Heredity (1978), **40** (2), 299-303

NOTES AND COMMENTS

GENETIC NATURE OF WHITE IVORY (w^i) IN THE WHITE LOCUS OF *DROSOPHILA MELANOGASTER* AS RESOLVED THROUGH CROSSING-OVER TESTS

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Received 22.vi.77

Summary

The presumed duplicational nature of white ivory (w^i) of *Drosophila melanogaster* was tested by crossing over. It was observed that w^i neither reduces nor enhances recombination in the intervals marked by subsites 1 and 3 and 4 and 5 of the *white* locus. The duplicational nature of this mutant site is questioned.

1. Introduction

The sex-linked white ivory (w^i) mutant of *Drosophila melanogaster* was the subject of extensive investigation in the past because of a variety of unusual properties of this genetic site (Lewis, 1959; Bowman, 1965, 1969). As an outgrowth of these studies, high spontaneous reversion of w^i in the females was interpreted by Bowman (1965) as evidence for a serial tandem duplication of a portion of the *white* locus. A similar conclusion was also reached by Rasmuson (1962) on the basis of phenotypic interaction of various mutants with *zeste*. Crossing-over tests involving w^i showed that it is located to the left of w^{ch} (Subsite 4). The low frequency of the recovery of cross-over products from w^i/w and w^i/w^{ch} heterozygotes (Lewis, 1959) and the absence of recombination derivatives from w^a/w^i heterozygotes (Rasmuson, 1962) were reconciled with the assumption that w^i suppressed recombination. On the contrary, Judd (1965) showed that intra-locus duplication of the subsite 4 of the *white* locus greatly increased crossing-over in this genetic interval. Moreover, Bowman (1965) hypothesised that the reversion occurred by crossing-over within a double loop formed by intrachromosomal pairing of the duplicated segment unaccompanied by exchange of outside markers. Indeed, in the absence of flanking marker exchange, a direct genetic proof is hard to visualise (Bowman and Green, 1966). Gabay and Laughnan (1973) could not unequivocally confirm such a model in the case of the *Bar* duplication in the modified attached-X system which affords an opportunity to recover both the products of such recombination events in the same individual. These seemingly conflicting reports led us to undertake crossing-over tests with w^i and other mutants of the *white* locus. This paper deals with the nature of w^i as revealed through crossing-over tests within the *white* locus.

2. Materials and methods

A synopsis of the mutants used, their symbols, phenotypes and linkage is given in table 1. Flies were raised on standard corn meal-molasses-agar

medium at 25±1°C. All heterozygous females carried SM1/+ and *Ubx*^{130}/+ rearrangements in the second and third chromosomes respectively to increase crossing-over in the distal portion of the X-chromosome. Additional information regarding these mutants was obtained from Lindsley and Grell (1968).

TABLE 1

Synopsis of mutants with symbols or reference included in text

Symbol	Phenotype	Linkage
y	Yellow body colour	X-0·0
spl	Split bristle	X-3·0
SM1	Lewis and Mislove, 1953	inversions autosome II
Ubx^{130}	Ultrabithorax halteres	inversions autosome III
w	white eye colour	X-1·5
w^a	white-apricot, allele of *w*	
w^{ch}	white-cherry, allele of *w*	
w^i	white-ivory, allele of *w*	
w^{Bwx}	white-Brownex, allele of *w*	
w^{sp}	white-spotted, allele of *w*	

The method of conducting genetic crosses was essentially similar to that of earlier workers (Green, 1959; Judd, 1959). The coupled mutant derivative of reciprocal recombination was analysed through the *spotted-white* test (Green, 1959, 1969) and other tests, noted in the text. In the present study, three double mutants were employed which divided the *white* locus into three different sectors. $w^{Bwx}w^a$ marked subsites 1 and 3, w^aw^{ch} marked subsites 3 and 4 and $w^{ch}w^{sp}$ marked subsites 4 and 5, respectively (Judd, 1964).

3. RESULTS

In the first cross with w^+, four recombinants were recovered from 20,000 flies screened, of which two were yw^a and the other two were $w^{ch}spl$ (table 2, cross *a*). In the second cross with w^i (table 2, cross *b*) three recombinants were recovered from 42,000 flies screened, one was yw^a and the other two were $w^{ch}spl$. These three exceptional chromosomes yielded near wild eye colour in females when compounded individually with w^{sp}. To test whether these three recombinants were coupled mutants or not, further

TABLE 2

Recombination between double mutants at the white *locus and* w^i *or* w^+

Cross	Number of recombinants	Total flies screened	Frequency
(a) $yw^aw^{ch}spl/+$	4	20,000	1/5,000
(b) $yw^aw^{ch}spl/w^i$	3	42,000	1/14,000
(c) $yw^{Bwx}w^aspl/+$	4	41,600	1/10,400
(d) $yw^{Bwx}w^aspl/w^i$	5	40,500	1/8,100
(e) $yw^{ch}w^{sp}spl/+$	2	57,400	1/28,700
(f) $yw^{ch}w^{sp}spl/w^i$	3	81,600	1/27,200

genetic tests were adopted. This was done by constructing females of the genotypes yw^a (?)+/*spl*; SM1/+; Ubx^{130}/+., +(?)w^{ch1}*spl*/*y*; SM1/+; Ubx^{130}/+ and +(?)w^{ch2}*spl*/*y*; SM1/+; Ubx^{130}/+ and mated to *ywspl* males. It was assumed that if w^a and w^{ch} were phenotypically similar to w^aw^i and w^iw^{ch} respectively, only w^i exceptions originating from reciprocal crossing-over would be recovered. The possibility of crossing-over between w^a and w^i arose from the fact that the recovery of w^{ch}*spl* amongst the offspring of yw^aw^{ch}*spl*/w^i females could plausibly be explained as due to reciprocal crossing-over in this genetic interval. Out of 89,000, 94,200 and 120,000 flies screened (table 3) in three separete crossing-over tests, no w^i exception was recovered. This proved that the recombinants were not coupled mutants. The reason for the failure to recover w^aw^i and w^iw^{ch} from the original cross remained unresolved at this stage of the experiment. However, occurrence of yw^a and w^{ch}*spl* indicated that w^i is located to the left of w^{ch} but right of w^a. Although the frequency of recombinants derived from this cross was less

TABLE 3

Crossing-over tests to determine the genotypes of exceptionals originated from yw^aw^{ch}spl/w^i *heterozygotes*

Cross	Number of recombinants	Total flies screened
yw^a(?)+/*spl*	—	89,000
+(?)w^{ch1}/*y*	—	94,200
+(?)w^{ch2}/*y*	—	120,000

when compared to the control value, it did not guarantee that suppression of recombination occurred since we encountered crossing-over. This point remained unsettled and needed further crossing-over tests involving other mutants of subsites 3 and 4.

In the crossing-over test with $w^{Bwx}w^a$ (table 2, crosses *c* and *d*), the possibility existed that the w^a recombinants were overlooked due to their phenotypic resemblance to the double mutant $w^{Bwx}w^a$ (Judd, 1959). In the fourth cross as in table 2, cross *d*, five recombinants were sorted out from a population of 40,500 flies screened. Out of the five recombinants, four appeared with a pale eye colour distinguishably lighter than w^{Bwx} flies. Recovery of a single *spl* male in this cross was significant enough to indicate that crossing over took place between w^a and w^i. The resultant triple mutant as a result of such a crossing-over remained unrecognised. However, from the spotted-white test, the four exceptions having pale eye colour indicated that the compounded females manifested near wild type eye colour. Hence the possibility existed of their being coupled mutants. These four exceptionals were tested separately for mutational activity, *i.e.* the presence of w^i was ascertained through the recovery of single mutants due to its frequent reversion to w^+. A homozygous stock was established for each recombinant as it was recovered. Periodic examination was carried out for the recovery of w^{Bwx} flies as the consequence of reversion of w^i to w^+. All four recombinants produced exceptions with clear w^{Bwx} eye colour. The frequency of recombination events did not provide any evidence for suppression of recombination when compared to the control value.

Phenotypically $w^{ch}w^{sp}$ flies possessed very faint eye colour in males. The females heterozygous for the double mutant and w were of darker eye colour. Interestingly, the dark eye colour in females is indistinguishable from that of w^{ch}/w. In the crossing-over tests indicated in table 2, crosses *e* and *f*, the yw^{ch} recombinant remained unrecognised in females and it was only possible to recover them in the males. In the case of crossing-over tests with w^i, three recombinants were recovered from a population of 81,600 flies screened; one was a yw^{ch} male and the remaining two were " w " *spl* (table 2, cross *f*). The latter two exceptions yielded w^{sp} eye colour in females when compounded with w^{sp} implicating that they were coupled mutants. Further mutational analysis, as stated earlier, resulted in clear w^{sp} eye colour exceptions. Recovery of $w^iw^{sp}spl$ from this cross indicated that the location of w^i was to the left of w^{sp}. Comparing recombination frequency obtained from the crosses indicated in table 2, crosses *e* and *f*, it was inferred that w^i did not eliminate recombination in the genetic interval delimited by subsites 4 and 5.

4. Discussion

The foregoing data do not provide support for the earlier contention (Bowman, 1965; Rasmuson, 1962) that w^i is a small serial tandem duplication within the *white* locus of *Drosophila melanogaster*. Instead, the interallelic crossing-over tests with the double mutants employed *viz*, $w^{Bwx}w^a$ and $w^{ch}w^{sp}$ pointed to w^i being a point mutation. No increase or decrease of recombination was encountered in the genetic intervals marked by subsites 1 to 3 and 4 to 5. Obviously, if w^i really represented a duplication, absence of effects on recombination events, as evident in the present study, is contrary to the expectations based on the findings of Judd (1965). Thus, in the absence of any support for the duplicational nature of w^i, we believe that this mutant reverted by some other mechanism rather than the one proposed by Bowman (1965). Nevertheless, we confirm that w^i is located to the left of w^{ch} as earlier indicated by Lewis (1959). Our data strengthened further this notion by offering evidence for the location of w^i to the right of w^a but to the left of w^{sp}.

Acknowledgments.—Grants received from C.S.I.R. (No. 7/15 (32)/75—E.M.R. 1) and U.G.C. (Grant No. F-33-95/71 Se-1) to S. K. S. are acknowledged herewith.

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