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.*

Castro-Vargas, Cynthia. Biodiversification Genomics Laboratory, CINVESTAV-LANGEBIO, Guanajuato, Mexico.

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

not appear to correlate with any other known traits so that the variation in copulation duration remains a great enigma. Copulations that last longer expose the couple to risks of predation, however, so we assume that there is some important advantage to mating longer that offsets this risk.

Successful copulation involves the transfer of the ejaculate, including seminal fluid and sperm. But the timing of the transfer of the components is not well-known. For example, in *D. melanogaster*, which copulates for 20 minutes, early reports (LeFevre and Johnson, 1962) indicate that sperm transfer does not take place during the first 10 minutes, while some other studies dispute this (Gilchrist and Partridge, 2000). If sperm transfer is not taking place during the entire 20 minutes, and copulation is only a few minutes in other species, the function of longer copulations is enigmatic.

Stock of flies and their rearing

I used two species, *D. melanogaster*, with a 20 minute copulation duration, and *D. hydei*, with a 2.5 minute copulation duration (Markow 1996) to examine the timing of sperm transfer during copulation. For the experiments with *D. melanogaster*, two strains from different geographic regions were used: SD-5, from San Diego, California, and S13 from Sinaloa, Mexico. For the experiments with *D. hydei*, only one strain was used: GTO 813-5, from Guanajuato. All flies were reared on banana medium with live yeast at 24 ± 1°C with a 12 hour photoperiod.

Timing of sperm transfer during copulation

Virgin females and males were separated and allowed to mature in separate medium vials until used in the experiments. Mature virgin females and males were paired and allowed to mate. At specific time points after copulation began (1, 3, 5, 10 minutes for *D. melanogaster* and 15, 20, 30, 60, 120 seconds for *D. hydei*) the pairs were separated. The female reproductive tract was removed and examined for sperm under the microscope. A minimum of 10 matings per time point were tested in order to reveal whether sperm are transferred continually or whether they are transferred early or late in the copulation.

Results

Because female *D. melanogaster* had sperm at 10 minutes but not 5 minutes, I performed some additional matings and interrupted them between 5 and 10 minutes (Table 1). Although *D. melanogaster* mates for 20 minutes, sperm transfer clearly does not begin until 7 minutes and all females are not inseminated until 10 minutes have elapsed in both strains. In *D. hydei*, in which copulations last for only a few minutes, sperm transfer does not begin immediately either (Table 1).

Table 1. Sperm present in female reproductive tract after interruption at different time points during copulation.

Sperm present in dissected female?	Minutes after copulation is initiated (Number of females/ 10 sperm)								
(*min*)	1	3	5	6	7	8	10	15	20
D. melanogaster SD_5	0/10	0/10	0/10	0/10	7/16	5/10	10/10	10/10	10/10
D. melanogaster S13	0/10	0/10	0/10	2/10	5/10	9/10	10/10	10/10	10/10
(*sec*)	15	20	25	30	60	120			
D. hydei GTO 813-5	0/10	2/10	7/10	10/10	10/10	10/10			

Discussion

Long lasting copulations exhibit costs in terms of time, energy, and predation vulnerability. In some Drosophilid species such as *D. melanogaster*, copulation duration has been reported to be determined by the male (MacBean and Parsons, 1967). Because sperm are not the only thing transferred during copulation, it is

possible that males are initially transferring seminal fluid components (proteins and other molecules) that are necessary for successful fertilization, storage, and use of their sperm. For instance, seminal fluid proteins (SFP) elicit post-mating changes in female physiology and behavior to the male´s advantage, reducing female receptivity, increasing ovulation and egg production, or changing feeding behavior (Markow and Ankney, 1988; Ávila *et al*., 2011; Chapman, 2001); therefore, males increase their offspring number by delaying remating in females. This clearly is not the case for *D. hydei*, because females remate up to four times in a single morning (Markow, 1985).

On the other hand, males could prolong copulation in order to increase their fertilization success by displacing the sperm from former copulations (LeFevre and Johnson, 1962). Given that females copulate with multiple mates, sperm belonging from different males interacts in the female reproductive tract, where sperm competition for predominance occurs (Gromko *et al.*, 1984; Gilchrist and Partridge, 2000; Clark, 2002). Accessory gland proteins (Acp's) present in the seminal fluid have shown to play an important role in this interaction (Harshman and Prout, 1994; Gilchrist and Partridge, 1995; Ram and Wolfner, 2007); however, the mechanisms by which Acp's help mediating sperm competition and sperm displacement remain unknown.

In comparison with *D. melanogaster*, *D. hydei* exhibits a short copulation duration. This represents a big advantage for females, who can increase their genetic variability by remating with different males. Males in many species, however, have developed strategies that offset the female remating. In many *Drosophila* species, a mating plug is formed within the female reproductive tract during or after copulation, which acts as a physical barrier for subsequent sperm or to prevent the loss of sperm (Bairati, 1968). Likewise, in some species of the repleta group, an insemination reaction mass is formed (Patterson, 1946; Markow and Ankney, 1988), and in other species, males transfer nutritional components to females (Markow *et al*., 1990). Again, this is not the case in *D. hydei* so, if the chances of remating in this short copulation species are high and the males are not transferring any nutritional content in the sperm or forming a mating plug, why does this species exhibits a short copulation duration? It could be argued that given the giant sperm size in *D. hydei* (Pitnick and Markow, 1994a), males only transfer a few sperm to females (Pitnick and Markow, 1994b), therefore reducing the time *in copula*.

My results show no differences among *D. melanogaster* strains. Since copulation duration was recorded for only one strain of *D. hydei*, it would be interesting to analyze and compare another strain from a different location.

While the present study clearly demonstrates that sperm transfer does not begin immediately, the nature of the male-female interactions that take place prior to sperm transfer and the nature of long copulations remain to be studied.

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