176: 1945-1955; Simmons, M.J., M.W. Thorp, J.T. Buschette, L. Peterson, E.W. Cross, and E.L. Bjorklund 2010, Genet. Res. 92: 261-272; Simmons, M.J., M.W. Meeks, E. Jessen, J.R. Becker, J.T. Buschette, and M.W. Thorp 2014, Genes, Genomes, Genetics 4: 1417-1427; Stuart, J.R., K.J. Haley, D. Swedzinski, S. Lockner, P.E. Kocian, and M.J. Simmons 2002, Genetics 162: 1641-1654.

Impairment of piRNA-mediated regulation of *P*-element mRNAs in *D. melanogaster* females heterozygous for a mutant allele of *argonaute3* or *caravaggio.*

Thorp, Michael W., and Michael J. Simmons. Department of Genetics, Cell Biology and Development, University of Minnesota, Twin Cities, St. Paul, MN. E-mail: simmo004@umn.edu

The proteins encoded by the genes *argonaute3* (*ago3*), *aubergine* (*aub*), and *piwi* are involved in processing small RNAs that regulate transposable elements in the *D. melanogaster* genome. As a group, these proteins are called Piwi-type proteins, and the RNAs associated with them are called Piwi-interacting (or pi) RNAs (Aravin *et al.*, 2007; Brennecke *et al.*, 2007; Li *et al.*, 2009). piRNAs are generated from many different loci, including one in the Telomere Associated Sequences (TAS) at the left end of the X chromosome. This locus has been identified as the source of the piRNAs that regulate the *P* family of transposable elements (Brennecke *et al.*, 2008). *P* elements are mobilized by an enzyme, the P transposase, which is encoded by structurally complete members of the *P*-element family (Engels, 1989). *P*-element activity is restricted to the germ line, because the mRNA for the transposase is produced only in that tissue (Laski *et al.*, 1986); in the soma, the last of the introns—the one denoted as the 2-3 intron because it lies between exons 2 and 3—is not removed from *P* transcripts. When translated, these incompletely spliced *P* transcripts produce a polypeptide that is unable to catalyze transposition.

In the germ line, *P* elements are regulated by piRNAs generated from *P*-elements that have fortuitously inserted into the TAS of XL (Ronsseray *et al.*, 1991; Marin *et al.*, 2000; Stuart *et al.*, 2002). These piRNAs can be passed from mother to offspring through the egg cytoplasm (Brennecke *et al.*, 2008). Thus, a female that carries a telomeric *P* element can endow her offspring with the ability to regulate *P*-element activity, even if the offspring do not inherit the telomeric *P* element itself (Simmons *et al.*, 2012). This maternal effect is one of the hallmarks of *P* regulation. Engels (1979) coined the term "P cytotype" to encompass all the components of *P* regulation—both chromosomal and cytoplasmic. Recent analyses have revealed that the chromosomal component consists of the *P* elements themselves—especially cytotype-anchoring telomeric *P* elements—and the cytoplasmic component consists of maternally transmitted piRNAs.

At the molecular level, *P* regulation is characterized by a reduction in the amount of germ-line *P* mRNA (Jensen *et al.*, 2008). This reduction could either be due to repression of *P* transcription or to post-transcriptional destruction of *P* transcripts; in either case, the mechanism must be mediated by piRNAs generated from a telomeric *P* element. However, *P*-element regulation might also involve other factors. One candidate is heterochromatin protein 1 (HP1), which is encoded by the *Suppressor of variegation 205* [*Su(var)205*] gene (Eissenberg *et al.*, 1990), and another is the HP1/ORC-associated protein (HOAP), which is encoded by the *caravaggio* (*cav*) gene (Cenci *et al.*, 2003). These two proteins are involved in chromatin organization, notably at telomeres, where they could influence the expression of piRNA-generating *P* elements. The proteins Ago3, Aub, and Piwi are thought to be involved directly in the production of piRNAs from these *P* elements. Depleting any of these chromatin-organizing or piRNA-processing proteins might impair the production of piRNAs, and thereby allow *P*-element mRNAs, especially transposase-encoding mRNAs, to accumulate in the germ line.

To test this hypothesis, we used reverse transcription and the polymerase chain reaction (RT-PCR) to assess the levels of mRNAs from a telomeric *P* element, denoted *TP5*, and a transgenic complete *P* element, denoted *H(hsp/CP)2*, in females that were heterozygous for these elements and a mutant allele of the *ago3* or *cav* gene. Females that are homozygous for the $ago3^{t1}$ or $ago3^{t3}$ alleles are sterile (Li *et al.*, 2009), and flies

that are homozygous for the mutant *cav* allele are lethal (Cenci *et al.*, 2003). All these mutant alleles are functionally null, and in heterozygous condition, they would be expected to deplete the amount of wild-type Ago3 or HOAP protein by 50%, as has been demonstrated with *ago3*^{t3}/+ heterozygotes (Li *et al.*, 2009). RNA was isolated from four samples of 20 adult females of each genotype with the Trizol reagent, and then reverse transcribed using the ThermoScript Reverse Transcriptase and an oligo-dT primer. In addition to the samples from the mutant genotypes, we obtained samples from *TP5*/+; *H(hsp/CP)2*/+; +/+ and +/+; *H(hsp/CP)2*/+; +/+ control females. The cDNAs from each sample were amplified by PCR with appropriate primers and the products were analyzed by electrophoresis in 1% agarose gels. The procedures for RNA isolation, reverse transcription, and PCR have been described by Jensen *et al.* (2008).

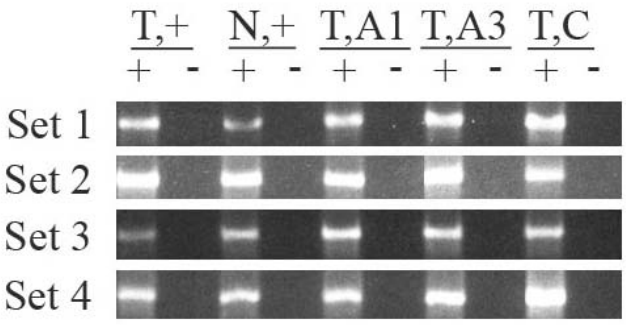

Figure 1. RT-PCR analysis of "somatic" *CP* mRNA in sets of samples from mutant and control female genotypes carrying the *H(hsp/CP)2* transgene (here abbreviated *CP*). Sample T,+: *TP5*/+; *CP*/+; +/+. Sample N,+: +/+; *CP*/+; +/+. Sample T,A1: *TP5*/+; *CP*/+; *ago3*^{t1}/+. Sample T,A3: *TP5*/+; *CP*/+; *ago3*^{t3}/+. Sample T,C: *TP5*/+; *CP*/+; *cav*/+. All the samples came from independent RNA isolates. The 1539 bp RT-PCR products were obtained by amplifying the cDNAs with the primers PΔ0/1-d and P2075-u; see Jensen *et al.*, (2008) for details.

Figure 1 shows the analysis of *CP* mRNAs that retain the 2-3 intron. These mRNAs are produced in somatic cells, where the 2-3 intron cannot be excised from *CP* transcripts; hence, we refer to them as "somatic" mRNAs. However, they may also be produced in germ-line cells if the 2-3 intron is not removed with 100% efficiency. Figure 1 shows no consistent differences in the intensities of the RT-PCR products among the genotypes that were analyzed. Thus, the presence or absence of the cytotype-anchoring *TP5* element or the presence of an *ago3* or *cav* mutation does not seem to affect the abundance of the "somatic" mRNA derived from the *CP* element. These findings fit with the absence of cytotype regulation of *P* elements in somatic cells (Stuart *et al.*, 2002; Simmons *et al.*, 2004). Thus, piRNAs do not operate to diminish *P*-element mRNAs in the soma.

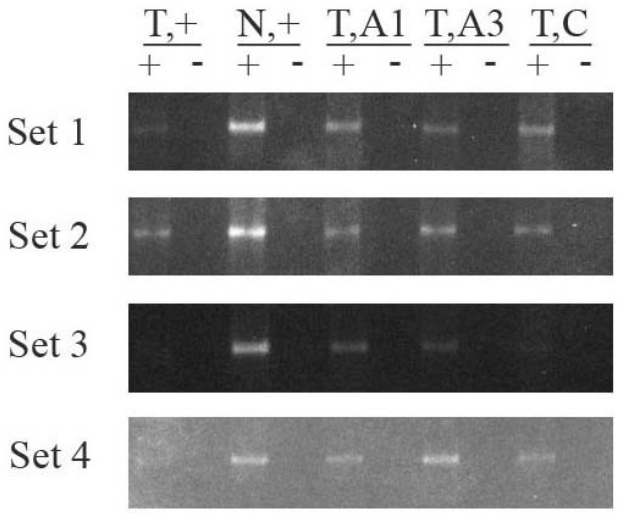

Figure 2. RT-PCR analysis of germ-line CP mRNA in sets of samples from mutant and control female genotypes. The genotypes are the same as those in Figure 1. The 1495 bp RT-PCR products were obtained by amplifying the cDNAs with the primers PΔ0/1-d and PΔ2/3-u (Jensen *et al.*, 2008). The latter primer is specific for mRNAs that do not have the 2-3 intron, which is removed from *P* transcripts only in germ-line cells.

Figure 2 shows the analysis of *CP* mRNAs that have lost the 2-3 intron. These mRNAs are produced exclusively in germ-line cells. In each set of samples, the most intense RT-PCR product is the one from the flies that did not carry the *TP5* element (denoted N,+). Without this element, there is no cytotype regulation of the *P*-element family and the abundance of germ-line mRNAs from the *CP* element is undiminished. All the other samples—from flies that carried *TP5* (denoted with a T)—show less intense RT-

PCR products. Thus, in these samples, the abundance of germ-line *CP* mRNA has been reduced. In sets 1, 3, and 4, the reduction is clearly strongest in the samples from flies that did not carry an *ago3* or *cav* mutation (denoted T,+), whereas in set 2, the reduction is about the same for all the *TP5*-bearing genotypes. These observations indicate that the *TP5* element is responsible for reducing the amount of germ-line *CP* mRNA, and that in three of the four sets, the *ago3* mutations (A1 = $ago3^{t1}$; A2 = $ago3^{t3}$) or the *cav* mutation (C) ameliorate this reduction. Heterozygous *ago3* or *cav* mutant alleles therefore appear to impair the *TP5*-anchored mechanism that prevents *CP* mRNA from accumulating in the female germ line.

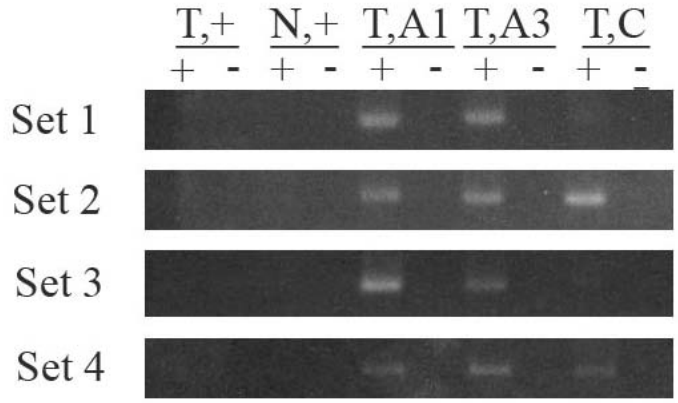

Figure 3. RT-PCR analysis of sets of germ-line TP5 mRNAs from mutant and control female genotypes. The genotypes are the same as those in Figure 1. The 471 bp RT-PCR products were obtained by amplifying the cDNAs with the primers TP5-d, which is specific for the *TP5* element, and PΔ2/3-u (Jensen *et al.*, 2008).

Figure 3 shows the analysis of germ-line mRNA from the *TP5* element. Of course, the flies that did not carry *TP5* (the sample denoted N,+) could not—and did not—produce this mRNA. The flies that carried *TP5* but that did not carry a mutant *ago3* or *cav* allele (T,+) also did not produce any germ-line *TP5* mRNA. In these flies, the *TP5* element evidently generates only piRNAs. However, some germ-line *TP5* mRNA does appear when a mutant *ago3* or *cav* allele is present in the genotype (samples denoted T,A1; T,A2 and T,C). Thus, heterozygous mutant alleles of *ago3* or *cav* alter the expression of the *TP5* element. The appearance of *TP5* mRNA in these heterozygous mutant genotypes suggests that expression of the *TP5* element has shifted from generating piRNAs to producing mRNAs, likely diminishing the power of the piRNA mechanism to repress *P*-element activity. This expectation is consistent with the impaired ability of *TP5*-bearing flies that are heterozygous for a mutant *ago3* or *cav* allele to prevent the accumulation of *CP* mRNA in the germ-line (Figure 2). At the molecular level, depleting the Ago3 or HOAP proteins by mutating one copy of the relevant genes therefore seems to weaken the regulatory system that is anchored in the telomeric *TP5* element. However, the accompanying note by Elston and Simmons (2015) indicates that heterozygous *ago3* or *cav* mutations have no effect on repression of *P*-element excisions from an X-linked locus in males. Thus, even a regulatory system weakened by the maternal (and zygotic) effects of heterozygous *ago3* or *cav* mutations is capable of repressing *P* activity in the male germ line.

A similar analysis has been reported for females heterozygous for a mutant allele of the *aub*, *piwi*, or *Su(var)205* genes (Simmons *et al.*, 2010). Heterozygous *aub* alleles and one *piwi* allele ($piwi^1$) allowed *CP* mRNA to accumulate in the germ line, and they also increased the expression of *TP5* mRNAs. The $piwi^2$ allele, which is thought to be less severe than $piwi^1$ because of the nature of the molecular lesion and the absence of any homozygous effect on male fertility, did not allow *CP* mRNA to accumulate, and it did not increase the expression of *TP5* mRNA. $Su(var)205^{04}$ also did not allow *CP* mRNA to accumulate, but it did increase the expression of *TP5* mRNAs. When heterozygous, the *aub* alleles, $piwi^1$ and $Su(var)205^{04}$ disrupted repression of *P*-excisions in the male germ line, whereas $piwi^2$ did not. All these results suggest that *P*-element regulation may involve the collaboration of the Piwi-type proteins Ago3, Aub, and Piwi, the chromatin-organizing proteins HP1 and HOAP, and *P*-specific piRNAs.

Acknowledgments: Phillip Zamore kindly provided the *ago3* mutants and Maurizio Gatti kindly provided the *cav* mutant. The Department of Genetics, Cell Biology and Development of the University of Minnesota provided financial support.

References: Aravin, A.A., G.J. Hannon, and J. Brennecke 2007, Science 318: 761-764; Brennecke, J., A.A. Aravin, A. Stark, M. Dus, M. Kellis, R. Sachidanandam, and G.J. Hannon 2007, Cell 128: 1089-110; . Brennecke, J., C.D. Malone, A.A. Aravin, R. Sachidanandam, A. Stark, and G.J. Hannon 2008, Science 322:

1387-1392; Cenci, G., G. Siriaco, G.D. Raffa, R. Kellum, and M. Gatti 2003, Nature Cell Biology 5: 82-84; Eissenberg, J.D., T.C. James, D.M. Foster-Hartnett, T. Hartnett, V. Ngan, and S.R.C. Elgin 1990, Proc. Natl. Acad. Sci. U. S. A. 87: 9923-9927; Elston, J., and M.J. Simmons 2015, Dros. Inf. Serv. 98: 1-3; Engels, W.R., 1979, Genet. Res. 33: 219-236; Engels, W.R., 1989, in *Mobile DNA* (eds. D.E. Berg and M.M. Howe), pp 437-484, Washington, DC: American Society for Microbiology Publications; Jensen, P.A., J.R. Stuart, M.P. Goodpaster, J.W. Goodman, and M.J. Simmons 2008, Genetics 179: 1785-1793; Laski, F.A., D.C. Rio, and G.M. Rubin 1986, Cell 44: 7-19; Li. C., V. V. Vagin, S. Lee, J. Xu, S. Ma, H. Xi, H. Seitz, M. D. Horwich, M. Syrucka, B. M. Honda, E. L. W. Kittler, M. L. Zapp, C. Klattenhoff, N. Schulz, W. E. Theurkauf, Z. Weng and P. D. Zamore. Cell 137: 509-521. Marin, L., M. Lehmann, D. Nouaud, H. Izaabel, D. Anxolabéhère, and S. Ronsseray 2000, Genetics 155: 1841-1854. Ronsseray, S., M. Lehmann and D. Anxolabéhère 1991, Genetics 129: 501-512. Simmons, M. J., J. D. Raymond, J. B. Niemi, J. R. Stuart and P. J. Merriman 2004, Genetics 166: 243-254. Simmons, M. J., M. W. Thorp, J. T. Buschette, L. Peterson, E. W. Cross and E. L. Bjorklund 2010, Genet. Res. 92: 261-272. Simmons, M. J., L. M. Ragatz, I. R. Sinclair, M. W. Thorp, J. T. Buschette and C. D. Grimes 2012, Genet. Res. 94: 339-351. Stuart, J. R., K. J. Haley, D. Swedzinski, S. Lockner, P. E. Kocian and M. J. Simmons 2002, Genetics 162: 1641-1654.

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

Gredler, Jennifer N., and Mohamed A.F. Noor. Biology Department, Duke University, Durham, NC 27708 USA; E-mail: jngredler11@gmail.com

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).