was tested extensively for spontaneous reversion to w^+. Here a frequency of 24 w^+/308,961 chromosomes scored was found, a rate equivalent to the collective w^i reversion rate noted above. On the basis of these reversion data it appears not unreasonable to conclude that the w^i mutants derived from w^c are identical to the w^i mutant from which w^c arose.

Having no *a priori* basis for interpreting the nature of the w mutants derived from w^c, an analytical scheme equivalent to that employed in the analysis of w^i was adopted. Six independent w mutants were crossed to a *sp-w* tester stock such

TABLE 5

Intragenic recombination and spontaneous reversion of w^i *mutants derived from* w^c. *(type cross females* w^i/y w^{ch} spl; Cy/+; Ubx/+; × *males* y^2 w spl*)*

w^i mutants	Number w^+ = reversions	Number $y\ w^+$ = recombinants	Total flies scored
w^{i1}	.	2	74,965
w^{i2}	4	2	58,329
w^{i4}	2	3	55,234
w^{i5}	6	2	84,931
w^{i8}	2	3	66,908
w^{i9}	3	.	47,993
w^{i10}	.	1	42,406
w^{i11}	2	.	85,027
Total	19	13	515,793

that females *y sp-w spl/w* and heterozygous for the autosomal inversions *Cy* and *Ubx*^{130} were obtained. For each experiment a total of 23 mating bottles were made up. In each, ten heterozygous females were crossed to an excess of *y*^{2} *w spl* males. Females were allowed to oviposit for 5 days, transferred to fresh media for 4 days and transferred a second time for a final 4 day period. The results of these experiments are given in Table 6. Two distinctive results were obtained. Two *w* mutants produced reversions to *w*$^{+}$ but only in association with recombination, a result which suggests they are mutationally stable. The remaining four *w* mutants produced numerous mutants without crossing over including those with *w*$^{+}$, *w*^{c} and *w*^{dc} phenotypes. The numbers listed in Table 6 represent a minimal estimate of the frequency of these mutants. Since ten females were used per mating a conservative scoring plan was adopted. Each phenotypic change per bottle was counted as a single mutational event irrespective of cluster size, and clusters of 1 to 7 per bottle occurred. Note, too, that for the mutable *w* genes the frequency of reversion to *w*$^{+}$ associated with recombination, the *y w*$^{+}$ in Table 6, is comparatively low, not unlike that observed for *w*^{i}. Since the *y w*$^{+}$ individuals recorded represent the only exceptions found in a particular bottle, they are very likely recombinants and not premeiotic reversions followed by a meiotic crossover. Needless to say, it is clear from these data that some *w* mutants derived from *w*^{c} appear to be as mutationally unstable as *w*^{c} itself.

Because of clustering, a more precise estimate of the frequency and variety of phenotypic classes of mutants derived from unstable *w* mutants can be better obtained by scoring the progeny of single females. Accordingly, a reinvestigation of the mutability of *w*^{5}, *w*^{8} and *w*^{9} was undertaken after first building for each two stocks: one carrying the *y* marker and the second carrying the *spl* marker. Crosses between the two stocks produce *y w/w spl* females which were crossed individually to *y*^{2}*w spl* males. Since crossing over in the *y-spl* interval is about 2%, the markers serve to identify which of the two *w* genes mutated. This is especially desirable in the case of large clusters. In addition to the three *w* mutants, parallel experiments with *w*^{c} and with *w*^{dc} derived from *w*^{c} were carried out. The results of all these experiments are tabulated in Table 7. For *w*^{5}, *w*^{8} and *w*^{9} the results confirm the data of Table 6 and demonstrate that each has a high

TABLE 6

Intragenic recombination and mutation of w *mutants derived from* w^{c}.
(*type cross females* w/y sp-w spl; Cy/+; Ubx/+ × males y^{2}w spl)

	Frequency of exceptions of the type				
w mutant	*y w*$^{+}$	*w*$^{+}$	*w*^{c}	*w*^{dc}	Total progeny
w^{1}	5	.	.	.	62,864
w^{2}	2	8	1	.	70,242
w^{5}	2	5	3	1	81,714
w^{7}	2	.	.	.	57,693
w^{8}	.	4	7	6	15,465
w^{9}	.	4	.	.	98,869

TABLE 7

Frequency and phenotypes of mutations occurring in females homozygous for w^c *or a mutable derivative of* w^c. (*type cross female* y w^c/w^cspl × *male* y^2w spl)

Mutable gene	Number of independent mutations to							Total mutants	Total genes tested
	w^+	$w^{"+"}$	w^{dc}	w^c	w^{di}	w^i	w		
w^c	26	..	1	—	..	14	15	56	192
w^5	15	..	6	..	..	1	—	22	236
w^8	7	1	9	25	4	..	—	46	122
w^9	19	..	..	8	..	17	—	46	292
w^{dc}	6	..	—	12	1	7	11	37	120
w^{di}*	9	..	4	5	—	..	15	33	150
$w^{"+"}$†	2	..	1	23	..	1	9	36	154
w^c‡	14	1	..	—	..	6	13	34	130

* Derived from w^{dc}.
† Derived from w^8.
‡ Derived from w^5.

mutability. At first glance it would appear that w^8 mutates more frequently than w^5 or w^9, but this could be misleading. Since there is at present no efficient way of measuring the mutation of w^5 or w^9 to a mutationally stable w type, it is quite conceivable that the mutation frequencies of both are underestimated. It does appear that among those unstable mutants in which ostensibly all possible mutant progeny can be phenotypically scored, the mutation frequency in females is approximately one per four genes tested.

There does appear to be a difference, as judged by phenotypes, in the spectrum of mutants to which w^5, w^8 and w^9 mutate. Note the numerous w^{dc} types recovered from w^8 and the numerous changes to w^i recovered from w^9. Whether these differences are real cannot for the moment be determined since the data available are too limited.

Included in Table 7 are mutation data on the w^{dc} found in the original experiment described in Table 2; on a w^{di} recovered from w^{dc} and phenotypically intermediate between w^i and w^c; on a $w^{"+"}$ derived from w^8, essentially wild type in phenotype and separable from w^+ by virtue of the deep maroon eye color manifest in the heterozygote $w/w^{"+"}$. Together with w^+, w^c, w and w^i the types w^{di}, w^{dc} and $w^{"+"}$ represent the total range of phenotypic classes identified thus far. The data in Table 7 demonstrate that each is mutable, at a rate approximating one per four genes tested.

Finally, there are included in Table 7 mutation data on a w^c derived from w^5. Phenotypically the derived w^c is indistinguishable from the original w^c and mutationally it appears also to be indistinguishable.

In Table 8 a distribution of the mutant cluster sizes derived from the mutation data of Table 7 is presented. It will be noted that by and large the distribution of cluster sizes is the same for each mutable gene analyzed.

Three independent w^+ reversions derived from w^c were crossed to the *sp-w* tester described above and their mutability was tested. Precisely the same procedure was followed as that outlined above for the w mutants. Thus, for each

TABLE 8

Size and number of mutant clusters derived from different mutable genes

Gene tested	Cluster size														
	1	2	3	4	5	6	7	8	9	10	11	....	17	....	20
w^c	17	7	9	4	3	3	8	9	1	2	1	.	.	.	1
w^5	9	4	2	2	3	.	.	2	.	.	.	.	.	.	.
w^8	9	4	7	7	2	2	4	3	.	.	.	.	1	.	.
w^9	17	10	5	6	1	1	2	1	.	1	.	.	.	.	.
w^{dc}	18	6	1	2	3	4	1	2	.	.	.	.	.	.	.
w^{di}	14	4	4	4	2	4	.	.	.	1	.	.	.	.	.
w^{+}	12	8	6	2	3	1	4	2	.	.	.	.	.	.	.

w^+ about 230 w^+ chromosomes were tested for mutation among over 50,000 progeny scored. All three w^+ reversions appear to be stable for no mutations at the w locus were found.

Brief mention should be made here of somatic mutations detected in the eyes of w^c and lighter eye color derivatives such as w^8. While no systematic study was undertaken, occasional mutations of w^c to w or w^8 to w^c or w^+ were detected as small spots in the eyes just as was reported for w^i by LEWIS (1959).

The data presented thus far are consistent with the hypothesis that the mutational instability of w^c is an inherent property of the w^c gene. A variety of outcrosses of w^c to stocks of widely differing genetic constitutions had no effect on the instability of w^c. This fact makes it difficult to account for the mutability of w^c as a function of an independently segregating mutator gene which specifically controls the mutation of w^c much as *Dt* controls a_1 in maize. Thus, in spite of the enormously high mutation rate, there is nothing in the data which points to an alternative hypothesis. However, in the course of testing individual homozygous w^8 females, a wholly unexpected class of exceptions was recovered which are germane to any explanation for the mutation of w^c and its derivatives. The unexpected class of exceptions was females of the typical Notch wing (N) phenotype, and a description of their occurrence and their genetic analysis follows.

In addition to the exceptions listed in Table 7, the $y\ w^8/w^8 spl$ females produced 13 independent N mutants. Five N exceptions occurred in $y\ w^8$ chromosomes, in four instances as single N females and in one instance as a cluster of five N females. Eight N exceptions occurred in $w^8 spl$ chromosomes, in five cases as single N females and as clusters of two, three and four N females in one instance each. With the exception of one cluster of three, all N females were white-eyed in compound with w. In the one exception, all three N females were associated with a simultaneous mutation of w^8 to w^c. The N phenotype is well known, being associated with either mutation or loss of a gene lying 0.8 crossover units to the right of w. Since a N bearing X chromosome is lethal to males, it is conceivable that the frequency and cluster size of N exceptions derived from homozygous w^8 females was minimal. Therefore, the experiment was repeated with the slight variation that N and other mutations were sought as events occurring in $w^8 spl$

males and recovered in their female progeny. Each of 45 $w^8 spl$ males was crossed individually to ten $y^2 w$ *spl* females. From each cross 842 to 1282 female progeny were scored, among which the following exceptions were found: one w^+ as a single female; nine independent cases of w^c including four as single females and five as clusters of 8, 14, 33, 46 and 94 females respectively; three mutations to w^{dc} all as single females; and 12 separate occurrences of *N*, nine as single females and three as clusters of 2, 22 and 25 females each. These data fully confirm the results obtained with w^8 females.

A reexamination of earlier results and subsequent experiments with w^c and other mutable derivatives of w^c including w^{c+}, w^5 and w^9 showed that *N* exceptions occur regularly among their female progeny proving that *N* is not an exclusive derivative of w^8. Those *N* mutants recovered from w^c and w^{c+} were especially instructive for in addition to being *N* they were white-eyed in compound with *w* suggesting that the *N* mutation was associated with a deficiency including both the *w* and *N* genes. These observations motivated a systematic genetic analysis of *N* mutants derived from the various mutable genes described here with the aim of defining more precisely the genetic nature of each *N*. Should the *N* mutants uniformly prove to be deficiencies, there would be good reason to believe that the property of high mutation attributed to w^c and its derivatives is not an autonomous property but dependent on some other cause.

Balanced lethal stocks of 22 independent *N* mutants were obtained. Each *N* was subjected to a series of genetic tests designed to determine whether or not it is associated with a deletion and, if so, the extent of the loss. As a first test, each *N* was compounded to a deficiency whose lethal effect is located between *w* and *N*. The rationale of this test is as follows. If the *N* is lethal in compound with the tester deficiency, it is in all probability associated with a deficiency greater than the *N* gene alone. If the *N* is viable in compound with the tester deficiency, at most it is associated with a deficiency of the *N* gene. The deficiency chosen is the one derived by crossing over between the near identical inversions w^{m4} and rst^3. This deficiency, designated here $w^- rst^3$, is obtained by recombining the left half of w^{m4} and the right half of rst^3 thereby producing a loss of *w* and a lethal just to the right of *w* and to the left of *N* (LEFEVRE and WILKINS 1966). The compound of $w^- rst^3$ and a point mutation *N* is viable. The results of this test are included in Table 9. As noted, 19 of the 22 *N* mutants tested were lethal in compound with

TABLE 9

Genetic characterization of N *mutants*

Number *N* mutants	♀ heterozygote $N/w^- rst^3$	*N* ♂ in combination with w^+Y	$Dp\ w^{+51b7}$
3	V	V	V
9	L	V	V
2	L	L	V
8	L	L	L

(V = viable, L = lethal).

w^-rst^3 which means they are associated with a chromosome loss which extends at a minimum from w to N, possibly including both.

A second series of crosses was made with the aim of more precisely defining genetically the extent of the chromosome loss associated with the 19 N mutants lethal in compound with w^-rst^3. For this purpose two duplications of different genetic lengths but including the w^+ and N^+ loci were employed. One duplication w^+Y, extends from *kz*, 0.8 units to the left of w, to and including N (BROSSEAU *et al.* 1961). A second duplication, $Dp\,w^{+51b7}$, extends from w to an including *dm*, 1.6 units to the right of N (LEFEVRE 1952, RATTY 1954). Each N was separately combined with each duplication to determine whether the combination confers viability on males. Both duplications are known to confer viability on males carrying a point mutant N. The results of these tests are also included in Table 9 and show that the 19 N mutants fall into three classes. One class includes nine N mutants lethal in compound with w^-rst^3 but male viable with both duplications. This result is interpreted to mean that the deficiency associated with each does not extend beyond w on the left nor beyond N on the right. A second class includes two N mutants male viable in combination with $Dp\,w^{+51b7}$ but male lethal in combination with w^+Y. This is interpreted to mean that both N mutants extend from w to a point beyond N. Since $Dp\,w^{+51b7}$ includes the *dm* locus, these N mutants extend either to or just to the right of *dm*. Heterozygotes of both N mutants and *dm* were obtained and proved to be in both cases *dm* in phenotype. Thus, the loss in both N mutants extends from w to just to the right of *dm*. The third class includes eight N mutants which are male lethal when combined with either duplication. This suggests that all are rather long deficiencies. All eight N mutants were tested in compound with w^{258-45}, a deficiency which includes w and a lethal just to the left of w. Since all females N/w^{258-45} were viable it can be concluded that no N extends to the left of the w gene. Therefore, all probably extend beyond *dm* to the right of N since none are "covered" by $Dp\,w^{+51b7}$. Accordingly, all eight N mutants were separately compounded to *dm*, to *cho* which lies one unit to the right of *dm* and to *ec* just to the right of *cho* as a way of more exactly determining the extent of the deficiency in these eight N mutants. As expected, all compounds with *dm* were *dm* in phenotype meaning that the deficiency includes the *dm* locus. Six N mutants were cho^+ in compound with *cho* indicating that the loss extends beyond *dm* but not including *cho*. Two N mutants were *cho* phenotype in compound with *cho* and *ec* in compound with *ec* demonstrating that both involve deficiencies extending beyond *ec*, i.e. are equivalent to 4 crossover units in length. Considered together, these data mean that at a minimum five different N deficiencies have been recovered. In addition to the foregoing deficiencies, one deficiency including w and *loci* to the left of w but none to the right has been recovered. Thus, a variety of chromosome losses have been recovered in association with w^c and its mutable derivatives.

To date only a cursory examination of the salivary gland chromosomes of the N mutants has been undertaken. It will suffice to note here that in the two N mutants where no loss is indicated, the banding in the w-N interval is intact. In those

where a loss is indicated, bands, especially that of *N* locus, are absent. Detailed cytological study is underway.

DISCUSSION

The all-important question posed by these data, for which an answer must be sought, is the following. Is the mutability of w^c and its mutable derivatives an autonomous function of the gene itself or is mutability nonautonomous, that is under control of some agent other than w^c? Before attempting an answer, it would be appropriate to summarize the results presented thus far. The facts are these: (1) w^c mutates spontaneously at an inordinately high rate to a number of different states as judged by the associated phenotypes. (2) Mutations occur in both females and males. (3) The clustering of the mutants suggests that most, perhaps all, mutants occur premeiotically with the size of the cluster depending upon how early before meiosis the mutational event occurred. (4) Mutation occurs in the absence of recombination; neither meiotic or mitotic nor inter- or intrachromosomal recombination appears to be involved. (5) The mutational derivatives of w^c appear to be two types: those that are mutationally stable and those that are unstable. (6) The mutable derivatives mutate at the same general frequency as does w^c and, in general, to the same kinds of stable and unstable derivatives. (7) All unstable genes produce deficiencies of varying lengths which extend both to the left and right of the *w* locus. The deficiencies are recovered both as single individuals or as clusters.

What arguments militate for an explanation of autonomous mutability of w^c and some of its derivatives? The most compelling argument favoring this explanation is that the mutational event appears to be delimited to the *w* gene and that no specific genetic mechanism has been associated with the mutational event. In the absence of a specific genetic mechanism, by elimination, the simplest explanation is autonomous mutation.

There are, however, several reasons for doubting the plausibility of the aforementioned explanation. First, the frequency of mutation is of such a magnitude as to raise serious doubts that the mutation event could be an inherent property of the gene itself. While there are no fixed limits within which spontaneous mutation can occur, there is, nonetheless, no precedence for the mutation frequency found for w^c. Certainly the frequency of reversions of w^c to w^+ are outside the realm of reversion rates reported thus far in Drosophila, with the possible exception of the mutable miniature gene reported by DEMEREC (1941). An observation which is more difficult to reconcile with an autonomous mutational event is the recovery of the *N* mutants from w^c and its mutable derivatives. It is most difficult to see how a gene mutation event can account for the origin of deficiencies of the genetic length found in association with the bulk of the *N* mutants analyzed. Clearly, some other explanation must be sought. Since the pattern of occurrence of the *N* mutants and the pattern of recovery of the *w* gene mutants from each mutable gene parallels one another, i.e. both occur in clusters of varying size, it seems reasonable to believe that one underlying mode of origin is common to both.

An alternative to autonomous mutation is the control of mutation by "foreign" regulatory elements localized at the *w* gene and comparable to the controlling elements described by McCLINTOCK and others in maize. The most compelling argument favoring a controlling element interpretation comes after comparing the properties of the system described here and those of the maize mutable systems. In a number of respects the parallelisms between the two are remarkably close. Those properties which critically characterize the mutable system described here, namely (1) high mutation rate, (2) occurrence premeiotically or in somatic cells, (3) mutation to a variety of states which may be stable or unstable, (4) mutation to deficiencies—all have been thoroughly documented as properties of controlling-element regulated mutable systems in maize (see McCLINTOCK 1965). The one property of the maize systems not as yet demonstrated for the w^c system and one which would complete the parallelism, is transposition of the "foreign" controlling element from one site to another. There are at least suggestions that transpositions do occur albeit no critical proof has been possible thus far. On two separate occasions observations were made which suggest that transposition of a controlling element might have been involved. In one, a female homozygous $w^{di}spl$ produced, in addition to the expected progeny, nine males carrying a new mutant inseparable phenotypically from lz^s. In a second, a female homozygous $w^s spl$ produced, in addition to the expected progeny, nine males carrying a new *r* mutant. The occurrence of new mutants in clusters is precisely the kind of situation which would be anticipated should transposition of a controlling element take place to a new site. In maize, transposition of a controlling element to a new gene locus is often accompanied by a gene mutation at the new site. The new mutation may be stable or mutable. The mutability of the new lz^s and *r* mutants is currently under study.

In the absence of definite proof, any discussion of the origin and nature of a controlling element associated with w^c falls into the category of speculation. Nonetheless, it may be of some value to indulge briefly in speculation if for no other reason than to point out those salient facts and observations which must be accounted for in any hypothesis concerning the w^c system. Assuming a controlling element, two sources of its origin appear possible. One possibility is that this element has always been intimately associated with w^i and is, in fact, the basis for the two unusual genetic properties of w^i, namely reduced intragenic recombination and relatively frequent reversion to wild type. How then is this element associated with the properties of w^c? Conceivably as a consequence of X-irradiation the element became altered such that it is now more unstable, i.e. it can move from one point to another within the *w* gene. Such movement, it can be speculated, is associated with phenotypic change to w^c, w^{dc}, etc., each depending on the new location of the element. Or the element might revert to its original place and state producing w^i. Or it might be entirely lost producing w^+. The origin of several deficiencies described can be accommodated into this scheme if one argues that occasionally when the element is lost it behaves much as does phage lambda of *E. coli* following induction. Sometimes when the element is lost it leaves its chromosome location more or less intact, equivalent to lambda, but on

other occasions on leaving its chromosome location it takes some of the host chromosome along, much as does lambda-dg (CAMPBELL 1964).

A second possible source of the controlling element is that this is a wholly foreign agent which became incorporated into the chromosome following the X-irradiation, and the association with w^i is purely coincidental. It is difficult to make a choice between these alternatives, in fact while in the realm of speculation a choice seems unnecessary. However, it should be emphasized that the nature of w^i is to date unresolved and superimposing yet another unknown on w^i appears to confuse the genetical situation unnecessarily. For the moment, therefore, the simplest working hypothesis seems to be that w^i and w^c are related in that the same foreign element underlies the genetical properties of both. Precisely what the mechanism associated with each is remains an interesting and stimulating mystery.

SUMMARY

The origin of the frequently mutating gene, w^c, as a derivative of w^i is described. w^c mutates spontaneously in both females and males to a number of different allelic states the phenotypes of which range from w^+ to w. The mutation event appears to occur primarily, if not exclusively, before meiosis and appears not to involve any recombination or similar event. Alleles derived from w^c may be stable or mutable. In addition to apparent point mutations, w^c and its mutable derivatives produce deficiencies which include loss of the w gene and adjacent loci to the right or left of w. The causes of the high mutation rate are discussed in terms of an autonomous event and of an event controlled by a "foreign" element akin to the controlling elements demonstrated for maize.

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