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Genetic Mapping a Meiotic Driver That Causes Sex Ratio Distortion in the Mosquito *Aedes aegypti*

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An endogenous meiotic driver in the dengue and yellow fever vector mosquito *Aedes aegypti* can cause highly male-biased sex ratio distortion in crosses from suitable genetic backgrounds. We previously selected a strain that carries a strong meiotic drive gene (*D*) linked with the male-determining allele (*M*) on chromosome 1 in *A. aegypti*. Here, we performed segregation analysis of the M^D locus among backcross (BC_1) progeny from a driver male and drive-sensitive females. Assessment of sex ratios among BC_2 progeny showed ~5.2% recombination between the M^D locus and the sex determination locus. Multipoint linkage mapping across this region revealed consistent marker orders and recombination frequencies with the existing reference linkage map and placed the M^D locus within a 6.5-cm interval defined by the LF159 locus and microsatellite marker 446GAA, which should facilitate future positional cloning efforts.

Key words: *gene drive, linkage map, microsatellite, segregation distortion, sex linkage, SSCP*

Meiotic drive is one of the best known examples in which a selfish genetic element can increase in frequency and eventually become fixed in the population regardless of its phenotypic effect because its own fitness is partly uncoupled from that of the "host"' individual (Hurst and Werren 2001). Meiotic drivers violate Mendel's first law of independent transmission of alleles to gametes, and instead, particular alleles are preferentially transmitted following meiosis. Such drive systems have the potential to also impact population dynamics of closely linked neutral polymorphisms due to a selective sweep or hitchhiking effect (Maynard-Smith and Haigh 1974). This phenomena has been confirmed in investigations of natural populations including, for example, the segregation distorter (SD) system of *Drosophila melanogaster* (Palopoli and Wu 1996), the mouse *t*-complex (Hammer and Silver 1994), and more recently for a sex-linked meiotic driver in *D. simulans*, wherein sex ratio drive induced a strong selective sweep in populations where the distorter was in high frequency (Derome et al. 2004).

The existence of an endogenous sex-linked meiotic drive system has been reported in 2 mosquito species, *Aedes aegypti* and *Culex pipiens* (Hickey and Craig 1966a; Sweeny and Barr 1978). *Aedes aegypti* is the primary vector of dengue, yellow fever, and Chikungunya viruses to humans in many sub-tropical and tropical regions. Given the general failure in development of vaccines or drugs to effectively prevent most mosquito-borne diseases, considerable interest has emerged in exploring the potential for employing transgenic insect approaches to disease control. This would entail introducing antipathogen effector genes into mosquitoes, thereby making them incompetent to transmit disease to humans followed by their release into natural populations. Inherent to the success of such population replacement efforts would be the identification of suitable gene drive systems that would ensure rapid transgene introgression into natural populations as well as persistent linkage disequilibrium between the driver locus and the antipathogen effector gene (James 2005). We previously reported selection for an *A. aegypti* strain (T37) from a population in Trinidad, West Indies that carries a strong meiotic driver (Mori et al. 2004) and subsequently observed evidence for a hitchhiking effect in controlled cage studies (Cha, Mori, et al. 2006). Computer simulations also suggested that under some conditions, for example, uniform population sensitivity and low fitness costs of associated genes, the *A. aegypti* drive system could facilitate near-fixation of an antipathogen transgene in natural populations (Huang et al. 2007).

The meiotic drive system in *A. aegypti* is known to be tightly linked with the sex determination locus on chromosome 1 (Hickey and Craig 1966a). Of note, sex determination in *A. aegypti* and other mosquitoes in the subfamily Culicinae does not involve an XY chromosome system but instead is determined by an autosomal gene with the male-determining allele (*M*) being dominant to the

female-determining allele (*m*) and males being the heterogametic sex (Gilchrist and Haldane 1947). Because the meiotic drive gene (*D*) only functions when in *cis* configuration with the male-determining allele, it is generally symbolized as a superscript (M^D) after Hickey and Craig (1966a). Furthermore, the M^D gene product acts in *trans* with a responder locus tightly linked with the *m* allele, which shows variable response to M^D from sensitive (m^s) to insensitive (m^i) (Suguna et al. 1977; Wood and Newton 1991; Cha, Chadee, et al. 2006), to promote breakage of m^s allele–carrying chromosomes during meiosis (Newton et al. 1976). This results in a highly male-biased sex ratio in the resulting offspring (Hickey and Craig 1966b), for example, with appropriate crosses with T37 strain males, we obtain ~85% male progeny (Mori et al. 2004).

At present, little is known about the molecular basis for the M^D system in *A. aegypti*. However, other meiotic drive systems have been well characterized. The *SD* system in *D. melanogaster* has been shown to be determined by a duplicated but truncated *RanGAP* gene (Merrill et al. 1999), a regulatory gene of the small GTPase Ran. The mouse *t*-complex contains multiple distorters (*Tcd* loci) that act in *trans* on a single responder locus (*Smok1*). The *Tcd1* locus encodes a Rho GTPase-activating protein, whereas the*Tcd2* locus contains the *Fgd2* gene that encodes a truncated guanine nucleotide exchange factor (GEF) for Rho small G proteins (Bauer et al. 2005, 2007). These likely regulate different pathways in the Rho signaling cascade. Therefore, meiotic drive in both *D. melanogaster* and mouse has been associated with components of the Ras superfamily of regulatory small GTPases (Wennerberg et al. 2005). We recently employed suppressive subtractive hybridization to isolate transcripts enriched in testes from the *A. aegypti* T37 strain and identified a number of genes associated with signal transduction and cell cycle that included several Ras-related genes (Shin et al. 2011). Here, we take advantage of the existing linkage map (Severson et al. 2002) and whole genome sequence (Nene et al. 2007) for *A. aegypti* to localize the genetic map position for the M^D locus as a foundation for ultimately isolating the M^D gene and responder locus using positional cloning techniques.

Materials and Methods

Genetic Crosses

Two *A. aegypti* strains were used to generate mapping populations segregating for the M^D locus. The T37 strain carries a strong M^D gene and insensitive m^i allele (Mori et al. 2004). The RED strain is fixed for highly sensitive m^s alleles (Hickey and Craig 1966a; Mori et al. 2004). Mosquitoes were reared and maintained in an environmental chamber following our standard procedures (Schneider et al. 2007). Adult females were blood fed on anesthetized rats ~1 week after emergence. Our protocol for maintenance and care of experimental animals was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Notre Dame.

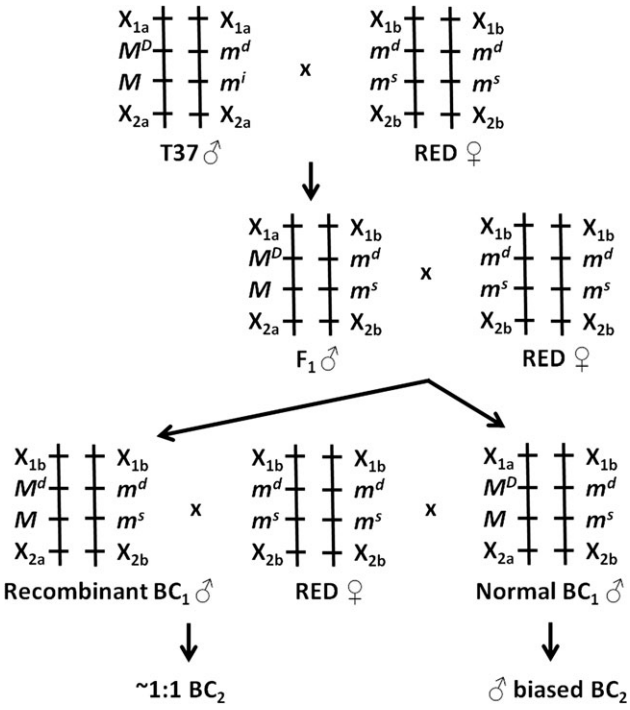

Figure 1. Crossing scheme for segregation analysis of meiotic drive (M^D) with the sex determination locus (*M*) and DNA-based genetic markers X_{ij}. Male-determining (*M*) alleles are dominant to female-determining alleles (*m*). Female-determining chromosomes carry drive sensitive (m^s) or drive insensitive (m^i) responder loci. Recombination between M^D and the *M* locus was revealed by sex ratios observed among BC_2 progeny.

To prepare an F_1 generation from pairwise matings, a single T37 strain male and 5 virgin RED strain females were placed in a 450 ml mesh-covered carton for 4 days. After blood feeding, individual females were transferred to 12.5 ml mesh-covered glass vials containing 1 ml water and a strip of paper towel as the oviposition substrate. F_1 progeny was reared from eggs from a single pairwise mating, and backcross (BC_1) populations were prepared by mating each F_1 male with 5 virgin RED strain females as previously described. A single BC_1 population was subsequently selected for genetic mapping. To assess the M^D phenotype of individual BC_1 males, the sex ratios observed in BC_2 populations were determined. BC_2 populations were prepared by mating each BC_1 male with 5 virgin RED strain females as previously described. Sex ratios were determined for progeny from each of the females and tested for departure from the expected 1:1 using the X^2 test. All parental F_1 and BC_1 adults were frozen and stored at −80 °C. The general crossing scheme is shown in Figure 1.

DNA Extraction and Genotyping

Genomic DNA was extracted from individual carcasses using our standard protocol (Severson et al. 1993). DNA from each

mosquito was resuspended in tris–ethylenediaminetetraacetic acid (10 mM Tris–HCl, 1 mM EDTA, pH 8.0).

Single-strand conformational polymorphism (SSCP) marker analysis was performed following Bosio et al. (2000). Previously, reported individual loci across the region flanking the sex determination locus on chromosome 1 (Severson et al. 2002) were initially screened for polymorphism with BC_1 parental individuals from the T37 and RED strains. Markers showing strain-specific polymorphism were then used to genotype BC_1 male progeny.

Preliminary screenings and linkage analysis of microsatellite marker polymorphisms were performed using denaturing polyacrylamide gel electrophoresis following Chambers et al. (2007). In silico identification of putative single-copy microsatellite loci within *A. aegypti* genome supercontig assemblies (Nene et al. 2007) that were determined by BLASTn analysis at VectorBase (http://aaegypti.vectorbase.org) to carry genetic markers on chromosome 1 (Severson et al. 2002) were identified using the Tandem Repeats Finder program (Benson 1999) and evaluated as described in Lovin et al. (2009).

Linkage Analysis

SSCP and microsatellite marker genotypes and M^D phenotypes for individual BC_1 males were scored, and multipoint linkage analysis was performed using the MAPMAKER computer program (Lander et al. 1987) with an LOD of 3.0 as the threshold for significance. Pairwise recombination distances were converted to Kosambi centiMorgans (Kosambi 1944).

Results and Discussion

The observed segregation ratio for the F_1 generation from the T37 male × RED female cross was 0.54:0.46 (male:female) among 102 total progeny. Because T37 males carry an insensitive responder allele (m^i) and *A. aegypti* progeny from individual females tend to normally be slightly male based, these results were expected. For example, RED strain by RED strain crosses typically produce ~54.5% male progeny (Mori et al. 2004). However, for the BC_1 generation, we obtained 203 progeny that showed a highly male-biased sex ratio (0.81:0.19). Each of the BC_1 males was allowed to mate with up to 5 RED strain females, and the BC_2 generation eggs were then collected and hatched separately for each female and sex ratios determined.

The M^D phenotype of 135 BC_1 males was determined based on sex ratios observed among 1–5 BC_2 families per male (Supplementary Table 1). An arbitrary threshold of at least 20 progeny per BC_2 family was used for statistical comparisons of sex ratios. Most BC_2 families for individual BC_1 males showed significant male-biased sex ratio distortion, but for 7 of the 135 BC_1 males, the BC_2 families did not show sex ratio distortion, thus indicating 5.19% recombination between the M^D gene and the male-determining locus.

Multipoint linkage mapping of the M^D locus was successfully performed relative to 2 SSCP and 2 microsatellite loci (Figure 2). The M^D locus is located within a 6.5-cm interval defined by the SSCP marker LF159 (GenBank accession T58320) located within supercontig 1.388 and the microsatellite marker 446GAA (primers—F: TGACTTCCCTGGGCATAGAG, R: GCCCCATCATG-CAACATAGT). The 446GAA locus is located within supercontig 1.446, which also contains the previously reported LF284 locus (BM005502) (Severson et al. 2002). Microsatellite locus AC2 was identified previously (Slotman et al. 2007), and BLASTn analysis placed it within supercontig 1.650, which also contains the *NaK* locus (AF393727) (Severson et al. 2002). As indicated in Figure 2, linear orders and map distances between markers were consistent with the composite linkage map (Severson et al. 2002). None of the other loci known to reside within the interval containing the M^D locus were informative in the mapping population, nor were we able to identify informative microsatellite loci within supercontigs containing these loci.

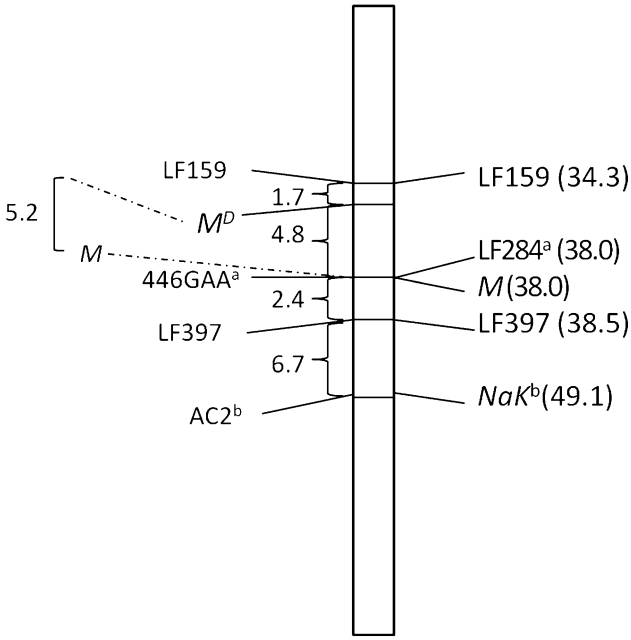

Figure 2. Linkage map of the meiotic drive (M^D) locus on chromosome 1. Map distances are listed in Kosambi centiMorgans. The left side of the map shows the markers utilized and map distances from this study. Observed recombination between the sex determination locus (*M*) and M^D is shown to the far left. The right side shows marker information from the existing reference map (Severson et al. 2002). [a]Both loci are in supercontig 1.446; [b]both loci are in supercontig 1.65.

In general, SDs are often found in genome regions with low recombination, such as heterochromatin associated with pericentromeric regions or with chromosomal inversions (Lyttle 1991). This results in reduced recombination between the distorter gene and the responder locus. This is particularly important as linkage disequilibrium across the drive system complex is critical to its function and long-term

stability. For example, the *SD* system in *D. melanogaster* and *t*-haplotype in mouse each shows reduced recombination caused by inversions that promotes strong linkage disequilibrium across broad genome regions around the drive complex (Hammer and Silver 1994; Palopoli and Wu 1996). Although the presence and genome structure of potential inversions in the *A. aegypti* genome is largely unknown, there is evidence for differences in heterochromatin structure between the male- and female-determining chromosomes that could impact recombination frequency around the genome region containing the sex determination locus and the M^D system (Wallace and Newton 1987). The sex determination locus is tightly coupled with the centromere (Newton et al. 1974), and the M^D locus is associated with an intercalary band when present (Newton et al. 1974, 1976).

In conclusion, our mapping efforts have placed the M^D system to a 6.5-cm interval in the *A. aegypti* genome, thus defining physical landmarks for future positional cloning efforts. A more fine-scale resolution was not possible due to low polymorphism observed among known genetic markers in this region and the incomplete status of the present *A. aegypti* genome assembly. Assignment to and orientation of supercontig assemblies to their respective chromosome positions is presently limited (Nene et al. 2007). Enhancement of the genome assembly is needed for identification of candidate genes for the M^D locus within a minimum genome region for functional analysis. Ongoing efforts to identify physical positions and relative orientations of supercontigs on *A. aegypti* chromosomes (Sharakhova et al. 2011) coupled with availability of a well-characterized bacterial artificial chromosome library (Jiménez et al. 2004) should facilitate this process. Successful molecular characterization of the meiotic drive system would promote efforts to better understand the observed phenotypic outcomes and to evaluate mechanisms for effectively coupling effector genes with the drive system as a novel mechanism for disease control via population replacement.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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