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Fine-scale crossover rate and interference along the XR-chromosome arm of *Drosophila pseudoobscura.*

Fitzpatrick, Courtney L., Laurie S. Stevison, and Mohamed A.F. Noor. Biology Department, Duke University, Durham, NC, USA; NOOR@DUKE.EDU.

Although broad-scale variation in recombination rates across *Drosophila* genomes is well established, recent studies have identified that fine-scale crossover rate variation also exists within the *Drosophila pseudoobscura* genome. The first study to explore such fine-scale variation focused on a 2-MB region of the XL chromosome arm and identified where crossovers fell among 370 progeny known to have had a crossover event between two phenotypic mutant markers (Cirulli *et al.*, 2007). Fine-scale recombination rates ranged from 1.4 to 52 cM/megabase. A later work examined variation across the second chromosome in crossover rate and correlated fine-scale recombination rate with patterns of nucleotide diversity within species and divergence between species (Kulathinal *et al.*, 2008). This latter study employed an illumina bead genotyping approach, but assumed that crossovers in adjacent windows likely represented an erroneous genotype (or a gene conversion event) in one of the windows.

Both of these studies assumed that crossover interference exists within *D. pseudoobscura*, an assumption that has not been tested in this species. The first study would have missed all double-crossover events within the 2-MB window because of the methods employed, while the second study would have erroneously excluded adjacent crossovers, hence underestimating overall crossover rates. One can best address this deficiency by examining many closely linked markers in a very large panel of backcross progeny.

Here, we directly measured crossover rate between markers within a 3 MB region on the XR chromosome arm of *Drosophila pseudoobscura* in a very large panel of backcross progeny. We identified fine-scale crossover rate variation in this region of the genome. We also used these results to obtain estimates of the coefficient of coincidence and interference in this species.

Materials and Methods

We crossed two strains of *Drosophila pseudoobscura*, Flagstaff 1993 and Mather 17, and backcrossed the F_1 females to males from the Flagstaff 1993 line. To achieve a sufficiently large sample size of F_2 backcross progeny, we performed two such crosses identically. We extracted DNA from and genotyped 1208 F_2 backcross individuals from the first cross and 2057 F_2 backcross individuals from the second cross, totaling 3265 F_2 backcross individuals, and we characterized crossover rate in a 3 MB region on the XR chromosome arm in these backcross progeny. Because here we examined this region exclusively, our further use of the terms "recombinant" and "non-recombinant" refers only to crossover events between XR_group8 position 5,051,027 and position 7,973,182 (Richards *et al.*, 2005).

We used a two-step process to characterize the crossover rate. First, we identified recombinants in the backcross sample using four microsatellite markers dispersed across the region (hence splitting it into three 1-megabase regions dubbed "A" through "C") as well as one additional marker in the center of region "B". We then genotyped the recombinant sample using six additional markers. Because we identified only two double recombinants in the initial scan of the first cross, we used the two outermost flanking markers, DPSX037N and DPSX021B1 (previously used in Ortiz-Barrientos *et al*., 2004), to differentiate recombinants from non-recombinants in the second cross for further genotyping with all of the other microsatellite markers. We assessed whether the distribution of crossovers deviated from a random distribution by bootstrapping (accounting for the sizes of the regions- see Cirulli *et al.,* 2007).

PCR was performed in 10 μL reactions containing 1 μL of DNA (Gloor and Engels, 1992), using the following touchdown cycling protocol: 1 min at 95°C, 3× (95° for 30s, 56°C for 30 s, 72°C for 30 s), 3× (95°C for 30 s, 53°C for 30 s, 72°C for 30 s), 30× (95°C for 30 s, 50°C for 30 s, 72°C for 30 s). PCR products were visualized on a polyacrylamide gel using LiCor 4300 DNA sequencer/ analyzers. The complete list of markers surveyed and primer sequences are available upon request.

Results and Discussion

We found significant crossover rate heterogeneity along three megabases of the XR chromosome arm ($p < 0.0001$, see Figure 1), a result consistent with studies that reported such heterogeneity along the XL and 2-chromosome arms of this species (Cirulli *et al*., 2007; Kulathinal *et al.*, 2008). Recombination rates in this XR region ranged from 1.8 to 19.8 cM/megabase. This heterogeneity was significantly associated with GC-content and simple repeats (multiple regression $r = 0.81$, overall $p = 0.02$, $P_{GC} = 0.04$, $P_{repeats} = 0.0087$), again confirming findings for the XL chromosome arm (Cirulli *et al.*, 2007).

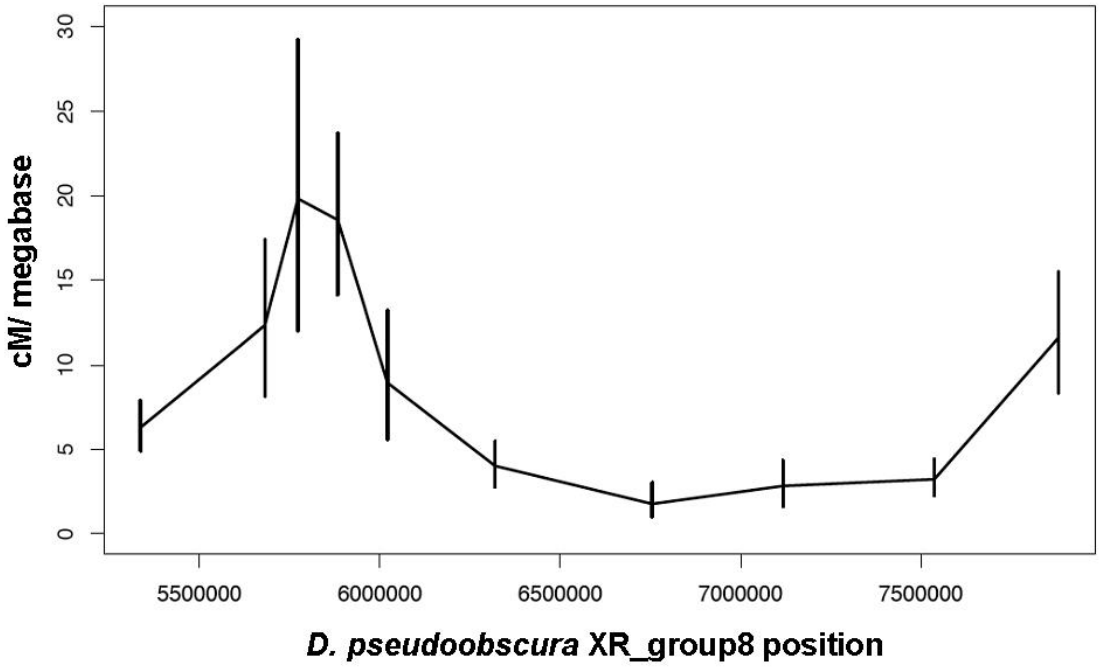

Figure 1. Fine-scale recombination rate across 3MB of the *D. pseudoobscura* XR chromosome arm.

Interference (I) is defined as I = 1-coefficient of coincidence (c.o.c.), where the c.o.c. reflects the ratio of observed to expected double crossover frequencies. The expected frequency of double crossovers between two endpoints was calculated from the RF of individual regions. Any interval completely lacking double crossovers will have a c.o.c. equal to 0 and can, therefore, be characterized as having complete crossover interference (I = 1). Because we only observed double crossovers between intervals A and C, we present the calculation of interference for that pair only. All other closer windows (2 megabases or less) exhibited complete crossover interference.

Of the 1208 flies that we genotyped at all 5 markers in the first scan of backcross progeny, 230 were recombinant. This recombinant frequency of 0.19 yields an estimated probability of double crossovers of 0.036 and, with no interference, predicts 43.8 double crossovers between the two outermost markers, DPSX037N and DPSX021B1. However, we only observed two double crossovers in that region. Therefore, the c.o.c. = 0.046 and I = 0.954 for markers three megabases apart.

Overall, these results demonstrate fine-scale crossover rate variation along a 3-MB region of the XR chromosome arm, strong crossover interference in this region, and complete (or nearly complete) interference in closer windows. Many questions remain about the molecular mechanism underlying this phenomenon, and the strength of interference may well vary throughout the genome. If this is the case, a more thorough characterization of genomic interference patterns will strengthen the overall understanding of recombination.

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Sperm storage and nuptial gifts in *Drosophila paulistorum.*

Ehrman, Lee., and Richard Wrancher. Natural Sciences, State University of New York at Purchase College: lee.ehrman@purchase.edu and richard.wrancher@gmail.com

Theodosius Dobzhansky, doctoral mentor, and colleague for some three decades, used to speculate that sperm entering the two pigmented sophophoran spermathecae could not exist. The single ventral receptacle, he maintained in our routine Saturday morning chats, was the primary sperm storage organ. So perhaps spermathecal sperm were digested, constituting protein-rich nuptial gifts granted needy gravid females.

Many male insects donate nuptial gifts to help insure successful copulation and offspring, a form of paternal investment. While nuptial gifts come in various forms in arthropods, evolutionary origins are obscure. Gifts range from inanimate objects to balls of silk to sacrificing their own life, all to insure copulation with the females and the production of progeny. Greater numbers or masses of a nuptial gifts seem to correlate with greater numbers of offspring (…success for fruit flies, 2009).