MAPPING A *DROSOPHILA MELANOGASTER* "CONTROLLING ELEMENT" BY INTERALLELIC CROSSING OVER*

M. M. GREEN

Department of Genetics, University of California, Davis, California 95616

Received July 16, 1968

CONTROLLING element is the name given by McCLINTOCK to those chromosomal entities associated with inordinately high mutation rates at specific gene loci (cf. detailed reviews by McCLINTOCK 1965 and 1967). Although originally described in *Zea mays*, the occurrence of controlling elements has been adduced for organisms so widely separate as *Escherichia coli* (TAYLOR 1963) and *Drosophila melanogaster* (GREEN 1967). While the induced mutation in *E. coli* is directly attributable to the integration of the phage, mu-1, at the site of mutation, the precise nature of the mutation inducing agents of maize and Drosophila is to date obscure. Nonetheless, the presence of controlling elements in maize and Drosophila leads to a number of cytogenetic changes which parallel in many details the behavior of temperate phages involved in lysogeny. Accordingly, it is not unreasonable to extend the parallelism and speculate that controlling elements are, in fact, integrated into the chromosomes at the gene sites associated with high mutability. That controlling elements are intimately associated with specific chromosomes is indisputable; that they are integrated into and replicate concurrently with chromosomes is still an open question. Evidence supporting integration would follow if controlling elements could be mapped by conventional crossing over. In at least one instance controlling elements have been successfully mapped. NELSON (1968) has mapped three controlling element regulated mutants of the *Wx* (waxy) gene of maize by interallelic recombination. In each the controlling element can be assigned to a specific location on the gene map, thereby supporting the idea of integration.

Evidence for a controlling element associated high mutation rate of the white (w) eye color gene in *D. melanogaster* has been previously documented (GREEN 1967). Since independent w mutants have been mapped by interallelic crossing over (GREEN 1959; JUDD 1959), experiments were undertaken to apply the same methods to map the Drosophila mutable gene, w^c. These experiments are the subject of this report.

MATERIALS AND METHODS

Standard Drosophila culture methods were employed throughout. Details of each cross will be given, where appropriate, in the text. A synopsis of the mutants used with their assigned symbols and linkage relationships is included in Table 1.

* Supported by grant GB 6127 from the National Science Foundation.

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*	
w	white eye color	*X*-1.5
w^a	white-apricot, allele of *w*	
w^{ch}	white-cherry, allele of *w*	
w^c	white-crimson, allele of *w*	
w^{col}	white-colored, allele of *w*	
w^i	white-ivory, allele of *w*	
sp-^{2}*w*	spotted-white-2, allele of *w*	
spl	split bristles, rough eye	*X*-3.0
sn^3	singed-3 bristles	*X*-21.0
Cy	Curley wing	Inversion of *2*
Ubx	Ultrabithorax-130	Inversion of *3*

EXPERIMENTAL

The rationale of the interallelic recombination experiments can be stated as follows. If w^c contains an integrated controlling element, it should be possible to localize this element by interallelic crossing over. For this purpose it is necessary to recover crossovers which couple w^c with *w* tester alleles which flank both sides of the w^c locus. To be significant, such crossovers must carry a mutable w^c. The reciprocal wild type recombinants can be ignored since, lacking the controlling element, they provide no precise information on its location.

Since w^c apparently arose from w^i (GREEN 1967), it was assumed that most likely the locus immediately to its right is marked by w^{ch}, and the locus immediately to its left is marked by w^{col} (GREEN 1959). Thus, the predicated allele sequence is: w^{col}-w^c-w^{ch}. It was further assumed that the origin of w^c from w^i meant that w^c could manifest some of the genetic properties peculiar to w^i. One property, relevant here, is the significant reduction in interallelic recombination associated with w^i (LEWIS 1959; BOWMAN 1965; BOWMAN and GREEN 1966). Anticipating that w^c might similarly reduce interallelic crossing over, exploratory crossing over experiments were first undertaken using the mutant *sp*^{2}-*w* localized just to the right of w^{ch}. Based upon its location and earlier experience, it was anticipated that interallelic crossing over should be maximal in females of the genotype w^c/sp^2-*w*.

The phenotypic identification of the putative $w^c sp^2$-*w* crossover was predicated upon earlier experience with interallelic crossing over between *w* mutants. Invariably where *w* mutants containing some eye pigment are studied, the coupling (cis) compound is phenotypically lighter than the eye color of each individual mutant and is usually white. Thus, for example, the coupled mutants $w^a sp^2$-*w* result in a white eye color when homo- or hemizygous. It was, therefore, assumed that individuals of the coupled compound $w^c sp^2$-*w* would similarly manifest a white eye color phenotype.

Since w^c mutates premeiotically to w with great frequency, it is quite conceivable that this event followed by a subsequent meiotic crossover involving the marker genes flanking the w locus could produce a white-eyed individual with the appropriate marker gene. This individual would be mistakenly identified as the putative crossover. In an attempt to minimize such misidentification, the following procedure was adopted. As a routine in interallelic crossing over experiments, separate crosses consist of mating ten heterozygous females with an excess of males per individual half-pint milk bottles containing about 50 ml of medium. 23 such crosses are made concurrently; females are allowed to oviposit for four days and transferred to fresh medium for three more days. Two more transfers are made at three day intervals such that progeny for each group of females are scored in four separate bottles. Each bottle yields about 1,000 offspring. If a white-eyed exception occurred with the appropriate marker gene as the sole exceptional individual in the four bottles of a set, it was saved as a putative recombinant and crossed for further testing. Where more than one white-eyed exception occurred in a set of four bottles of which one or more carried the expected crossover marker gene, they were interpreted to be the result of mutations of w^c to w followed by crossing over and were, therefore, discarded.

Accordingly, crossing over between w^c and sp^2-w was sought among the progeny of females $y\ sp^2$-$w\ spl/w^c$; $Cy/+$; $Ubx/+$ crossed to males $y^2w\ spl\ sn^3$. The expected crossover should be phenotypically $w\ spl$. In a total of ca. 30,000 F_1 scored one $w\ spl$ male, presumably the desired w^csp^2-$w\ spl$ crossover, was found. After establishing a stock, this chromosome was further tested by crossing to sp^2-w. It has been earlier demonstrated that coupled mutants, e.g. w^asp^2-w when compounded to sp^2-w (= females w^asp^2-$w/+\ sp^2$-w) are sp-w in phenotype. This in contrast to w^a/sp^2-w which results in a complementary (near wild type) phenotype and in contrast to w^-/sp^2-w (where w^- represents a white deficiency) which produces a white eye with faint spots indisputably lighter than sp^2-w. Thus, in agreement with expectation, females possessing the presumed w^csp^2-w X chromosome and an homologous X carrying only sp^2-w were clearly sp-w in phenotype.

A second test designed to establish the genotype of the presumed w^csp^2-w recombinant was predicated on the following assumption. If, in fact, this chromosome carries both an unaltered w^c and sp^2-w, w^c should still be mutable. Where w^c mutates to w^+, the resultant chromosome will contain only the sp^2-w allele, and thus sp^2-w individuals will occur among the progeny of w^csp^2-w homozygotes. To test this possibility females homozygous for w^csp^2-$w\ spl$ were crossed individually to $y^2w\ spl\ sn^3$ males and $sp^2w\ spl$ exceptions sought among their progeny. A number of such experiments were carried out of which the following is representative. Among a total of 59 w^csp^2-$w\ spl$ females tested, eight produced sp^2-$w\ spl$ exceptions: five females produced single sp^2-$w\ spl$ exceptions; two females produced three sp^2-$w\ spl$ exceptions and one female produced four sp^2-$w\ spl$ exceptions. In other experiments even larger "clusters" of sp^2-$w\ spl$ exceptions were found. These data mean that by interallelic crossing over, the coupled compound

$w^c sp^2$-w has been recovered and that following the crossover event, the high mutation property of w^c is retained.

A more precise localization of w^c was carried out using w^{ch} as the tester mutant. Crossing over was scored among the progeny of $y\ w^{ch}\ spl/w^c$; $Cy/+$; $Ubx/+$ females mated to $y^2 w\ spl\ sn^3$ males. The crossover sought was expected to be phenotypically "w" *spl* (white-eyed, split). Among about 120,000 F_1 scored, three "w" *spl* exceptions, 2 males and 1 female were recovered and bred as possible $w^c w^{ch}$ *spl* recombinants. The "w" *spl* female proved on progeny testing to carry a parental $y^2 w\ spl\ sn^3$ X chromosome and an homologous X which was male lethal. Thus, the "w" *spl* X was in all probability a w deficiency (w^-) known to occur as a function of the controlling element (GREEN, unpublished). Stocks were established of the "w" *spl* chromosomes recovered as males and were tested as follows. It has been previously shown that sp^2-w in compound with w mutants results in a complementary phenotype even in the case of females $w^a w^{ch}/sp^2$-w. Therefore, using both exceptional chromosomes females of the genotype "w" *spl*/sp^2-w were obtained. In one case the females produced a complementary (near wild type) eye color implying that the "w" *spl* chromosome carried both w^c and w^{ch}. In the second case the females manifested a faint *sp*-w phenotype characteristic of w^-/sp^2-w heterozygotes implying that the "w" *spl* chromosome was a male viable w deficiency, a type which had been previously recovered as a consequence of the controlling element behavior (GREEN, unpublished).

Subsequently, the genotype of the presumed $w^c w^{ch}$ recombinant was proved by assuming that in this chromosome w^c retained its property of frequent mutation to w^+. When such mutations occur, w^{ch} individuals should be recovered. Accordingly, females presumptively homozygous $w^c w^{ch}$ *spl* were tested individually by crossing to $y^2 w\ spl\ sn^3$ males and mutations of w^c to w^+ were sought by recovering w^{ch} *spl* exceptions. In a typical experiment, in which 58 females were tested, the following results were obtained: 14 females produced w^{ch} *spl* progeny; 3 produced single w^{ch} *spl* exceptions; 8 produced two w^{ch} *spl* exceptions; 2 produced nine w^{ch} *spl* exceptions and 1 produced ten w^{ch} *spl* exceptions. These results confirm the recovery of the $w^c w^{ch}$ crossover demonstrating that w^c is localized to the left of w^{ch} and that the property of mutation was not lost as a consequence of the crossover event.

It should be further noted that the recovery of but a single $w^c w^{ch}$ crossover among 120,000 progeny indicates that w^c like w^i markedly reduced interallelic crossing over. On the basis of crossing over between w^{ch} and pigmented w mutants located to the left of w^{ch}, such crossovers are expected in a frequency of about 1/20,000 progeny (GREEN 1959). Furthermore, in interallelic crossing over tests involving w^{ch} and w^i, BOWMAN and GREEN (1966) found no $w^i w^{ch}$ crossovers in 54,000 progeny scored.

Since by interallelic crossing over it had been previously shown that w mutants can be seriated in the order w^{col}–w^a–w^{ch}–*sp*-w, one more interallelic crossing over experiment with w^c was undertaken. While the aforementioned results localize w^c to the left of w^{ch}, they are ambiguous as to its precise location. Accordingly, experiments were undertaken to localize w^c with respect to w^{col}. The relatively

low crossover frequency between w^c and w^{ch} suggested that w^c is probably localized to the right of w^{col}, i.e. between w^{col} and w^{ch}. Since w^{col} conditions the formation of relatively highly pigmented eyes, it was anticipated by virtue of previous experience that the coupled w^{col} w^c genotype might not necessarily be white-eyed but rather have a slight amount of pigment. The desired crossover, carrying the *spl* marker, was sought among the progeny of the cross females y^2w^{col} *spl*/w^c × y^2w *spl* sn^3 males. Among a total of approximately 225,000 progeny, twelve F_1 exceptions, eight *w spl* and four *spl* with a small amount of eye pigmentation equivalent to that of w^i were found. All were tested for complementation with sp^2-*w*. The eight *w spl* exceptions when compounded to sp^2-*w* gave a slight but discernible level of complementation characteristic of *w* mutants derived by mutation from w^c. These were assumed to be *w* mutants derived from w^c and discarded. The remaining four all gave a high level of complementation. Of these, three proved on more careful examination to be phenotypically inseparable from w^i, while one, called w^{tinged}, was clearly darker. One presumed w^i was tested for spontaneous reversion to w^+ by scoring the progeny of homozygous females. Since spontaneous reversions of w^i to w^+ occur with a frequency of about 1/20,000 in homozygous w^i/w^i females and a comparable frequency found for the derived w^i, it was concluded that these three exceptions were the result of mutations of w^c to w^i. It was, therefore, inferred that the exception, designated w^{tinged}, was the w^{col} w^c *spl* crossover. Accordingly, homozgyous w^{col} w^c *spl* females were individually tested for mutation of w^c to w^+ evidenced by the recovery of w^{col} progeny. Among 58 females tested, 19 produced w^{col} exceptions, 4 females yielding single w^{col} exceptions; 5 females two w^{col} exceptions each; 5 females three w^{col} exceptions each; 4 female six w^{col} exceptions; 1 female seven w^{col}; and 1 female sixteen w^{col} exceptions. These data prove the recovery of the crossover chromosome w^{col} w^c *spl* and together with the other data demonstrate that w^c is localized together with its controlling element between w^{col} and w^{ch}.

DISCUSSION AND CONCLUSIONS

Two points are established by the foregoing data. First, w^c can be assigned by interallelic crossing over to a specific site. Second, the controlling element accompanies w^c in the course of the crossing over event which couples w^c to the particular *w* tester. This association favors the conclusion that the controlling element is literally integrated at the w^c site. Integration implies that the controlling element contains DNA and that this DNA is attached or bound to the chromosomal DNA such that both replicate in concert. It should, however, be pointed out that other explanations for the localization of the controlling element are possible. Conceivably, the controlling element could be attached without integration to the *X* chromosome at the *w* gene in such a way that its replication accompanies chromosome replication. The recombination data do not exclude this possibility. Rather, integration is preferred to attachment because it appears to be a simpler, more plausible explanation.

While the data are too scanty to permit a clear-cut decision, they are, nonethe-

less, suggestive that the presence of the controlling element in some way impedes interallelic crossing over. In contrast to the frequency of interallelic crossing over with conventional *w* mutants, great difficulty was encountered in recovering recombinants with w^c. This fact is further suggestive of integration of the controlling element and stands in contrast to the results of NELSON (1968) for controlling element mediated *wx* mutants in maize which appear not to reduce intralocus crossing over. The reduced crossing over associated with the Drosophila controlling element implies that its size is such as to locally affect pairing enough to decrease interallelic crossing over.

SUMMARY

The highly mutable white-crimson gene and its controlling element of Drosophila have been mapped by interallelic crossing over. The results support the interpretation that the controlling element is integrated at a particular site in the white gene.

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