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LINKAGE DATA SUPPORTING A MATHEMATICAL EXPLANATION FOR SOME EMPIRICAL *CIS-TRANS* EFFECTS

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1. Introduction

Clark and co-workers reported sizable *cis-trans* differences in recombination frequencies (Clark and Feldman, 1981*b*) and fitness components (Clark *et al.*, 1981; Clark and Feldman, 1981*a*) associated with genetically marked non-overlapping inversions of the second chromosome of *Drosophila melanogaster.* Turelli (1982) pointed out that such differences are expected when the markers scored are in linkage disequilibrium with polymorphic loci that affect the traits considered. Originally, this proposed explanation of Clark *et al.*'s data was supported only by general observations concerning disequilibrium between inversions and allozymes and the fact that the *cis-trans* differences varied with the genetic background used. Here we present linkage data that support the linkage disequilibrium hypothesis and resolve a paradox concerning the recombination rates reported in Clark and Feldman (1981*b*).

In their experiments, Clark *et al.*, used the simple inversions *Cy*: *In*(2*L*)22*D*1-2; 33*F*5-34*A*1 and Pm^2 (synonymous with bw^{V32g}): *In*(2*LR*)40*F*; 59*E*. Each exhibits a dominant visible phenotype and is recessive lethal. Pm^2 produces a plum eye color through position-effect variegation of the *brown* locus (Lindsley and Grell, 1968). In contrast, the *Curly* wing phenotype is produced by an allele at a locus, denoted *Cyg* to distinguish it from the inversion, just inside the left break of the *Cy* inversion. As originally demonstrated by Tinderholt (1961), the mutant at *Cyg* can be removed from *Cy* by a double crossover. By showing that the recombination frequencies reported in Clark and Feldman (1981*b*) correspond to the genetic distance between *Cyg* and Pm^2 rather than *Cy* and Pm^2, we will argue that the *Curly* marker probably "escaped" from the *Cy* inversion during the synthesis of the lines analysed by Clark *et al.* This rare event would create the linkage disequilibrium necessary to explain the observed *cis–trans* differences by the mechanism proposed in Turelli (1982). This finding does not affect the conclusions of Clark and Feldman (1981*a*) or Clark *et al.*, (1981) concerning estimation of selection components, population predictions, or the existence and implications of epistatic interactions in selection components.

To interpret the recombination data below and in Clark and Feldman (1981*b*), it is useful to have at least approximate genetic map positions for *Cyg* and the ends of the inversions. Tinderholt (1961) placed *Cyg* at

2–6·1 ± 0·9. The inversions can be assigned approximate genetic map positions using figure 3 of Ising and Block (1981), which relates the cytological map of chromosome 2 to its genetic map. The left end of *Cy* is roughly at map position 2–5·0, its right end is roughly at 2–48·0. The left end of the Pm^2 inversion is at 2–55·0. The approximate map distance separating *Cy* and Pm^2 corresponds to a recombination frequency of roughly 0·07 assuming no double crossovers in this interval. However, in double heterozygotes both inversions would be expected to suppress crossing over in the relatively short interval between them (Lucchesi, 1976). In fact, using double heterozygotes for the inversions *Cy* (as above) and *Cy*(2*R*):*In*(2*R*)42*A*23; 58*A*4–*B*1, Graubard (1932, tables 1 and 2) reported a recombination frequency of 0·005. In contrast, in their original description of Pm^2, Schultz and Dobzhansky (1934) found that in Pm^2 heterozygotes the recombination frequency between *aristaless* (2–0·01) and *Bristle* (2–54·8) was 0·444 ± 0·015. From this one would expect a recombination frequency between *Cyg* and Pm^2 of roughly 0·38. The estimates of recombination frequency between *Cy* and Pm^2 in Clark and Feldman (1981*b*) range from 0·357 ± 0·012 to 0·414 ± 0·010, depending on linkage phase and genetic background. Clearly these correspond better to the recombination frequency expected between *Cyg* and Pm^2. This conjecture was checked by a simple mapping experiment.

2. Materials and Methods

The most direct test of the hypothesis that the *Curly* allele was removed from its inversion in the stocks of Clark *et al.*, would be to examine polytene chromosomes. Unfortunately, the *Curly* bearing stocks were lost, forcing us to use the less direct argument based on recombination frequencies. *Trans* double heterozygotes with the *Curly* allele on a noninverted second chromosome and Pm^2 on its homolog were obtained by mating *Cy*(*no Ins*)*ed*/$ds^{33k}Pm^1$ flies supplied by the Bowling Green Stock Center to Pm^2 flies from the "homozygous" genetic background described in Clark and Feldman (1981*a*). Virgin *Cy*(*no Ins*)/Pm^2 females were mass mated to Oregon-R males.

3. Results and Discussion

Among 2005 progeny scored, there were 674 *Curly*, 573 *Plum*, 371 *Curly-Plum*, and 387 wild type individuals. Ignoring viability differences, these give 0·378 ± 0·022 as an approximate 95 per cent confidence interval for the recombination frequency between *Cyg* and Pm^2. Because of differences in the *Curly* bearing chromosomes used and the overall genetic backgrounds, this estimate is not exactly comparable to any of the four in Clark and Feldman (1981*b*). Nevertheless, it is statistically consistent with the estimate 0·395 ± 0·005 obtained from the most similar stock used by them, namely their *trans* double heterozygote with "homogeneous" genetic background. It is also consistent with the prediction based on the work of Schultz and Dobzhansky (1934). These results suggest that during the stock construction, there was a double crossover event that transferred the *Cyg* marker allele to a chromosome without the *Cy* inversion. The spontaneous frequency of such an event must be very low, but its probability was probably

enhanced by the use of multiply inverted chromosomes during the stock construction (see Clark *et al.*, 1981, Lucchesi, 1976, and Tinderholt, 1961). The homogeneity of recombination frequencies within each background strongly implies that throughout the experiments, the *Cy* chromosomes were free of inversions. Because the crossover event was probably unique, it would have generated substantial linkage disequilibrium between the *Cyg* marker and flanking loci.

Clark and Feldman (1981*b*) proved mathematically that *cis–trans* differences in recombination frequencies can generate stable linkage disequilibrium. Based on the assumption that they had found such a difference between linked inversions, they argued that this phenomenon might contribute to the linkage disequilibrium frequently observed between linked inversions in wild populations. Essentially their argument was that linked inversions somehow intrinsically generate *cis-trans* differences in recombination frequencies. However, based on the findings reported here, their data do not address this question. Nevertheless their mathematical results may still have relevance to naturally occurring inversions, because the linkage disequilibrium commonly observed between inversions and flanking loci may be sufficient to generate *cis–trans* differences in recombination between linked inversions (Turelli, 1982). This possibility remains to be demonstrated experimentally.

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