Reinvestigation of an Endogenous Meiotic Drive System in the Mosquito, *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT We have initiated efforts to determine the molecular basis for the M^D meiotic drive system in the mosquito, *Aedes aegypti*. The effect of the M^D gene is a highly male-biased sex ratio, but varies depending on the frequency and sensitivity of a susceptible responder m^s allele. The M^D system has potential as a mechanism for driving trangenes for pathogen resistance into natural *Ae. aegypti* populations. Because all previously existing laboratory strains carrying the M^D gene have been lost, we have selected for a new strain, T37, that carries a strong driver. Matings between T37 males and drive-susceptible m^s females result in progeny with highly biased sex ratios, wherein only ≈14.7% females are produced. We discuss the potential for identifying M^D candidate genes based on comparisons with the well-described *Drosophila melanogaster* segregation distorter (*SD*) meiotic drive system and considerations for release of transgenic *Ae. aegypti* into natural populations where M^D and insensitive m^i alleles are likely segregating.

KEY WORDS sex ratio, population replacement, transgenic insects, genetic control, selection

THE POTENTIAL FOR CONTROLLING arthropod-borne diseases through the genetic manipulation of the arthropod host has been recognized and advocated for over three decades (Knipling et al. 1968). Proposed genetic control strategies for mosquitoes have included sterile male release programs, cytoplasmic incompatibility, hybrid sterility, and population replacement (Knipling et al. 1968, Asman et al. 1981, Curtis 1985). However, to date, none of these strategies has been successfully implemented, despite repeated attempts. In general, the failure of genetic control strategies has related directly to the reduced reproductive fitness among mosquitoes to be released, relative to natural mosquito populations. The less fit genome is subject to strong purifying selection and is quickly lost in a natural environment. Considerable progress has, however, been made in the development and application of transgenesis technology in mosquitoes, with a goal to produce transgenic insects that carry stable antipathogen effector genes (Adelman et al. 2002, Moreira et al. 2002, Robinson et al. 2004). Still, successful genetic control will, by necessity, involve a population replacement strategy wherein the released mosquitoes also carry a genetic system that facilitates a rapid selective sweep of the target transgene (Collins and James 1996).

One potential mechanism for promoting population replacement is through agents that promote distortion of Mendelian segregation by meiotic drive (Lyttle 1993, Braig and Yan 2001, Taylor and Ingvarsson 2003). In such systems, a "selfish" genome segment is able to destroy or disable its alternate form, thereby increasing its frequency among potential gametes. Genic meiotic drive involves the interaction between two genetic elements, the driver locus and the responder locus, that results in the asymmetric disabling of gametes carrying sensitive alleles at the responder locus. This effect itself can be detrimental, as it can result in a loss of functional gametes that is proportional to the strength of the driver. However, under conditions where gamete loss does not necessarily result in a proportional loss of individual fecundity, genic meiotic drivers can increase in frequency in natural populations, despite their overall deleterious effect (Hamilton 1967). Meiotic drivers are most commonly active in males of a species, where relatively high levels of sperm disfunction have no or little effect on fecundity. Theory further suggests that a strong autosomal drive system could become fixed without major detrimental effects on the population (Lyttle 1991). To become established and maintained, meiotic drive genes must remain linked with an insensitive allele at the responder locus or the effect of the drive gene will be autolethal (Charlesworth and Hartl 1978). Therefore, once a drive mutation arises in a population, there is

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strong balancing selection for it to remain physically associated with an insensitive responder allele.

The best-studied example of genic meiotic drive is the segregation distorter (*SD*) system of *Drosophila melanogaster*. Males heterozygous for the *SD* chromosome and the wild-type SD^+ chromosome normally produce in excess of 95–99% *SD*-carrying sperm (Hartl et al. 1967). *SD* chromosomes have been observed in nearly every *D. melanogaster* natural population examined (Hiraizumi and Nakazima 1967, Temin and Marthas 1984). The *SD* complex consists of three loci straddling the centromeric region of chromosome 2 (Lyttle 1991). These loci include (1) *SD*, the *Segregation distorter* gene; (2) *E(SD)*, the *Enhancer of Segregation Distortion*, required for full expression of drive; and (3) *Rsp*, the *Responder*, which represents the target site for the action of *SD*. The *SD* and *E(SD)* loci are located on the left arm of chromosome 2, ≈1 map unit apart. The *Rsp* locus is located on the right arm of chromosome 2. All *SD* chromosomes isolated from natural populations have been found to carry *SD*, *E(SD)*, and highly insensitive *Rsp* alleles (Sandler and Golic 1985, Temin et al. 1991) and reflect much less polymorphism than SD^+ chromosomes (Palopoli and Wu 1996). Furthermore, it seems that strong epistatic selection within the *SD* complex results in a large genetic hitchhiking effect around the entire pericentric region of chromosome 2, and this region represents at least 10% of the *D. melanogaster* genome (Palopoli and Wu 1996).

Endogenous meiotic drive systems have been described in two mosquito species: *Aedes aegypti* (Hickey and Craig 1966a) and *Culex pipiens quinquefasciatus* (Sweeny and Barr 1978). In both species, a sex-linked gene coupled with the male-determining allele at the sex determination locus drives the normally equal sex ratio in favor of males (e.g., is analogous to Y-linked drivers). Note that sex determination in these mosquitoes is determined by a single autosomal gene, located on chromosome 1, with maleness being the dominant allele (Gilchrist and Haldane 1947). Males represent heterozygotes (*Mm*) at the sex locus, whereas females represent the homozygous recessive (*mm*) condition. A meiotic drive (M^D) gene product acts in *trans* with a locus indistinguishable from *m* (e.g., likely tightly linked with the *m* allele at the sex locus) to cause breakage of the *m* allele carrying chromosomes in *Ae. aegypti* (Newton et al. 1976). The *m* allele carrying chromosomes can be sensitive (m^s) or insensitive (m^i) to M^D gene effects. Additionally, *m* allele sensitivity to distortion varies considerably over a range of haplotypes (Hickey and Craig 1966a, Wood 1976, Suguna et al. 1977 Owusu-Daaku et al. 1997). The *Ae. aegypti* meiotic drive system, therefore, reflects similarity with and has been likened to the *SD* complex in *D. melanogaster* (Wood and Newton 1991). That is, the M^D and $m^{s/i}$ loci seem analogous with *SD* and *Rsp*, respectively. Additionally, several modifiers of M^D have been identified. One locus, designated *t* (*tolerance of Distorter*), is located near the *red-eye* (*re*) locus on chromosome 1 and results in a reduction in sex ratio distortion (Wood and Newton 1991). This likely reflects the effects of a suppressor of M^D; similar suppressor gene effects have been observed in *D. melanogaster*. The effects of two autosomal loci also have been observed: (1) another suppressor of M^D linked with the *spot abdomen* (*s*) locus on chromosome 2 and (2) an apparent enhancer of M^D linked with the *black tarsus* (*blt*) locus on chromosome 3 (Wood and Ouda 1987).

While considerable progress has been made in elucidating the molecular basis for meiotic drive in *D. melanogaster* (Kusano et al. 2003), nothing is known about the molecular nature of meiotic drive in mosquitoes. Unfortunately, all previously identified *Ae. aegypti* strains carrying the endogenous meiotic driver have been lost. Here we report the results of our efforts to reselect for meiotic drivers among a diverse sampling of *Ae. aegypti* laboratory and field populations. We have developed a strain, T37, from field collections obtained in Trinidad, that carries a strong meiotic driver that likely represents the M^D gene; T37 males mated with females from a sensitive strain produce ≈14.7% female progeny. Availability of the T37 strain should facilitate genetic and molecular efforts to isolate and characterize the major components for the *Ae. aegypti* meiotic drive system.

Materials and Methods

Mosquitoes. Mosquitoes were reared in an environmental chamber at 26°C, 84% RH, and under a 16-h light/8-h dark cycle, with a 1-h crepuscular period at the beginning and end of each light cycle. Larvae were reared on a bovine liver powder suspension. Adults were maintained on a 5% sucrose solution. Female mosquitoes were blood fed on anesthetized rats. *Ae. aegypti* genetic stocks were obtained from several sources and included (1) a laboratory strain from Bangkok, Thailand; (2) field samples collected from Akomadan and Fante, Ghana; (3) a laboratory strain from Mombasa, Kenya; (4) a laboratory strain, RED, that carries multiple morphological mutant markers and was previously shown to be highly sensitive to meiotic drive (Wood and Ouda 1987); (5) a laboratory strain, Rockefeller; and (6) field samples collected from Trinidad. The number of generations in laboratory culture is unknown for each laboratory strain.

Genetic Crosses. Crosses to identify males carrying the M^D gene were prepared generally following the mating scheme outlined by Hickey and Craig (1966a). Approximately 10–15 individual males from each stock to be tested were each mated with 3–5 RED strain females. In addition, males from field-collected stocks were also mated with three to five females from the same source. Field-collected females were included as a potential mechanism to capture male drive chromosomes in an insensitive m^i chromosome background (based on an arbitrary assumption that females within such stocks would reflect strong purifying selection for drive insensitivity). Next, three to seven F_1 males from each testor male were each sib-mated with three to five F_1 females, and one F_2 family per F_1 male was reared, and its sex ratio was determined. After

mating and blood feeding, females from all crosses were transferred individually to oviposition vials containing water and an oviposition substrate (paper toweling strip). After oviposition, the substrates were removed, allowed to dry at room temperature, and stored until hatched. Departures from the expected 1:1 sex ratio were determined using the χ^2 test. A minimum threshold value of $P < 0.01$ was used to identify families with significant departures from expected, because *Ae. aegypti* crosses generally reflect a slight bias for male progeny under nondriving conditions and could show significance at the $P = 0.05$ level.

Strain Selection. With selected testor males that showed strong meiotic drive, F_1 progeny were reared from one of the matings with a testor strain female and evaluated for sex ratio. A stable colony was established by allowing the F_1 progeny from a single driving testor male to mass sib mate. Seven male progeny from the resulting colony were tested for drive against RED strain females as previously described.

Results

We identified and isolated male-determining chromosomes carrying a putative meiotic driver by testing 74 individual males from six laboratory and field populations against females from the drive-sensitive RED strain (Table 1). As anticipated, results from testing the RED strain against itself generally exhibited the expected 1:1 ratio, with a mean of 45.5% females among the 82 F_2 families examined. Similar results were observed with males from the Mombasa strain, with a mean of 49.7% females among the 73 F_2 families examined. With the 10 males each from the Bangkok strain and Ghana field population, we identified one male per stock with which the majority of F_2 families exhibited a significant male bias, averaging 37.8 and 33.0% females, respectively. Among 15 males from the Rockefeller strain, the majority of F_2 families from 2 (13.3%) exhibited a significant male bias, averaging 24.6 and 26.9% females, respectively. The greatest levels of sex ratio distortion were observed among 14 males from the Trinidad field population; for 6 males (42.9%), the majority of F_2 families showed a significant male bias. We observed a mean of 23.3% females in the F_2 progeny from these males, with a range from 15.3 to 35.9%.

Because the Trinidad field population showed the best evidence for carrying the M^D meiotic drive gene, we examined F_1 progeny from matings with three of the males showing significant sex ratio distortion and females from the Trinidad field population (T8, T23, and T37 with a mean of 22.7, 23.1, and 15.3% females, respectively, in the F_2 progeny after mating with RED strain females). The observed sex ratios suggested that the Trinidad field population females that mated with these males each carried insensitive m^i chromosomes, because the ratios were not significant at $P = 0.01$ (50.0, 36.8, and 38.3% females with the T8, T23, and T37 males, respectively).

The T37 male × Trinidad female F_1 progeny were mass sib mated to initiate a stable laboratory colony carrying the putative Distorter (M^D) locus and insensitive m^i chromosomes (Table 2). We also examined sex ratios among two F_3 families prepared by pair-wise sib matings of progeny from the no. 4 F_2 family from the original T37 male x× RED female cross (Table 1). The observed sex ratios were highly biased, and yet fecundity remained high, with 5.9 and 10.7% females among 101 and 121 total progeny, respectively. Seven males from the first-generation T37 colony were tested for presence of the M^D locus by examining F_2 progeny from pair-wise matings with RED strain females (Table 2). Sex ratios observed among F_1 progeny from four of these males were not significant at $P = 0.01$, whereas that from one male was marginally significant at $P = 0.01$, providing further evidence that the T37 colony was initiated with insensitive m^i chromosomes (no data were collected for two males). Results from the F_2 progeny provide strong support for the presence of the M^D locus in the T37 colony, because 45 of 47 F_2 families examined showed highly significant ($P < 0.0001$) sex ratio distortion. We observed a mean of 14.7 females across all 47 F_2 families, with 15 families (31.9%) containing <10% females.

Discussion

The possibility of mosquito-borne disease control through population replacement with individuals carrying a stable transgene that confers pathogen resistance has gained considerable support over the past decade. Laboratory studies already have validated the concept of stable germline transgene insertion and expression of foreign genes or gene knockouts in mosquitoes that results in the prevention or reduction in their susceptibility to various pathogen systems (Blair et al. 2000, Adelman et al. 2002, Moreira et al. 2002). An essential understudied component is the identification and development of effective and efficient methods for promoting the successful spread of a desired transgene construct into natural mosquito populations.

We have initiated an effort to ultimately characterize completely the M^D meiotic drive system in *Ae. aegypti.* Because all previously described laboratory strains carrying M^D have been lost, we tested 74 males from six laboratory and field populations for their ability to produce highly male-biased progeny when mated with a strain (RED) that is highly sensitive to the effects of M^D (Wood and Ouda 1987). We found no evidence for sex ratio bias in males tested from a Mombasa laboratory strain and only limited evidence in males tested from a Bangkok laboratory strain and two Ghana field populations. Two (13.3%) of the males tested from the Rockefeller laboratory strain showed highly male-biased sex ratios, suggesting that the M^D gene is present but not fixed in this strain. Indeed, presence of the M^D gene in this strain was previously reported (Hickey and Craig 1966b). The M^D gene seems to be prevalent among the Trinidad field population, with ≈42.9% of males tested showing highly male-biased sex ratios. Of note, the previously described but now lost T30 strain that carried the M^D

Table 1. Sex ratios observed among crosses between *Aedes aegypti* populations and the drive-sensitive RED strain

Strain	Male ID	F_1		F_2 families (% female)						
		n	% female	1	2	3	4	5	6	7
Bangkok	B1R1	95	45.3	44.3	50.6	52.1	41.2	46.9	51.9	
	B2R1	105	50.5	31.3*	44.5	43.1	44.8	42.4	32.0*	
	B3R3	42	47.6	53.0	45.3	45.1	49.2	48.0	50.0	
	B4R3	138	52.9	51.5	29.8*	25.8**	50.4	47.8	40.3	
	B5R1	130	46.9	55.1	57.9	48.6	44.3	49.4	47.8	
	B6R2	117	53.8	39.4	47.1	51.4	52.7	46.6	52.8	
	B7R2	92	43.5	41.0	33.3	51.5	54.1	38.3	15.5***	
	B8R2	58	60.6	45.2	43.0	47.2	52.8	53.4	45.9	
	B9R3	34	32.4	46.0	36.9	49.5	57.9	48.0	36.8	
	B10R1	117	47.9	33.7*	27.3***	57.6	29.8***	45.9	32.3***	
Ghana[a]	G1RF	125	51.2	52.6	46.3	41.4	44.8	50.5	50.3	
	G2RF	34	47.1	50.0	42.9	52.5	46.7	44.1	44.9	
	G4RF	79	45.6	36.4	56.1	44.6				
	G5RF	80	52.5	31.9***	34.2***	38.6*	23.3***	34.2***	35.6**	
	G9RF	41	48.8	39.7	58.2	50.7	50.0	38.0	39.8	52.4
	G10RF	94	45.7	40.7	43.0	40.6	33.7*	35.1*	36.7*	
	G11RA	90	62.2	36.8	51.7	40.8	45.2	56.0		
	G12RA	107	50.5	50.7	55.4	47.2	44.8	49.0	48.7	
	G13RA	97	50.5	57.1	52.0	50.6	44.9	50.7	48.3	
	G14RA	55	45.5	47.8	44.0	47.5	29.6*	34.8*	50.6	
Mombasa	M1R2	105	61.3	38.5	46.0	49.5	39.6	55.4		
	M2R1	115	47.8	42.2	48.4	46.6	55.9	45.0		
	M3R1	140	47.1	50.9	57.7	43.9	52.0	58.8	48.7	
	M4R1	81	55.6	42.6	59.5	63.2	54.1	71.9	53.7	
	M5R1	112	50.9	56.9	46.9	47.1	43.9	40.8	51.5	
	M6R2	110	40.0	41.1	49.0	52.2	50.0	54.2		
	M7R1	94	59.6	50.0	51.4	49.0	47.8	52.7	41.8	
	M8R2	117	42.7	50.6	44.8	42.6	48.7	63.0	61.5	
	M9R1	101	42.6	51.3	48.6	48.0	49.0	43.9	52.2	
	M10R2	127	54.3	42.6	39.4	56.9	40.0	60.4		
	M11R2	122	50.8	51.4	50.6	52.9	42.5	45.7	41.4	
RED	1R2	98	46.9	48.6	38.9	35.9	44.0			
	2R2	98	48.0	53.1	36.8	32.9*	40.6	50.0	57.9	
	4R3	78	41.0	42.6	54.4	43.1	46.0	39.4		
	5R2	131	55.7	56.4	46.5	48.5	59.8	51.5		
	6R2	132	53.0	41.8	34.2	43.2	48.6			
	7R2	137	44.5	48.6	43.0	49.6	44.4	45.1	42.9	
	8R2	112	49.1	41.4	36.1	52.4	39.3	50.0		
	9R1	134	53.7	50.0	50.6	46.7	48.6	53.6	44.8	
	10R2	109	39.4	44.8	46.3	45.2	34.8*			
	11R1	142	43.7	27.3**	42.4	46.1	41.2	50.6	35.1*	
	12R1	113	49.6	49.3	58.6	43.4	48.3	45.9	38.2	
	13R3	107	45.8	40.9	57.1	43.6	48.8	48.0	40.5	
	14R1	107	36.6*	39.4	51.5	45.4	47.0	51.1	46.0	
Rockefeller	R1R3	68	60.3	48.5	51.1	50.4	56.2	53.9	47.5	
	R2R3	124	43.5	49.3	50.0	41.9	43.3	51.9	49.3	
	R3R2	127	46.5	43.0	40.8	56.2	45.4	55.6	45.4	
	R4R1	125	52.0	60.1	50.6	45.2	49.1	50.0		
	R5R2	104	41.3	16.7**	27.0***	19.3***	44.7	31.9**	8.3***	
	R6R1	45	35.6	25.6***	15.5***	22.5***	28.6***	41.5	28***	
	R7R2	135	47.4	58.8	50.8	47.5	48.5	50.8		
	R8R1	117	41.0	37.9	38.5	52.4	51.8	26.7***	38.8*	
	R9R1	65	60.0	61.1	48.5	44.6	46.5	34.5**	33.3**	
	R10R2	119	52.9	48.0	37.7*	42.4	38.2	40.6		
	R11R3	29	34.5	51.2	45.3	30.6***	38.3	36.3*		
	R12R3	103	40.8	36.7	51.5	44.4	60.4	57.7		
	R14R2	130	53.1	37.4	58.9	49.1	50.0	59.1		
	R15R3	132	47.0	53.3	39.0*	36.6*	49.7	55.2	50.5	
	R16R1	64	45.3	45.7	38.7	38.0	39.3*	35.0**	49.3	
Trinidad	T1R	20	50.0	42.3	56.6	50.3	29.7**	31.4**		
	T5R	43	38.6	30.7***	38.5*	43.0	31.2***	39.2*	33.0**	
	T8R	56	51.8	21.9***	15.9***	21.3***	41.1	18.6***	17.3***	
	T9R	96	35.4*	20.5***	22.0***	12.8***	15.5***			
	T11R	80	40.0	50.0	52.9	55.3	54.3	54.4	57.1	
	T15R	125	56.0	47.4	40.7	25.7***	37.9	24.1***	38.2	
	T20R	100	65.0*	41.5	43.7	33.8*	33.6**	45.3	53.8	

(Continued on next page)

Table 1. ***(Continued)***

Strain	Male ID	F_1		F_2 families (% female)						
		n	% female	1	2	3	4	5	6	7
	T23R	92	50.0	12.6***	39.2	25.2***	16.8***	25.7***	19.0***	
	T25R	83	37.3	38.5	42.6	19.4***	60.0	50.0	46.5	
	T29R	79	44.3	31.3**	46.9	40.5	45.8			
	T30R	67	35.8	50.8	43.6	47.4	44.2	54.7		
	T35R	61	45.9	25.0***	19.4***	20.4***	24.7***	37.3*		
	T37R	135	36.1*	6.5***	23.2***	3.5***	29.5***	16.0***	18.8***	9.9***
	T40R	87	54.0	48.2	46.8	49.0	50.0			

[a] RF = Akomadan and RA = Fante. $*P < 0.01$, $**P < 0.001$, $***P < 0.0001$ for 1:1 expected sex ratio.

gene originated from the Trinidad population (Wood 1976).

We have successfully selected for a stable strain (T37) that carries the M^D gene from progeny resulting from pair-wise mating between one Trinidad male and one Trinidad female. Progeny from T37 strain males mated with RED strain females consistently reflect sex ratio bias with a mean of only 14.7% female progeny. As previously shown regarding the effects of M^D (Hastings and Wood 1978, Youngson et al. 1981), fecundity remained high in all crosses involving T37 strain males; for example, we obtained a mean of 108.7 adult progeny per F_2 family examined in Table 2 (data not shown).

Development of the T37 strain provides an excellent resource for efforts to characterize molecularly the M^D gene and its target $m^{s/i}$ locus. In addition, ongoing efforts to determine the complete genome sequence for *Ae. aegypti* (Severson et al. 2004) and advances in the characterization of the *D. melanogaster SD* system (Kusano et al. 2003) should greatly benefit this effort. The *SD* gene has been identified as a mutated *RanGAP* gene, which functions as a GTPase activator for Ran, a protein critical to nuclear transport (Merrill et al. 1999, Kusano et al. 2003). The *Rsp* locus has been shown to be a simple 120-bp AT-rich repeated array with the degree of sensitivity to drive correlated with an increase in copy number (Wu et al. 1988, Pimpinelli and Dimitri 1989). *SD* causes affected sperm to be dysfunctional, apparently because of a failure of chromatin condensation. Although the exact mechanism is unknown, it has been suggested that disruption of nuclear transport by mutant RanGAP could interfere with developmental changes associated with sperm maturation, such as histone replacement or transition through cell cycle check-points (Kusano et al. 2003). With *Ae. aegypti*, M^D causes chromosome breakage during meiosis among developing sperm carrying the m^s allele (Newton et al. 1976, 1978). The possibility exists, therefore, that meiotic drive in *Ae. aegypti* may also be caused by mutations in genes associated with the Ran signaling pathway. Anticipated complete genome sequence for *Ae. aegypti* will allow for the identification of Ran pathway genes that are found to reside near the sex locus and their subsequent evaluation as candidates for the M^D locus.

Although we are assuming that the T37 strain carries an M^D gene that is representative of the Trinidad population as well as other geographic regions, there may be allelic variants that differ in drive strength segregating in *Ae. aegypti* populations. It is also possible that other drive genes that are independent of M^D could exist and remain to be discovered. The T37 strain provides the opportunity to begin investigating these possibilities. The impact of a strong M^D gene is also influenced by the degree of sensitivity associated with the $m^{s/i}$ locus, because sensitivity varies considerably among *Ae. aegypti* populations (Hickey and Craig 1966a, Wood 1976, Owusu-Daaku et al. 1997, Suguna et al. 1977). By determining the molecular basis for the M^D phenotype, we may be able to design

Table 2. Sex ratios observed among crosses with the Trinidad T37 male and T37 colony

Cross		F_1		% females in F_2 families (total n)[a]						
		n	% female	1	2	3	4	5	6	7
T37 male × Trinidad female		131	38.3[b]							
	MaleID									
T37 colony male × RED female	T37R2	111	45	5.0 (119)	17.5 (154)	22.7 (128)	20.6 (68)	31.8 (132)	5.8 (104)	2.0 (150)
	T37R3	107	37.4[c]	6.7 (45)	7.4 (136)	13.6 (88)	8.9 (180)	11.2 (151)	5.6 (161)	26.7 (101)
	T37R4	nd		0.8 (124)	22.6 (137)	18.1 (144)	4.8 (124)	27.9 (140)	16.4 (159)	
	T37R5	nd		16.5 (109)	24.4 (119)	15.5 (103)	0.0 (56)	10.1 (109)	34.4 (32)[b]	9.3 (129)
	T37R6	100	51	21.7 (143)	6.7 (105)	11.0 (118)	16.5 (115)	11.0 (146)	23.0 (100)	
	T37R7	63	38.1	5.8 (52)	1.2 (82)	11.5 (148)	1.8 (114)	3.3 (123)	7.8 (102)	5.3 (132)
	T37R8	52	59.6	39.5 (86)[b]	17.6 (68)	12.7 (102)	11.1 (90)	20.2 (84)	13.3 (128)	21.6 (97)

nd, no data.
[a] All families showed significant sex ratio distortion ($P < 0.0001$) except where indicated.
[b] $P > 0.01$.
[c] $P < 0.01$.

synthetic gene constructs with enhanced drive potential or to identify other candidate drive genes based on the determination of the exact drive mechanism.

While efforts to address the potential use of the M^D system for population replacement are dependent on its complete molecular characterization, we can envision two mechanistic strategies for its implementation. One strategy would be to couple a desired transgene with a drive insensitive m^i allele or the direct replacement of the m^i allele with a transgene through a yet to be elucidated nonhomologous recombination mechanism. Controlled releases of males fixed for the transgene construct on their female-determining chromosome and carrying a strong M^D gene would initially result in a reduction of females in field populations. However, because of strong purifying selection, females homozygous for insensitivity to drive and carrying the transgene would increase in number, progressively replacing sensitive individuals. The populations would eventually re-establish an approximate 1:1 sex ratio, but would carry the transgene construct at high frequencies. Another strategy would be to couple a desired transgene directly with the M^D gene. Because the native M^D system already functions in *trans*, the resulting construct could be stably inserted into one of the two *Ae. aegypti* autosomes. Controlled releases of males fixed for insensitivity to drive would result in the progressive replacement of both males and females carrying sensitive alleles (e.g., no sex ratio distortion would be observed). An obvious limitation of either strategy is that the native populations would need to be highly sensitive to the effects of M^D. This could involve natural sensitivity, or based on results from molecular characterizations, it may be possible to engineer an M^D gene that affects most or all native $m^{s/i}$ alleles.

While our present focus is on identifying the M^D gene and its associated genome factors, efforts to employ population replacement strategies for *Ae. aegypti*–transmitted diseases should also consider the natural population dynamics of the M^D system at the micro- and macro-levels as well as among laboratory strains used to develop stable transgenic lines destined for field release. That is, success of a released individual carrying a transgene of interest would be negatively impacted if they carried a susceptible m^s allele, and the M^D gene was segregating in the target field population. Negative selection effects of M^D could counteract positive selection effects of other drive systems that have been proposed as tools for population replacement, particularly if the preferred drive system is linked with the m^s allele. Indeed, the M^D gene and the m^i allele have been shown to be present nearly worldwide, but are not uniformly distributed (Wood and Newton 1991). Understanding the basis for this distribution is important to proposed genetic control strategies, because one might consider, for example, why M^D is so frequent in Trinidad compared with other geographic areas.

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References Cited

Adelman, Z. N., N. Jasinskiene, and A. A. James. 2002. Development and applications of transgenesis in the yellow fever mosquito, *Aedes aegypti.* Mol. Biochem. Parasitol. 121: 1–10.

Asman, S. M., P. T. McDonald, and T. Prout. 1981. Field studies of genetic control systems for mosquitoes. Annu. Rev. Entomol. 26: 289–318.

Blair, C. D., Z. N. Adelman, and K. E. Olson. 2000. Molecular strategies for interrupting arthropod-borne virus transmission by mosquitoes. Clin. Micro. Rev. 13: 651–661.

Braig, H. R., and G. Yan. 2001. The spread of genetic constructs in natural insect populations, pp. 251–314. *In* D. K. Letourneau and B. E. Burrows (eds.), Genetically engineered organisms: assessing environmental and human health effects, CRC Press, Boca Raton, FL.

Charlesworth, B., and D. L. Hartl. 1978. Population dynamics of the segregation distorter polymorphism of *Drosophila melanogaster.* Genetics. 89: 171–192.

Collins, F. H., and A. A. James. 1996. Genetic modification of mosquitoes. Sci. Med. 3: 52–61.

Curtis, C. F. 1985. Genetic control of insect pests: growth industry or lead balloon? Biol. J. Linn. Soc. 26: 359–374.

Gilchrist, B. M., and J.B.S. Haldane. 1947. Sex linkage and sex determination in a mosquito, *Culex molestus.* Hereditas. 33: 175–190.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science. 156: 477–488.

Hartl, D. L., Y. Hiraizumi, and J. F. Crow. 1967. Evidence for sperm dysfunction as the mechanism of segregation distortion in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. U.S.A. 58: 2240–2245.

Hastings, R. J., and R. J. Wood. 1978. Meiotic drive at the $D(M^D)$ locus and fertility in the mosquito, *Aedes aegypti.* Genetica. 49: 159–163.

Hickey, W. A., and G. B. Craig, Jr. 1966a. Genetic distortion of sex ratio in a mosquito, *Aedes aegypti.* Genetics. 53: 1177–1196.

Hickey, W. A., and G. B. Craig, Jr. 1966b. Distortion of sex ratio in populations of *Aedes aegypti.* Can. J. Genet. Cytol. 8: 260–278.

Hiraizumi, Y., and K. Nakazima. 1967. Deviant sex ratio associated with segregation distortion in *Drosophila melanogaster.* Genetics. 55: 681–697.

Knipling, E. F., H. Laven, G. B. Craig, R. Pal, J. B. Kitzmiller, C. N. Smith, and A.W.A. Brown. 1968. Genetic control of insects of public health importance. Bull. Wld. Hlth. Org. 38: 421–438.

Kusano, A., C. Staber, H.Y.E. Chan, and B. Ganetzky. 2003. Closing the (Ran)GAP on segregation distortion in *Drosophila.* BioEssays. 25: 108–115.

Lyttle, T. W. 1991. Segregation distorters. Annu. Rev. Genet. 25: 511–557.

Lyttle, T. W. 1993. Cheaters sometimes prosper: distortion of mendelian segregation by meiotic drive. Trends Genet. 9: 205–210.

Merrill, C., L. Bayraktaroglu, A. Kusano, and B. Ganetzky. 1999. Truncated RanGAP encoded by the Segregation

Distorter mutation in *Drosophila.* Science. 283: 1742–1745.

Moreira, L. A., A. K. Ghosh, E. G. Abraham, and M. Jacobs-Lorena. 2002. Genetic transformation of mosquitoes: a quest for malaria control. Int. J. Parasitol. 32: 1599–1605.

Newton, M. E., R. J. Wood, and D. I. Southern. 1976. A cytogenetic analysis of meiotic drive in the mosquito, *Aedes aegypti* (L.). Genetica. 46: 297–318.

Newton, M. E., R. J. Wood, and D. I. Southern. 1978. Cytological mapping of the *M* and *D* loci in the mosquito, *Aedes aegypti* (L.). Genetica. 48: 137–143.

Owusu-Daaku, K. O., R. J. Wood, and R. D. Butler. 1997. Selected lines of *Aedes aegypti* with persistently distorted sex ratios. Heredity. 79: 388–393.

Palopoli, M. F., and C.-I. Wu. 1996. Rapid evolution of a coadapted gene complex: evidence from the *Segregation distorter (SD)* system of meiotic drive in *Drosophila melanogaster.* Genetics. 143: 1675–1688.

Pimpinelli, S., and P. Dimitri. 1989. Cytogenetic analysis of Segregation Distortion in *Drosophila melanogaster:* the cytological organization of the Responder (*Rsp*) locus. Genetics. 121: 765–772.

Robinson, A. S., G. Franz, and P. W. Atlkinson. 2004. Insect transgenesis and its potential role in agriculture and human health. Insect Biochem. Mol. Biol. 34: 113–120.

Sandler, L., and K. Golic. 1985. Segregation distortion in *Droshophila.* Trends Genet. 1: 181–185.

Severson, D. W., D. L. Knudson, M. B. Soares, and B. J. 2004. Loftus. *Aedes aegypti* genomics. Insect Biochem. Mol. Biol. 34: 715–721.

Suguna, S. G., R. J. Wood, C. F. Curtis, A. Whitelaw, and S. J. Kazmi. 1977. Resistance to meiotic drive at the M^D locus in an Indian wild population of *Aedes aegypti.* Genet. Res. 29: 123–132.

Sweeny, T. L., and A. R. Barr. 1978. Sex ratio distortion caused by meiotic drive in a mosquito, *Culex pipiens* L. Genetics. 88: 427–446.

Taylor, D. R., and P. K. Ingvarsson. 2003. Common features of segregation distortion in plants and animals. Genetica. 117: 27–35.

Temin, R. G., and M. Marthas. 1984. Factors influencing the effect of segregation distortion in natural populations of *Drosophila melanogaster.* Genetics. 107: 375–393.

Temin, R. G., B. Ganetzky, P. A. Powers, T. W. Lyttle, S. Pimpinelli, P. Dimitri, C.-I. Wu, and Y. Hiraizumi. 1991. Segregation distortion in *Droshophila melanogaster:* genetic and molecular analyses. Am. Nat. 137: 287–331.

Wood, R. J. 1976. Between-family variation in sex ratio in the Trinidad (T-30) strain of *Aedes aegypti* (L.) indicating differences in sensitivity to the meiotic drive gene M^D. Genetica. 46: 345–361.

Wood, R. J., and N. A. Ouda. 1987. The genetic basis of resistance and sensitivity to the meiotic drive gene *D* in the mosquito *Aedes aegypti* L. Genetica. 72: 69–79.

Wood, R. J., and M. E. Newton. 1991. Sex-ratio distortion caused by meiotic drive in mosquitoes. Am. Nat. 137: 379–391.

Wu, C. I., T. W. Lyttle, M. L. Wu, and G. F. Lin. 1988. Association between DNA satellite sequences and the Responder of Segregation Distorter in *D. melanogaster.* Cell. 54: 179–189.

Youngson, J.H.A.M., H. M. Welsh, and R. J. Wood. 1981. Meiotic drive at the $D(M^D)$ locus and fertility in the mosquito, *Aedes aegypti* (L.). Genetica. 54: 335–340.

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