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Maternally inherited components do not detectably influence recombination rate variation in *Drosophila pseudoobscura*.

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Introduction

Genetic recombination during meiosis is essential for the successful segregation of chromosomes and acts as an important driver of evolutionary change via its roles in the processes of speciation and the maintenance and creation of genetic diversity. Despite its importance, recombination rate is known to respond to a variety of factors, both molecular and environmental, that can ultimately lead to substantial heritable variation in recombination rates amongst individuals, populations, and species (Smukowski and Noor, 2011). Such variation in recombination rates has been documented in a diverse range of taxa that includes humans, mice, *Drosophila*, yeast, and *Arabidopsis* (Comeron *et al*., 2012; Coop *et al*., 2008; Drouaud *et al*., 2006; Dumont and Payseur, 2011; Fledel-Alon *et al*., 2011; McGaugh *et al*., 2012; Petes, 2001).

Recently, parent-of-origin effects on gene transcription and expression were suggested to influence the location and formation of double stranded break precursors to recombination, thus potentially influencing patterns of variation in recombination rates across the genome (Adrian and Comeron, 2013). If true, maternal effects may affect recombination landscapes genome-wide and create variation in recombination rates among individuals within species. Maternal effects are known to play pivotal roles in body axis formation during embryogenesis in *Drosophila*; however, studies directly testing the role of maternal effects (*e.g*., through cytoplasmically inherited factors) on recombination rate variation have yet to be explored. This study aims to step away from the commonly studied influences on recombination rates, including sequence variation, epigenetics, protein binding sites, and plasticity in response to changing environments, to offer further insight about parental influences on recombination in their offspring.

In this study, we use two subspecies of *Drosophila pseudoobscura, D. ps. pseudoobscura* and *D. ps. bogotana*, to study how maternal effects impact variation in recombination rates. These two subspecies were chosen since they are genetically divergent (Prakash *et al.*, 1969), allowing more opportunity for potential parent-of-origin effects on recombination, and since they form fully fertile hybrid females. We set up reciprocal inter-subspecies crosses to generate F_1 hybrid females, differing only in maternal effects and organellar genomes, and use over 1400 F_2 progeny from each cross to infer the recombination rates in the F_1 females at two windows located on opposite ends of chromosome 2 (Muller's Element E).

Materials and Methods

Fly Stocks and Crosses:

Laboratory strains of *Drosophila pseudoobscura pseudoobscura* (genome strain MV2-25) and *Drosophila pseudoobscura bogotana ER white* obtained from the Drosophila Species Stock Center at UCSD in 2012 (stock number 14011-0121.152) were used to set up reciprocal inter-subspecies crosses.

Virgin adult female *D. ps. pseudoobscura* and *D. ps. bogotana* were collected and maintained for 5-6 days to allow for sexual maturity and mating receptivity. After 5-6 days, reciprocal crosses were set up in which four *bogotana* females were crossed with four *pseudoobscura* males, and four *pseudoobscura* (ps) females were crossed with four *bogotana* (bog) males. All parents were allowed to mate for 6-7 days before being removed. A total of 31 mating vials were set up (14 for bog female × ps male, and 15 for ps female × bog male). F_1 females from both crosses were then held for 5-6 days before being backcrossed to ps males. F_1 females and ps males were then confined for 4 days before being removed. From 90 crosses (45 for each reciprocal cross) of four F_1 females with four ps males, a total of 1435 F_2 progeny from the ps female × bog male cross and 1437 F_2 progeny from the bog female × ps male cross were generated and used to infer recombination rates in the F_1 females. A maximum of thirty-two F_2 progeny from each vial were used to avoid results being overly influenced by any single vial.

Recombination Rate Assay:

Recombination rate was measured at two windows located on opposite ends of chromosome 2 using four microsatellite markers (DPS2028, DPS2001, DPS2025, and DPS2003) that produce differentially sized products for the bog and ps alleles at each of the four loci. All primers pairs used can be found in Table 1 and were developed in either Noor *et al.* (2000) or Ortiz-Barrientos *et al.* (2006). The first window examined spanned from base pairs 1,515,714 through 4,807,929 of the assembly (Richards *et al.*, 2005), while the second window spanned from base pairs 22,978,798 through 29,200,493. Base pair positions are based on the November 2004 (Flybase 1.03/dp3) version of the *D. pseudoobscura* genome available on the UCSC genome browser.

Table 1. Primer pairs used to amplify variable microsatellite regions.

Primer Name	Forward	Reverse	Assembly Positions
DPS2028	tcagcctccgcttcgattg	cgcctacctcgtacctatacagcat	1515714-1515878
DPS2001	caaagacagagccaaagcct	tgggcattaaagtgcaatca	4807736-4807929
DPS2025	tgggcgatgttcaagtgtcaa	attatggaagcgatcgaagcg	22978798-22978990
DPS2003	catttcaagcagaagacgca	cctcgggtattatttcgggt	29200289-29200493

Genomic DNA from both parents and offspring was extracted using the single fly squish protocol (Gloor and Engels, 1992), and the four markers were amplified by PCR in 10 µl reaction volumes that consisted of 1× buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM forward primer with the M13 tag, 0.5 µM reverse primer, 700 or 800 IRD-labeled M13 tag, and 1U Taq polymerase. The touchdown PCR program consisted of a one minute 95°C denature step, followed by 3 cycles of 95°C, 56°C, and 72°C for 30, 30, and 45 seconds, respectively, 3 cycles of 95°C, 53°C, and 72°C for 30, 30, and 45 seconds, respectively, and 33 cycles of 95°C, 50°C, and 72°C for 30, 30, and 45 seconds, respectively. PCR products were then run on a 5% polyacrylamide gel using a LICOR 4300, and the genotypes were scored manually. Chi-square analyses were used to test for statistically significant differences in recombination rates between the two reciprocal crosses at both the DPS2028-DPS2001 and DPS2025-DPS2003 windows. A power analysis was also performed using a custom Perl script to determine the probability of finding a 4-5% or larger difference in recombination rate at each window given our sample size.

Results and Discussion

Based on our power analysis, our sample size gave us approximately a 98% chance of detecting a real difference of 5% or larger and approximately a 90% chance of detecting a real difference of 4% or larger in recombination for the DPS2028-DPS2001 window. Likewise, we had approximately a 84% chance of detecting a real difference of 5% or larger and approximately a 65% chance of detecting a 4% or larger difference in recombination for the DPS2025-DPS2003 window. However, we observed only a 1.8% difference in recombination rate for the DPS2028-DPS2001 window and a 0.65% difference in recombination rate for the DPS2025-DPS2003 window between the F1 hybrid females with either the *D. ps. bogotana* or *D. ps. pseudoobscura* cytoplasm (Table 2). Neither of the two differences in recombination rates were statistically significant upon chi-square analysis ($p < 0.10$ for both windows). Thus, even using crosses between strains from different subspecies, we fail to detect evidence that maternally-inherited cytoplasmic components contribute detectably to variation in recombination rates within *Drosophila pseudoobscura*.

Table 2. Recombination rates observed between the two reciprocal crosses of *D. ps. bogotana* and *D. ps. pseudoobscura*.

		DPS2028-DPS2001 window		DPS2025-DPS2003 window	
cytoplasm	# progeny	# recombinants	Kosambi centiMorgans	# recombinants	Kosambi centiMorgans
bogotana	1437	207	14.82 cM	431	34.65 cM
MV2-25	1435	181	12.89 cM	421	33.64 cM

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Sperm transfer and the enigma of copulation duration in *Drosophila.*

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Drosophila species exhibit tremendous variation in copulation duration (Markow, 1996). In some species, such as *D. arizonae*, a pair is *in copula* for only 1 or 2 minutes, while in others, such as *D. acanthoptera*, copulation lasts for 2 hours. Curiously, the differences among species in copulation duration do