CHROMOSOME PAIRING AND RECOMBINATION IN *DROSOPHILA MELANOGASTER*

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A study of recombination in *Drosophila melanogaster* indicated that pairing might be polarized (JUDD 1964). The relative frequency of crossover classes obtained from duplication/normal heterozygotes led to the postulation that pairing was discontinuous and that either of two pairing arrangements can be realized under such a condition but with one type occurring much more frequently than the other. ALTENBURG (1964) pointed out that the observed experimental results can be explained with only one pairing arrangement if crossover events take place at different rates at two points. In the latter model, pairing is continuous and strictly polarized.

Experiments have been carried out which show that two pairing arrangements do occur and that one produces larger numbers of recombination events than the other in the region studied.

The experimental design is based on the pairing schemes shown in Figure 1. The duplication utilized is one which arose by asymmetrical exchange within the white locus of the X chromosome (JUDD 1961). It has been demonstrated that it includes the white-cherry (w^{ch}) recombination site (Site 4) but not the one occupied by white-apricot (w^a) at Site 3 (JUDD 1964). Females heterozygous for the small duplication ($w^{+,dup}$) and the white mutants w^a and w^{ch} were constructed. They also carried the mutants γ^2 (yellow-2: 1-0.0; yellow body, dark bristles), sc (scute: 1-0.0+; missing scutellar bristles), fa (facet: 1-3.0; eye facets irregular), ec (echinus: 1-5.5; eye enlarged and rough) in heterozygous state as markers for detecting recombination events. The second and third chromosomes were made heterozygous for $SM1$ and Ubx^{150} respectively. These are complex chromosomal rearrangements which increase crossing over in the X chromosome.

The females of genotype $w^{+,dup} ec/\gamma^2 sc w^a w^{ch} fa; SM1/+; Ubx^{150}/+$ were mated to males of constitution $sc z w^z$. The mutant z (zeste: 1-1 $\pm$; yellow eye color) interacts with the white locus duplication and is used here to detect the presence or absence of $w^{+,dup}$. The white mutant, w^z , is white in phenotype and is not a suppressor of zeste. The $z w^z$ combination proved most successful in the detection of the desired recombination classes.

The apricot and cherry recombination products can be easily recognized among the male and female offspring since the parental types are phenotypically either white ($w^a w^{ch}/z w^z$) or light zeste mottled ($w^{+,dup}/z w^z$) as females, or white ($w^a w^{ch}$) or wild type ($w^{+,dup}$) as males. The division of the apricot products into two groups, those that contain the duplication and those which do not, is done by crossing each to z . The presence of the duplication causes females of genotype $z+ w^{a,dup}/z w^{+}$ to be mottled in phenotype. It should be noted that the normal apricot is a product of recombination at point a following pairing of type I while the apricot-duplication comes from a type II pairing arrangement.

Both the duplication-cherry ($w^{dup,ch}$) and the normal (w^{+}) products have a wild-type phenotype when seen as males or as females heterozygous for $z w^z$. Since the parental type, $w^{+,dup}$, is also wild type as a male these classes cannot be distinguished among the male offspring. They

MATERIALS AND METHODS

FIGURE 1.—Diagram to illustrate the discontinuous pairing model in duplication/normal heterozygotes. The recombination sites of the white locus are designated by the numbers 1 to 5. The mutants w^a and w^{ch} occupy sites 3 and 4 respectively. Type I shows rightmost elements paired to the point where homology is interrupted by the duplicated segment. Type II illustrates the alternate arrangement with leftmost elements paired. Regions of exchange are indicated by arrows; the resulting recombinant types and the phenotype of each are given. The numbers indicate frequency of recognizable classes among 137,598 offspring.

can be recognized as females, however, since the parental class, $w^{+,dup}/z w^z$, is light zeste mottled. Following the original recovery of these two classes, each was combined with the mutant z to distinguish between them. Males of type $w^{dup,ch}$ (i.e., 3 4 w^{ch} 5) in *cis* arrangement with z exhibit a phenotype similar to a dark spotted-white, that is, small dark spots on a cherry-like background. The normal chromosome (i.e., 3 4 5) when combined with z is wild type as a male and zeste as a female. The model predicts that the duplication-cherry product comes from a type Ia exchange while the normal recombinant arises from type IIb.

The one remaining recombinant class, $w^a w^{ch}$ 4 5, from a type IIb exchange is not recognized in this experiment. It is presumed to have a white phenotype similar to the $w^a w^{ch}$ parental combination.

RESULTS

Pairing pattern type I shown in Figure 1 yields two recognizable exchange products when crossing over occurs at point *a*. The normal apricot products (w^a 4 5) all carried the outside markers $y^2 sc ec$, indicating a recombination event of the expected type. The duplication-cherry products (3 4 w^{ch} 5) all carried the *fa* marker. Allowing for the fact that the apricot type can be recognized in both male and female offspring while $w^{dup,ch}$ is recovered only as females, a comparison of the numbers of exchange chromosomes recovered for each type shows them to be essentially equal as is expected of reciprocal classes.

In order to prove that the $w^{dup,ch}$ class contains the duplication with the w^{ch} mutant in the rightmost element, females of genotype $sc z w^{dup,ch} fa/+$; *SM1*/+; *Ubx*¹³⁰/+ were constructed and mated to $y^2 sc w^a w^{ch} fa$ males. Two putative

$w^{dup, ch}$ crossover products were chosen for use in this experiment. In both cases the expected $w^{ch} fa$ and $sc z w^{+, dup}$ products were obtained, thus confirming the constitution of this class. Six crossover products were recovered among 79,910 offspring.

Pairing pattern II shown in Figure 1 should yield four exchange products, three of which can be recognized. A crossover at point *a* gives an apricot-duplication strand which carries the markers $\gamma^2 sc ec$ and the reciprocal product is a normal chromosome containing the mutant w^{ch} and the marker *fa*. If pairing pattern II occurs with the same frequency as pattern I, these two classes should be recovered with frequencies as great or greater than the w^a and $w^{dup, ch}$ types described above because the region in which type IIa exchange can occur should be as long as or longer than the comparable Ia region, depending on the exact length of the duplicated segment. It is clear from the data given in Figure 1 and those reported earlier (Judd 1964) that type Ia products are recovered about five times more frequently than those from IIa. Combining data from all experiments where w^a , $w^{a, dup}$ and w^{ch} have been scored shows 48 w^a ; 7 $w^{a, dup}$; 11 w^{ch} .

Pairing arrangement II also yields crossovers in region *b*, one class of which can be recognized. It is the recovery of the wild-type class which proves that two pairing patterns do occur regardless of whether the model presented here is correct in detail. The wild-type class is the most frequently recovered type in this experiment, and gives a rough measure of the genetic length of the duplication proximal to w^{ch} . This is about 0.034 units when correction is made for the missing classes and the increase in crossing over of about threefold brought about by the presence of autosomal inversions. No correction is made for the approximately threefold increase caused by the duplication itself (Judd 1964). It is possible then that the duplication extends to the right of w^{ch} to include Site 5 but this in no way changes the interpretation of the pairing arrangements.

DISCUSSION

The model proposed for synapsis is based on a concept of discontinuous pairing between homologous chromosomes. If such a model is correct, the data could be taken to indicate a polarity in the pairing pattern with synapsis beginning at any point of homology between the chromosomes and proceeding distally for a short distance. This would account for the higher frequency of type I pairing where the rightmost elements of the white locus are effectively paired along the chromosomes to the point where homology is interrupted by the repeat of the segment containing the w^{ch} site. It could be postulated that type II pairing is accomplished through the initiation of pairing in the region designated IIb in Figure 1 and proceeding distally from that point.

The alternative pairing model proposed by ALTENBURG is shown as type III in Figure 2. It should be noted that four classes of recombinants are expected from crossover events at points *a* and *b*. No wild-type recombinant class is expected if only this type of pairing occurs, therefore, it is clear from the data presented here that if pairing is continuous, both type III and type IV arrange-

FIGURE 2.—Diagram to illustrate the continuous pairing model. Type III is that proposed by ALTENBURG (1964) resulting from strict proximal to distal polarization. Points of exchange are indicated by arrows. The resulting recombinant types and the phenotype of each are given. Type IV is predicted if pairing is continuous but not strictly polarized. Types III *b* and IV *b* exchanges give unique products which allow recognition of the two pairing arrangements.

ments must occur. Examination of the recombinant classes shows that crossovers at point *b* in type III yield products unique to that pairing arrangement and type IV *b* events are unique to type IV pairing. No estimate of the relative frequency of type III and IV arrangements can be made, however, since region III *b* is not strictly homologous to region IV *b*. The only statement which can be made from the available data is that both type III and type IV must occur if the continuous pairing model is correct. Under such circumstances it is not necessary to postulate any polarity of pairing whatsoever.

It has long been assumed that pairing necessary for recombination is continuous, but such a concept has no direct evidence to support it. On the other hand, discontinuous pairing has been postulated by PRITCHARD (1955, 1960) to account for the pattern of recombination events found in *Aspergillus*. Evidence for discontinuous pairing in *Drosophila* was presented by GREEN (1962). His work shows that recombination in regions adjacent to homozygous duplications is increased in excess of the added genetic length of the duplication itself. Data presented here and in an earlier publication (JUDD 1964) also support the hypothesis since even the small intralocus duplication in heterozygous state increases recombination in the w^a - w^{ch} interval over that found in normal chromosomes. In the light of this evidence, it is felt that greater weight should be given to the discontinuous pairing model. Also, it seems reasonable to assume that the very small loop which must form in pairing of type III and IV would interfere with exchanges closely adjacent to it. The opposite result is obtained.

In a system which is paired discontinuously but polarized, there could be specialized recognition sites at which pairing is initiated. If such is the case,

there must be more than one such initiating site within an effectively paired segment because the data presented here show that effectively paired regions need not have fixed boundaries. However, these data can be explained using a model which assumes fixed boundaries for effectively paired segments with the initiation of pairing at any point of homology within the region. There is no necessity to postulate polarity in such a model. In the situation presented here, all that need be assumed is that the region in which crossovers are selected lies nearest the left boundary of the paired region. Pairing which begins in the longer region to the right will result in type I pairing while type II will be obtained from pairing initiated in the shorter interval to the left.

It is clear that the need for invoking polarity appears only if pairing is discontinuous and extends over regions without fixed boundaries. If pairing is either continuous or occurs in regions with predetermined limits, there is no need to postulate polarity for the system. It is also possible that the small duplication itself is influencing the pairing pattern in such a way that type I is preferred over type II or that for some reason recombination in the w^u - w^{ch} region is more frequent when type I pairing occurs.

In general, the evidence favoring discontinuous pairing is strong enough for tentative acceptance of such a model but it is clear that another approach to the question of polarity is necessary before any conclusions can be drawn concerning its existence in *Drosophila*.

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SUMMARY

Evidence supporting discontinuous pairing at the time of recombination is presented. This is based on data showing that two patterns of pairing occur in duplication/normal heterozygotes. One pairing arrangement occurs about five times more frequently than the other. This may be explained by (1) invoking polarity in the pairing process, in this case from proximal to distal, or (2) assuming that effective pairing regions have fixed boundaries with the possibility of pairing beginning at any point of homology and extending proximal and distal from the point of initiation.

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