# CONTROLLING ELEMENT MEDIATED TRANSPOSITIONS OF THE *WHITE* GENE IN *DROSOPHILA MELANOGASTER*[1,2]

M. M. GREEN
*Department of Genetics, University of California, Davis, California 95616*

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AMONG the several mutable gene systems in *Zea mays* regulated by controlling elements, at least three features appear to be uniquely characteristic to all. (1) The controlling element appears to be integrated at the site of the gene manifesting the property of high mutability. (2) The kind or kinds of changes at a gene locus under controlling element regulation are particular and not random. (3) The controlling element is capable of transposition from one locus to another locus, the latter located either on the same chromosome or on a nonhomologous chromosome (see McCLINTOCK 1965, 1967 for reviews). Mutation of the inordinately mutable white-crimson ($w^c$) gene of *Drosophila melanogaster* has been attributed to a controlling element (GREEN 1967). To date the $w^c$ system shares two of the above mentioned maize characteristics, *viz.* apparent integration of the controlling element at the site of $w^c$ (GREEN 1969) and particular changes associated with mutation of $w^c$ (GREEN 1967 and unpublished). It is the purpose of this report to present data which demonstrate that the $w^c$ associated controlling element is capable of spontaneous transposition, i.e. it moves from its location on the *X* chromosome to a new location on an autosome where it is integrated. A novel feature associated with the $w^c$ controlling element is that when it transposes, it carries along a portion of the white gene which is similarly integrated at the new location. The implications of this transposition event for the nature of the controlling element and the genetical organization of the white gene will be considered in some detail.

## MATERIALS AND METHODS

Conventional Drosophila culture methods were employed throughout. Flies were reared in a controlled room temperature of 22–23°C. A synopsis of the several mutants used is included in Table 1.

## EXPERIMENTAL

The occurrence of four, independent transpositions, designated *Tp* $w^c$*-1*, *Tp* $w^c$*-2*, etc., will be documented.

*Origin of* Tp $w^c$-1: The first transposition of $w^c$ was recovered as a by-product of an interallelic crossing over experiment in which the crossover coupling $w^c$ and $w^{sp}$ was sought. One such putative crossover of the genotype $w^c w^{sp} spl$, which

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TABLE 1

*Synopsis of gene symbols used in text*

| Symbol | | Chromosome and location |
|---|---|---|
| *y* | yellow body color | *X*-0.0 |
| $y^2$ | allele of y | .... |
| *z* | zeste eye color | *X*-0.4 |
| *sa* | sparse arista | *X*-1.4 |
| *w* | white eye color | *X*-1.5 |
| $w^-$ | white deficiency | .... |
| $w^{Bwx}$ | white-Brownex, allele of *w* | .... |
| $w^a$ | white-apricot, allele of *w* | .... |
| $w^c$ | white-crimson, allele of *w* | .... |
| $w^{ch}$ | white-cherry, allele of *w* | .... |
| $w^{sp}$ | spotted-white, allele of *w* | .... |
| *spl* | split bristles, rough eye | *X*-3.0 |
| $sn^3$ | singed-3 bristles | *X*-21.0 |
| *f* | forked bristles | *X*-56.7 |
| *Cy* | Curly wings | Inversions of *2* |
| *Ubx* | Ultrabithorax-130 | Inversions of *3* |
| *TM3* | ..... | Inversions of *3* |
| *ve* | veinlet-wing veins | 3-0.3 |
| *h* | hairy | 3-26.5 |
| *Ly* | Lyra wings | 3-40.5 |
| *ri* | radius incompletus | 3-47.1 |
| *Sb* | Stubbe bristles | 3-58.2 |
| *sbd* | stubbloid bristles | 3-58.2 |
| $e^4$ | ebony-4 body color | 3-70.7 |
| *ro* | rough eye | 3-91.1 |
| *Ser* | Serrate wings | 3-92.5 |

when homozygous was white-eyed in phenotype, was found (GREEN 1969). Proving this genotype depended upon demonstrating the presence of mutable $w^c$ which following mutation of $w^c$ to $w^+$ would uncover $w^{sp}$. Accordingly, homozygous females $w^c w^{sp} spl$ were individually crossed to $y^2 w^- spl\ sn^3$ males. Two types progeny were expected: white-eyed individuals with a maternally derived *X* chromosome of the genotype $w^c w^{sp}$ and $w^{sp}$ individuals with a maternally derived *X* chromosome of the genotype $w^+ w^{sp}$, the result of premeiotic mutation of $w^c$ to $w^+$. The latter were found as single individuals or clusters proving the recovery of the $w^c w^{sp}$ crossover. In addition, one homozygous $w^c w^{sp}\ spl$ female produced both the expected white-eyed progeny and a single male of the phenotype approximating $w^c spl$ and equivalent to that of $w^c/w$. One possible contrived explanation for this exceptional male was to suppose that the controlling element moved from the site of $w^c$ to that of $w^{sp}$, leaving the former mutated and reverting the latter to wild type, thereby "uncovering" $w^c$. Since with this explanation of $w^c$ remains sex-linked, the $w^c spl$ male was crossed to attached-*X* females homozygous for the recessive, sex-linked mutants *y*, *w* and *f*. It was not altogether surprising to find that expectations of sex-linkage were not fulfilled. Approximately one-half the

male progeny of this cross were $w^e spl$, i.e. inseparable in phenotype from the parental male and one-half the males were *w spl*. The latter were relatively slow in development beginning eclosion some 48 hrs after their $w^e spl$ brothers. Similarly, the female progeny consisted of two approximately equal classes: those *y w f* in phenotype and those *y f* with a dilute $w^e$ eye color. These results are consistent with only one explanation: $w^e$ is no longer linked to an *X* chromosome but to an autosome. Crosses were, therefore, made to determine the new linkage of $w^e$. For this purpose males of the genotype *w*; $w^e/Cy$; *Ubx* were crossed to *w* females. The progeny of this cross proved the linkage of $w^e$ to chromosome 3 since $w^e$ segregated from *Ubx* but not from *Cy*. This transposed $w^e$ will henceforth be referred to as *Tp* $w^e$*-1*.

Subsequent crosses established a number of phenotypic properties of *Tp* $w^e$*-1*. First, *Tp* $w^e$*-1* is recessive to $w^+$ for males carrying a wild-type *X* chromosome and *Tp* $w^e$*-1* are wild type in eye color. Second, *Tp* $w^e$*-1* is by the criterion of complementation, inseparable from the conventional *X*-linked $w^e$. Just as females of the genotype $w^e/w^{sp}$ manifest a complementary eye color phenotype, so males of the genotype $w^{sp}$; *Tp* $w^e$*-1* (i.e., an *X* chromosome carrying $w^{sp}$, a chromosome 3 carrying *Tp* $w^e$*-1*) also manifest a complementary eye color. Third, homozygous *Tp* $w^e$*-1* individuals are not obtained presumably because the *Tp* $w^e$*-1* third chromosome carries a recessive lethal inseparable from *Tp* $w^e$*-1*. Subsequently, a balanced lethal stock of the genotype *w*/*w*; *Tp* $w^e$*-1*/*TM3, Sb Ser* was synthesized.

Since *Tp* $w^e$*-1* is recessive to $w^+$, its presence could be determined only where the *X* chromosome carried a mutated $w^+$ gene; for simplicity this was either *w* or $w^-$, where $w^-$ is a male viable *w* deficiency. Unless otherwise stated, it should be understood that all individuals possessing *Tp* $w^e$*-1* will also be homozygous for *w* or $w^-$.

Preliminary linkage experiments in which crossing over was determined among the offspring of females *Tp* $w^e$*-1*/*Ly Sb* placed *Tp* $w^e$*-1* in the left arm of chromosome 3 some 34 units to the left of *Ly*. More precise mapping was carried out using the markers *ve* and *h*. Crossing over results based upon scoring 2,832 progeny of females *Tp* $w^e$*-1*/*ve h* × males *ve h*/*ve h* localized *Tp* $w^e$*-1* between *ve* and *h* at about 3.8 units to the right of *ve*.

Although the very fact of *Tp* $w^e$*-1* and its composite phenotypic properties indicates that $w^e$ transposed and integrated into chromosome 3, a more or less unequivocal demonstration follows if *Tp* $w^e$*-1* manifests the unique property of $w^e$, *viz.*, high mutation frequency. Experiments designed to estimate the mutability of *Tp* $w^e$*-1* were conducted by individually crossing females *Tp* $w^e$*-1*/*TM3, Sb Ser*; $w^-/w^-$ to males +/+; $y^2 w^- spl\ sn^3$. Mutation data were derived from fertile females; a female was classified as fertile if she produced a minimum of 150 progeny. Those producing less were excluded from consideration. The following results were obtained in a typical experiment: of 60 females crossed, 57 were fertile. Among these females, 15 produced exceptional progeny in addition to the expected progeny carrying *Tp* $w^e$*-1*. Ten females produced white-eyed progeny including six females with single white-eyed exceptions, two females with two

exceptions each, one female with four exceptions and one female with five exceptions. Five females produced exceptions with an eye color distinctly lighter than *Tp* $w^c$*-1* and designated $w^{di}$. Among these females two produced single $w^{di}$ individuals, two produced two $w^{di}$ individuals each and one produced three $w^{di}$ exceptions. These results demonstrate that *Tp* $w^c$*-1* is mutable just as in the conventional $w^c$ and support the view that transposition involved the removal of $w^c$ from the *X* chromosome and its insertion into chromosome 3.

At this juncture it is appropriate to consider further the two mutational derivatives of *Tp* $w^c$*-1*. Those third chromosomes derived from *Tp* $w^c$*-1* which in combination with homozygous *w* are white-eyed will be referred to as *Tp w-1*, while those designated $w^{di}$ will be referred to as *Tp* $w^{di}$*-1*. Since $w^c$ was found previously to mutate most frequently to $w^+$, *w* and $w^i$ (GREEN 1967), it is worthwhile to ask whether the same mutational events account for *Tp w-1* and *Tp* $w^{di}$*-1*. An answer to this question was sought by answering the following three questions: (1) Are the *Tp w-1* and *Tp* $w^{di}$*-1* third chromosomes lethal when homozygous as is *Tp* $w^c$*-1*? (2) Do *Tp w-1* and *Tp* $w^{di}$*-1* complement with $w^{sp}$ as does *Tp* $w^c$*-1*? (3) Are *Tp w-1* and *Tp* $w^{di}$*-1* mutable?

Before attempting an answer to these questions, balanced stocks, *Tp w-1/TM3, Sb Ser* and *Tp* $w^{di}$*-1/TM3, Sb Ser* were synthesized. This was done by recovering such mutants from the cross of single female by single male both of the genotype *Tp* $w^c$*-1/TM3, Sb Ser* (the *X* chromosomes being homo- or hemizygous $w^-$). Since from this cross only heterozygotes survive, mutations are automatically recovered as balanced genotypes, and balanced stocks may be obtained with one further cross. Six independent *Tp w-1* and two independent *Tp* $w^{di}$*-1* mutants were obtained and balanced to *TM3, Sb Ser*. All proved to be lethal when homozygous and lethal in compound with *Tp* $w^c$*-1*. Thus the mutation event occurred without alteration of the *Tp* $w^c$*-1* associated recessive lethal.

Complementation was assayed by comparing the eye phenotypes of males *Tp w-1/+*; $w^{sp}$ or *Tp* $w^{di}$*-1/+*; $w^{sp}$ with those of brothers *TM3, Sb Ser/+*; $w^{sp}$ derived from the cross of females $w^{sp}$ by males *Tp w-1/TM3, Sb Ser* or *Tp* $w^{di}$*-1/TM3, Sb Ser*. Two results were obtained. For all six independently derived *Tp w-1* mutations, males of the genotype *Tp w-1/+*; $w^{sp}$ are $w^{sp}$ in phenotype, i.e. show no evidence of complementation and are phenotypically inseparable from their *TM3, Sb Ser/+*; $w^{sp}$ brothers. For the two *Tp* $w^{di}$*-1* mutants identical results were obtained. Males *Tp* $w^{di}$*-1/+*; $w^{sp}$ manifest a low level of complementation in that their eye colors are moderately but distinctly darker than those of their *TM3, Sb Ser/+*; $w^{sp}$ brothers. It should be noted that this level of complementation is relatively low when compared to the eye phenotype of *Tp* $w^c$*-1/+*; $w^{sp}$ males.

The mutability of two *Tp w-1* and one *Tp* $w^{di}$*-1* mutants was assayed by crossing individual females either *Tp w-1/TM3, Sb Ser;* $w^-/w^-$ or *Tp* $w^{di}$*-1/TM3, Sb Ser*; $w^-/w^-$ to $y^2 w$ *spl* $sn^3$ males. Mutations were sought in the non-*TM3* progeny. Females were classified as fertile if they produced a minimum of 100 non-*TM3* progeny. For each of the *Tp w-1* mutants, 95 fertile females were scored; for the

*Tp* $w^{di}$*-1* mutant, 96 fertile females were scored. No phenotypic evidence of mutation was uncovered among all three mutants tested, i.e. all appear to be mutationally stable.

The foregoing information is consistent with the interpretation that *Tp* $w^{c}$*-1* represents the transposition of a segment of the white region. Therefore, a definition of the genetic extent of this transposition becomes of prime interest. Since the coupled genotype $w^{c}w^{sp}$ determines a white eye color and *Tp* $w^{c}$*-1* results in the $w^{c}$ phenotype, it follows that the entire *w* segment was not transposed. Rather, it appears more likely that only a part of the *w* segment including $w^{c}$ was removed leaving a residual deficient *X* chromosome carrying the mutant $w^{sp}$. Two lines of evidence support this view: (1) the interaction of *Tp* $w^{c}$*-1* with *z*, and (2) the cytogenetics of the deficient *w spl* chromosome recovered in association with the original exceptional $w^{c}$*spl* male. Since both lines of evidence depend upon the genetic interrelationship of *z* and *w*, the salient features of this interrelationship will be briefly outlined here.

The apparent paradox of females homozygous *z* manifesting the characteristic zeste eye color while hemizygous *z* males are wild type in eye color was resolved by the demonstration that for its phenotypic manifestation, the *z* mutant depends upon the simultaneous dosage and mutational state of the *w* gene(s). Thus hemizygous *z* males manifest the zeste eye color if a duplication of the $w^{+}$ gene is included in their genomes (GANS 1953); this duplication need include only that part of the $w^{+}$ gene marked by the sites of $w^{ch}$ and $w^{sp}$ (GREEN 1959a, 1963). Conversely, if in homozygous *z* females, one of the two $w^{+}$ genes is deleted (and only the sites of $w^{ch}$ and $w^{sp}$ need be deleted), the resultant females manifest a wild-type eye color. In addition, the mutants $w^{ch}$ and $w^{sp}$ together with their isolocal alleles act as dominant suppressors of zeste whereas *w* mutants localized to the left of $w^{ch}$, e.g. $w^{a}$, do not so act. Thus females of the genotype *z* $w^{+}$/*z* $w^{ch}$ or *z* $w^{+}$/*z* $w^{sp}$ manifest a deep maroon rather than zeste eye color and females of the genotype *z* $w^{+}$/*z* $w^{ch}w^{sp}$ are inseparable from the wild type. Conversely, females *z* $w^{+}$/*z* $w^{a}$ are zeste in eye color. Interallelic crossing over experiments localized $w^{c}$ to the left of $w^{ch}$ (GREEN 1969) and consistent with this fact, females *z* $w^{+}$/*z* $w^{c}$ are zeste in eye color.

Based upon the foregoing facts, some idea of the genetic length of *Tp* $w^{c}$*-1* can be deduced from its interactions, if any, with *z*. If *Tp* $w^{c}$*-1* comprises the entire *w* segment with only $w^{c}$ as the mutated site, it would be expected to evoke the zeste phenotype when combined in males with an intact *X* chromosome carrying *z* and $w^{+}$. If *Tp* $w^{c}$*-1* includes $w^{c}$ and $w^{sp}$ together with the entire *w* region, it would be expected that males constituted of an intact *X* chromosome carrying *z* and $w^{+}$ plus *Tp* $w^{c}$*-1* would manifest a maroon eye color comparable to that of females of the genotype *z* $w^{+}$/*z* $w^{sp}$. Finally, if *Tp* $w^{c}$*-1* includes only a part of the *w* region, with that part marked by the $w^{ch}$ and $w^{sp}$ loci deleted, then males constituted of *Tp* $w^{c}$*-1* plus an intact *X* chromosome carrying *z* and $w^{+}$ would be expected to manifest a wild type eye color. Males of the genotype *z* $w^{+}$; *Tp* $w^{c}$*-1* were obtained from the cross homozygous *z* females by *Tp* $w^{c}$*-1/TM3, Sb*

*Ser* males. Without exception they are wild type in eye color which means that *Tp* $w^c$*-1* includes only a portion of the *w* region, with that portion marked by the $w^{ch}$ and $w^{sp}$ loci absent.

A parallel genetic analysis of the *w spl* chromosome recovered in combination with *Tp* $w^c$*-1* in the exceptional $w^c$*spl* male is consistent with the foregoing genetic definition of *Tp* $w^c$*-1*. There are a number of compelling reasons for regarding this chromosome, hereafter designated as $w^{-c}$, to be deficient for that part of the *w* region reciprocal to *Tp* $w^c$*-1*. First, $w^{-c}$*spl* has lost the high mutability characteristic of the $w^c w^{sp}$ chromosome from which it arose. In fact, it is mutationally stable which means $w^{-c}$ no longer includes $w^c$. Second, females of the genotype $w^{-c}/w^{sp}$ manifest the white eye color with faint spots characteristic of females compounded of a white deficiency and $w^{sp}$. Third, the developmental time of males and females homozygous $w^{-c}$ is delayed by about 48 hrs as compared to $w^c w^{sp}$ homozygotes under routine culture conditions. This delay is comparable to that described for homozygotes for a viable deficiency of the $w^{ch}$ and $w^{sp}$ sites of the *w* region (GREEN 1959b). Finally, there is genetic evidence suggesting that $w^{-c}$ contains the $w^{sp}$ mutant. This evidence stems from the suppressor action of $w^{-c}$ on *z*. As noted above females of the genotype *z* $w^+/z$ $w^{sp}$ are maroon in eye color while females *z* $w^+/z$ $w^-$ (where $w^-$ is a deficiency for both the $w^{ch}$ and $w^{sp}$ sites) are wild type in color. Females of the genotype *z* $w^+/z$ $w^{-c}$ were synthesized and their eye color is inseparable from that of *z* $w^+/z$ $w^{sp}$ females. These results are interpreted as follows: the deficiency associated with $w^{-c}$ does not extend to the $w^{ch}$ and $w^{sp}$ sites, hence $w^{-c}$ contains both the wild type allele of $w^{ch}$ and the $w^{sp}$ mutant.

*Origin of* Tp $w^c$-2: The discovery in the $w^c w^{sp}$*spl* stock of a single male carrying a new, spontaneous yellow body color mutant, designated $y^{67d}$, prompted the establishment of a homozygous $y^{67d} w^c w^{sp}$*spl* stock with the ultimate aim of testing the mutability of $y^{67d}$. This stock was maintained for several generations without selection in several mass cultures. In the course of checking this stock prior to testing the mutability of $y^{67d}$, three males of the phenotype $y^{67d} w^c$*spl* were found in one culture bottle. Each $y^{67d} w^c$*spl* male was crossed to several homozygous *y w f* attached-*X* females. The progeny of each cross were identical consisting of ca. one-half the males $y^{67d} w^c$*spl*, one-half $y^{67d} w$ *spl*; about one-half the females *y w f* and one-half *y f* with a dilute $w^c$ eye color. These segregation results are compatible only with the interpretation that $w^c$ is no longer sex-linked but linked to an autosome, the result of a transposition. As will be demonstrated, each of the exceptional $y^{67d} w^c$*spl* males carried the same transposition, henceforth designated *Tp* $w^c$*-2*. Crosses designed to establish the linkage relations of *Tp* $w^c$*-2* were carried out precisely as described for *Tp* $w^c$*-1*. All three examples of *Tp* $w^c$*-2* are linked to chromosome 3. In the course of these crosses a number of phenotypic properties of *Tp* $w^c$*-2* were established which are summarized as follows: *Tp* $w^c$*-2* like *Tp* $w^c$*-1* is recessive to $w^+$, i.e. males with a wild-type *X* chromosome and a third chromosome carrying *Tp* $w^c$*-2* are wild type in eye color. A complementary eye color results when *Tp* $w^c$*-2* is combined with $w^{sp}$ just as in the case of *Tp* $w^c$*-1*. In contrast to *Tp* $w^c$*-1*, *Tp* $w^c$*-2* is not lethal

when homozygous and from the cross *TM3, Sb Ser/Tp* $w^c$*-2;* $w^-/w^-$ females by *TM3, Sb Ser/Tp* $w^c$*-2;* $w^-$ males homozygous *Tp* $w^c$*-2* males and females are recovered in the expected numbers. The eye color of *Tp* $w^c$*-2* homozygotes, is as expected, distinctly darker than that of *Tp* $w^c$*-2* heterozygotes.

Preliminary mapping experiments localized all three occurrences of *Tp* $w^c$*-2* to the left arm of the third chromosome. More precise mapping of each *Tp* $w^c$*-2* was carried out using the markers *ve* and *h* precisely as described for localizing *Tp* $w^c$*-1*. The results of these crossing over experiments were consistent; all three transpositions were located at the same position between *ve* and *h*. For one *Tp* $w^c$*-2*, based on 4,121 individuals scored, a crossing over frequency of 10.0% was found for the interval *ve-Tp* $w^c$*-2* and 13.6% for the interval *Tp* $w^c$*-2–h*. A second *Tp* $w^c$*-2* gave crossing over frequencies of 9.5 and 13.1% respectively, for the same intervals based on 2,762 individuals scored. The third *Tp* $w^c$*-2* gave crossover frequencies of 10.3 and 13.0% respectively, for the two intervals based on scoring 2,549 individuals. These data mean that *Tp* $w^c$*-2* is located about 10 units to the right of *ve* and, therefore, to the right of *Tp* $w^c$*-1*. They also mean that in all probability all three occurrences of *Tp* $w^c$*-2* are identical, a fact consistent with origin as a premeiotic event.

Estimates of the mutability of *Tp* $w^c$*-2* were obtained by crossing single females of the genotype *Tp* $w^c$*-2/Tp* $w^c$*-2; w/w* to males *TM3, Sb Ser/+; w*. (The *TM3* balancer was included in the male's genotype to facilitate the recovery of mutations.) As in the *Tp* $w^c$*-1* mutation experiments, only those females producing a minimum of 100 progeny were included in an experiment. In a typical experiment the following results were obtained. Of 46 females tested, 42 were fertile. Of these, 20 produced in addition to *Tp* $w^c$*-2* progeny, white-eyed exceptions as follows: 9 females with single white-eyed exceptions; 4 females with two exceptions each; 3 females with three exceptions each; 2 females with four exceptions each, 1 female with eight exceptions and 1 female with ten exceptions. In addition, 2 females produced single exceptions of an eye color inseparable from that classified as $w^{di}$ in the case of *Tp* $w^c$*-1*. The two classes of exceptions are designated *Tp w-2* and *Tp* $w^{di}$*-2*.

Complementation tests of 13 independent *Tp w-2* mutants and 2 *Tp* $w^{di}$*-2* mutants were made by crossing homozygous $y^2w^{sp}$ females to *Tp w-2/TM3, Sb Ser* or *Tp* $w^{di}$*-2/TM3, Sb Ser* males and comparing the eye colors of the male progeny. No evidence of complementation was noted for any of the *Tp w-2* mutants tested; both the *Tp w-2;* $w^{sp}$ and *TM3, Sb Ser;* $w^{sp}$ males were $w^{sp}$ in phenotype and inseparable from each other. The two *Tp* $w^{di}$*-2* mutants gave a low level of complementation precisely as described above for the *Tp* $w^{di}$*-1* derivatives.

Tests of the mutability of the two *Tp w-2* and one *Tp* $w^{di}$*-2* derivatives of *Tp* $w^c$*-2* were carried out precisely as described above for *Tp w-1* and *Tp* $w^{di}$*-1* with the modification that homozygous *Tp w-2* and *Tp* $w^{di}$*-2* females were tested. For each type 96 homozygous females were tested and no evidence of mutation found.

The $y^{67d}$*w spl X* chromosome carried by each of the three exceptional $y^{67d}w^c$*spl* males was isolated and analyzed. Each proved to be of the genotype $y^{67}w^cw^{sp}$*spl* since each carried a mutable $w^c$ which on mutating to $w^+$ "uncovered" a residual

$w^{sp}$. Thus the *X* chromosome reciprocal to *Tp* $w^c$*-2* appears not to have been recovered.

*Origin of* Tp $w^c$-3: In an experiment designed to test the mutability of $y^{67d}$, single, homozygous $y^{67d}w^cw^{sp}spl$ females were crossed to $y^sw^-spl\ sn^3$ males. Among the progeny of one female was a single male of the phenotype $y^{67d}w^cspl$. This male was crossed to homozygous *y w f* attached-*X* females. The resulting progeny consisted of equal numbers of two types of males, *y* $w^c$*spl* and *y w spl* and equal numbers of two types of females, *y w f* and *y f* with a dilute $w^c$ phenotype. These segregation data are consistent with the linkage of $w^c$ to an autosome and this transposition is designated *Tp* $w^c$*-3*. Linkage analysis localized *Tp* $w^c$*-3* to the third chromosome. Preliminary crossing over experiments placed *Tp* $w^c$*-3* in the left arm of the third chromosome. Crossing over experiments using the *ve h* markers placed *Tp* $w^c$*-3* about 18 units to the right of *h*. A more precise localization was carried out by determining crossing over in females *Tp* $w^c$*-3/ri sbd;* $w^-/w^-$ × *ri sbd;* $w^-$ males. Based on experiments in which 2,463 progeny were scored, *Tp* $w^c$*-3* mapped at 0.9 crossover units to the left of *ri*.

So far as dominance, complementation, mutability, etc., are concerned, no difference could be discerned between *Tp* $w^c$*-3* and *Tp* $w^c$*-2*. Therefore, except for location these transpositions appear to be identical and no further comment is in order at this point.

*Origin of* Tp $w^c$-4: A critical feature of the $w^c$ mutable system—one that is diagnostic for the presence of a controlling element—is the regular recovery of deficiencies all of which are centered at the *w* locus and extend for varying lengths either to the right or left of this locus (GREEN 1967, unpublished). Subsequent to the discovery of the relatively long *N* deficiencies evoked by the $w^c$ controlling element, a comparatively large number of shorter deficiencies were recovered (GREEN, unpublished). Included among these shorter deficiencies are those which appear on genetical grounds to involve the loss of only a part or all of the *w* region. One such deficiency, designated $w^{-67c}$ was among several selected for more detailed study. A homozygous *y* $w^{-67c}sn^3$ stock was synthesized preparatory to answering the question of whether the controlling element was lost concurrent with the production of the $w^{-67c}$ deficiency. It was reasoned that if $w^{-67c}$ retained the $w^c$ controlling element, it should be possible to recover at a relative high frequency longer *N* deficiencies among the progeny of homozygous *y* $w^{-67c}sn^3$ females. If, however, the controlling element was lost concurrently with the origin of $w^{-67c}$, $w^{-67c}$ should be stable and *N* deficiencies should only rarely, if ever, occur. It will suffice to point out here that frequent *N* deficiencies, often as clusters, were recovered among the progeny of *y* $w^{-67c}sn^3$ females proving that $w^{-67c}$ contains the controlling element. Since the controlling element is located at the $w^c$ site, it was reasoned that at least part of the *w* region is intact in $w^{-67c}$ and the loss probably involves only the sites of $w^{ch}$ and $w^{sp}$. This conclusion is supported by the demonstration that females *z* $w^+/z\ w^{-67c}$ are wild type in eye color, a phenotype expected when both the $w^{ch}$ and $w^{sp}$ sites are deleted.

A homozygous *y* $w^{-67c}sn^3$ stock was maintained without selection by mass transfer. In the course of periodically checking this stock, two exceptional indi-

viduals were found: one male of the phenotype *y* $w^c sn^3$ and one female of the phenotype *y* $sn^3$ with a dilute $w^c$ eye color. The *y* $w^c sn^3$ male was crossed to homozygous *y w f* attached-*X* females and the progeny produced consisted of males one-half *y* $w^c sn^3$, one-half *y w* $sn^3$, females one-half *y w f*, one-half *y f* with a dilute $w^c$ eye color. These results are compatible with the interpretation of a transposition of $w^c$ to an autosome. The exceptional female was crossed to a *y* $w^{-67c} sn^3$ brother and produced the following offspring: females about one-half *y w* $sn^3$, one-half *y* $sn^3$ dilute $w^c$; males about one-half *y w* $sn^3$, *y* $w^c sn^3$. These results are also compatible with the transposition of $w^c$ to an autosome.

There are several reasons for concluding that the aforementioned two exceptions are not contaminations but arose directly from $w^{-67c}$. First, both exceptions carried the *y* and $sn^3$ markers. Second, genetic tests of the *y w* $sn^3$ *X* chromosome derived from both exceptions showed the *w* to be a genetic loss indistinguishable from $w^{-67c}$. Third, linkage tests demonstrated the new transposition, designated *Tp* $w^c$*-4*, to be different from the previous three described. (So far as can be determined the transposition derived from the exceptional male and female are identical and will be considered as such.) Linkage tests placed *Tp* $w^c$*-4* on the third chromosome. Preliminary crossing over experiments located *Tp* $w^c$*-4* in the right arm of the third chromosome. A more exact mapping of *Tp* $w^c$*-4* was obtained by determining the crossing over frequency in females *Tp* $w^c$*-4*/$e^4$*ro*; $w^-/w^-$. The results based on scoring 3,750 progeny showed that *Tp* $w^c$*-4* maps at 0.7 crossover units to the right of *ro*.

In general, *Tp* $w^c$*-4* appears not to differ from the three other transpositions described. It is recessive to $w^+$, complements with $w^{sp}$, and is mutable. From the cross *Tp* $w^c$*-4/TM3* females by *Tp* $w^c$*-4/TM3 Sb Ser* males, fully viable homozygous *Tp* $w^c$*-4* individuals are obtained. They are of interest because while homozygous *Tp* $w^c$*-4* females are fertile, homozygous *Tp* $w^c$*-4* males, extensively tested, are sterile.

## DISCUSSION

In Figure 1, the linkage relationships of the four transpositions are summarized and their approximate map positions given. Since all complement $w^{sp}$ and all are mutable, it is a reasonable conclusion that all transpositions are essentially alike and represent the removal of a segment of the *w* region including the controlling element and its integration at different places of the third chromosome. The very fact of integration raises a number of questions of which the following are judged to be the most important. (1) Were the transpositions mediated by the controlling element? (2) How much of the *w* gene was transposed? (3) By what mechanism

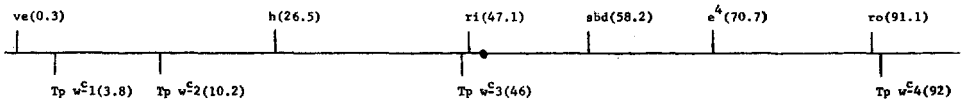


FIGURE 1.—Approximate map locations (in parentheses) of selected marker (above) and four $w^c$ transpositions (below) on chromosome 3. (●) = centromere.

was the segment of *w* gene integrated into the third chromosome? (4) Do the transpositions shed any light on the nature of the controlling element? (5) Are any insights into the mechanism of *w* gene action derived as a consequence of the transposition? An answer to each of the questions will be attempted within the framework of information presently available. Hence, it should be emphasized that for some questions reasonably clear-cut answers can be given, for others at best an educated guess, at worst only conjecture is possible.

Spontaneous transpositions of chromosome segments from one chromosome to another are indeed rare and such transpositions have only infrequently been accomplished by X irradiation. Thus, it is difficult to account for the transpositions documented here as spontaneous events. Moreover, the fact that all transpositions were derived from *w* genes containing a controlling element and that the controlling element was incorporated within each transposed segment makes it difficult to escape the conclusion that somehow the controlling element is responsible, directly or indirectly, for the transposition event. Precisely how the piece of *w* gene is excised from *X* chromosome and integrated into the third chromosome remains for the present a complete mystery. It is quite possible that the proposed model for transposition of the maize *Mp* (*Ac*) controlling element (GREENBLATT 1966, 1968) applies here as well. The data presented neither conflict with nor support this model which proposes, in part, that transposition occurs when the chromosomes are replicating. Moreover, intuitively this model has appeal because, in its simplest terms, transposition requires excision of DNA and integration into "foreign" DNA, a sequence of events most likely to occur during chromosome replication when presumably the DNA strand is temporarily free of its protein component.

The size of the transposed segment is obviously very small and there is ample reason to conclude that it represents only a portion of what has been called the white gene. The linear limits of the white region are defined by the $w^{sp}$ site on the right and $w^{Bwx}$ on the left (GREEN 1959a; JUDD 1964). In Figure 2, a micromap is given together with the postulated maximal genetic length of the trans-

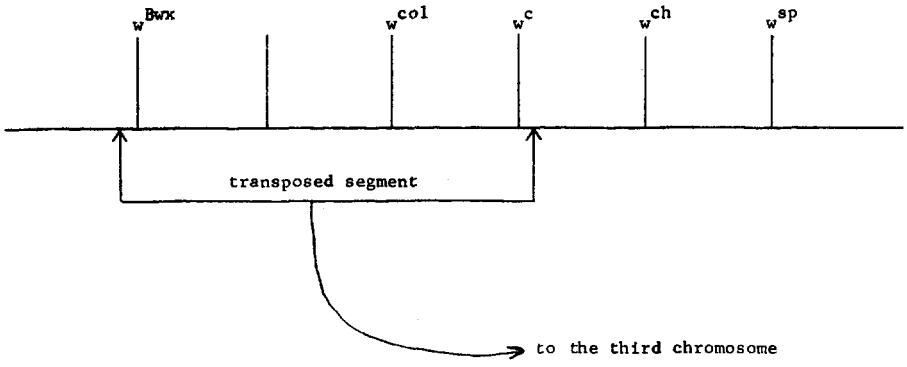


FIGURE 2.—A micromap of the *w* region indicating the postulated maximum genetic length of the transposed segment.

posed segment. The reasoning underlying this postulate is as follows. It will be recalled that three transpositions were derived from chromosomes of the genotype $w^c w^{sp}$. Since this genotype when homozygous leads to a white eye color as a result of the interaction between $w^c$ and $w^{sp}$, and since the transposed segment conditions the $w^c$ phenotype, it is reasonable to suppose that $w^{sp}$ was not included in the transposition segment. If the entire region were transposed, one would expect it to condition a white color in its new location just as it does in its normal location. That the $w^{ch}$ site is also not included in the transposition is supported by three lines of evidence. First, none of the transpositions promotes the development of the zeste eye color. This suggests that neither the $w^{ch}$ and $w^{sp}$ sites necessary for zeste eye color differentiation (GREEN 1959a) are present in the transposition. Since JUDD (1964) has submitted evidence that duplication of the $w^{ch}$ site alone promotes partial differentiation of the zeste eye color, it follows that this site is also missing in the transposed segment. Second, evidence was presented to show that the *w* deficiency reciprocal to *Tp* $w^c$*-1* carried the $w^{sp}$ mutant. Since this deficiency is only a partial not a complete zeste suppressor, this means that deficiency does not include the $w^{ch}$ site. Third, *Tp* $w^c$*-4* arose from a deficiency whose dominant suppression of zeste is consistent with the conclusion that both the $w^{ch}$ and $w^{sp}$ sites are deleted. These arguments favor the view that the transposed segments include the $w^c$ site but not the $w^{ch}$ and $w^{sp}$ sites.

The recent discovery of a new locus, *sa*, which lies immediately to the left of the *w* region (RAYLE and GREEN, 1968) fixes the leftmost genetic limit of the transposition at $w^{Bwx}$. Crossing over between *sa* and *w* is of the order 1/6600 indicating that *sa* and the *w* region are juxtaposed. When each *Tp* $w^c$ was combined with *sa* in males, their phenotype was *sa* demonstrating that a dominant $sa^+$ allele is not included in the transposition. Thus, the maximal genetic length of each transposition is that defined in Figure 2.

Based primarily on the genetic nature of deficiencies occurring in association with $w^c$ and its mutable derivatives, it was argued (GREEN 1967) that a controlling element must be somehow responsible for both the high mutation rate and the deficiencies. The intriguing fact that the deficiencies invariably have one end fixed at the *w* locus, i.e. rarely, if ever, overlap *w* (GREEN 1967, and unpublished) prompted the speculation that the controlling element is virus-like since it has properties analogous to those of temperate viruses. Thus, the reversion of $w^c$ to $w^+$ and the production of deficiencies have their counterpart in the induction of lysogenic bacteria. Temperate phage, lambda, of *E. coli* is a good example. Two consequences of induction of a lambda lysogen have been recognized: the release of the intact prophage and its subsequent maturation or the release of an altered prophage, lambda-dg, which includes an attached small piece of host chromosome. The former event is equivalent to the reversion of $w^c$ to $w^+$, i.e. the residual host chromosome is controlling element (virus!) free and, therefore, no longer mutable. The latter event is comparable to the formation of deficiencies, i.e. the reciprocal of a lambda-dg is a deficient host chromosome. This parallelism can now be carried one step further. The transpositions of $w^c$ are the equivalent of lambda-dg, i.e. these include not only the controlling element but an associated segment of

"host" $X$ chromosome. Taken together these facts imply that equating a controlling element to a temperate phage is no longer a fanciful speculation but a serious possibility. It remains now to be seen whether a "free" controlling element can be experimentally demonstrated.

Some comment is in order on the implications the transpositions have for the genetical organization and function of the $w$ region. On the basis of the subdivision of the $w$ region by interallelic recombination, this segment can be classified as a cistron, a genetic segment conditioning the synthesis of one polypeptide. However, it was earlier argued (GREEN 1959a, 1963), that such a classification is an oversimplification and ignores rather critical phenogenetic properties of particular mutants. Therefore, predicated, in part, on the correlation between suppression of zeste and the mapping of mutants by interallelic crossing over and, in part, on the genetic properties of duplications and deficiencies of the $w^{ch}$ and $w^{sp}$ marked segments of the $w$ region, it was argued (GREEN 1963) that at a minimum this region consists of two cistrons. The transpositions lend further support to this conclusion. Since the transposed segments do not include the $w^{ch}$ and $w^{sp}$ sites and the loss of these sites in a normal $X$ chromosome $w$ region results in a white eye color, i.e. no pigment synthesis, how can it be that the transposed segments condition pigment synthesis? This dilemma may be rationalized by borrowing from the operon model of gene function. Assume first that the $w$ region is composed of two parts: a structural part defined by the four sites between $w^{Bwx}$ and $w^{c}$ of Figure 2 and a regulatory part defined by the two sites designated $w^{ch}$ and $w^{sp}$. Assume further, by analogy with the *lac* operon (summarized by BECKWITH 1967), that control of the structural gene(s) while nominally the role of the designated regulatory sites may nonetheless be assumed by other regulatory genes. Thus, in the case of the genotype $w^{c}w^{sp}$ pigment synthesis fails because $w^{sp}$ is a malfunctioning regulator. When, however, the $w^{c}$ containing segment is freed from $w^{sp}$ and transposed to the site of some other normally functioning regulator gene, pigment synthesis is restored. It should be emphasized that this is a formal explanation of the functioning of the $w$ region; biochemical studies which test this (or other) explanations are long overdue.

## SUMMARY

Four independent transpositions of a segment of the white gene to the third chromosome in *Drosophila melanogaster* are described. A genetic analysis of the transpositions implicates a controlling element as the instrumental agent. It is concluded that the controlling element has properties equivalent to those of temperate viruses. The functional behavior of the transposed segment is consistent with the view that the white gene consists at a minimum of two cistrons.

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