Am. J. Trop. Med. Hyg., 75(1), 2006, pp. 70–77

POPULATION DYNAMICS OF AN ENDOGENOUS MEIOTIC DRIVE SYSTEM IN *AEDES AEGYPTI* IN TRINIDAD

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Abstract. An endogenous meiotic drive system was previously reported to be segregating in the yellow fever mosquito *Aedes aegypti* L. (Diptera: Culicidae) population in Trinidad. The meiotic driver (M^D) is tightly linked to the male determining locus and selectively targets sensitive responders linked to the female determining allele, causing fragmentation of female gametes. This results in highly male-biased progeny. The M^D system was initially studied as a genetic tool for population control with limited success, but recently interest has focused on its potential for population replacement. This study examines the distribution and dynamics of the M^D system in Trinidad natural populations. We obtained ovitrap samples from seven geographically distinct regions and determined the allele frequencies of the driver (M^D) and sensitive (m^s) versus insensitive (m^i) responders, respectively. Frequencies of the M^D allele ranged from 0.1 to 0.5 and were low at the two major port cities, Port of Spain and San Fernando, suggesting the effects of frequent immigration by non-driving genotypes. Frequencies of the m^i allele ranged from 0.4 to 0.7, suggesting the effects of strong selection by the driver. In addition, our results show that the driver and sensitivity of responders in the Trinidad populations are highly polymorphic. Continued studies of the dynamics of the M^D system in natural populations are critical to considerations of its use in population replacement.

INTRODUCTION

The remarkable advances in contemporary arthropod genomics offer incredible opportunities to develop and evaluate novel concepts for arthropod-borne disease control, including the genetic manipulation of the arthropod vector to render it incompetent to host and subsequently transmit the pathogen.[1] Implicit in efforts to develop strategies to replace highly competent arthropod vector populations with individuals carrying stable anti-pathogen effector genes is the need to identify and develop a genetic system that facilitates a rapid selective sweep of the target transgene. One potential mechanism for promoting population replacement is through agents that cause distortion of Mendelian segregation by meiotic drive.[2] In these systems, the meiotic driver product blocks maturation of gametes bearing a sensitive responder locus. The driver gene and responder locus are located on opposite members of homologous chromosomes, respectively. Because driver-carrying chromosomes selectively destroy their homolog, they have the potential for rapidly increasing in frequency in natural populations and theoretically could be engineered to also carry anti-pathogen effector genes, thereby implement population replacement.

Aedes aegypti and *Culex pipiens* L. mosquito populations have been shown to carry an endogenous meiotic drive system that can distort meiosis in favor of particular gametes.[3,4] No sex chromosome dimorphism exists in these species; instead, sex is determined by a single autosomal gene or a small chromosome segment on chromosome 1.[5,6] The meiotic drive gene (D) in *Ae. aegypti* is tightly linked with the male determining allele (M), and the responder locus is tightly linked to the female determining allele (m) on the homologous chromosome.[7–9] Alleles at the responder locus can be sensitive (s) or insensitive (i) to the driver gene product. In males heterozygous for the driver and a sensitive responder (M^Dm^s), the product of the M^D gene acts in *trans* to cause fragmentation of the m^s-bearing gametes during spermatogenesis. Neither the driver gene linked to a female determining allele (m^D) nor the responder linked to the male determining allele (M^s) functions properly to produce the drive phenotype.[10] Although the molecular basis for the M^D system is unknown, the observed phenotype in *Ae. aegypti* is a highly male-biased sex ratio.

Previous studies of the M^D system in *Ae. aegypti* focused on investigating its potential as a novel mosquito control strategy.[8,11–13] Based on the concept that the release of a strong meiotic driver into drive sensitive populations should result in increasingly male-biased populations, it was hypothesized that these populations could be driven to extinction. Curtis and others[12] combined the meiotic drive system with double translocation heterozygotes (T1T3) to test for *Ae. aegypti* population suppression in field cage experiments, and successfully achieved population eradication. Hickey and Craig[8] performed cage experiments designed to test the effect of the meiotic driver for genetic control of *Ae. aegypti*. Cage populations were manipulated to introduce strong driver males into sensitive female cage populations. Although male-biased sex ratios were observed for 10 generations, sex ratio distortion declined beginning at the F_3 generation and eventually reverted to a 1:1 ratio by the F_{11} generation. However, when they duplicated these experiments with a stronger drive strain and the same sensitive strain, they obtained population extinction after 43 weeks. To explain the reversion of sex ratio distortion in the former experiment, Hickey and Craig suggested the accumulation of drive suppressors in the female population,[7,8] and this was later supported by the identification of a *tolerance* gene in the RED strain by Wood and Ouda.[14] In addition to suppressors, driver insensitive responders exist in natural populations under non-driving conditions.[15] Therefore, selection for a drive suppressor or insensitive responder would inhibit population suppression by the meiotic driver. However, Wood and others[13] showed that the

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meiotic driver has strong population replacement potential. They introduced males containing the *red-eye* marker gene linked to a strong driver allele into sensitive black eyed mosquito populations. Although the *red-eye* marker alleles showed some unexpected tendencies for increasing, the M^D alleles showed strong allele replacement potential. By the F_{15} generation, the driver population consisted of 62% red-eyed mosquitoes. Recently, cage studies have shown that the M^D system can effect significant targeted allele replacement when introduced into sensitive populations.[16]

In conjunction with efforts to determine the feasibility of meiotic drive as a population replacement strategy, it is important to understand the population dynamics of the drive system in natural *Ae. aegypti* populations. Hickey and Craig[7] reported varying degrees of the strength of the meiotic driver and sensitivity of its responder in the *Ae. aegypti* population in Trinidad. In addition, Wood[17] categorized responder sensitivities in the T30 strain, derived from the Trinidad field population, into six groups based on the sex ratios. In previous studies, genetic polymorphism in the M^D system was apparent in a laboratory strain (WART).[7] Therefore, the dynamics of the M^D system in *Ae. aegypti* populations will be determined largely by the strength of the driver alleles, variability in sensitivity of the responder alleles, and their relative frequencies.

The existence of a strong meiotic driver within the *Ae. aegypti* Trinidad population has repeatedly been confirmed since the 1950s.[7,8,12,17–20] Trinidad is, therefore, one of the most ideal places to study the population dynamics of the *Ae. aegypti* meiotic drive system. The *Ae. aegypti* populations throughout the Caribbean experienced a significant genetic bottleneck in the early 1960s because of an intensive vector control program, largely through the widespread usage of DDT under the direction of the Pan American Health Organization (PAHO). By 1962, 18 continental countries and several Caribbean island countries had successfully achieved eradication.[21] Although the program officially ended in 1970 in the United States, after 1962, the number of *Ae. aegypti*–free countries declined quickly as rapid re-infestation by *Ae. aegypti* was reported.[22] However, after the end of the PAHO program, the *Ae. aegypti* eradication program in Trinidad and Tobago continued from 1976 to 1981 under the direction of the Ministry of Health.[23] Interestingly, presence of the meiotic driver was reported before and after the population bottleneck and still exists at a relatively high frequency in Trinidad natural populations.[8,18,19] To study the distribution and dynamics of the meiotic drive system in Trinidad, we selected seven locations and set up ovitraps for sampling *Ae. aegypti* populations. To determine drive genotypes, we followed the general mating scheme outlined by Hickey and Craig.[7] In our test crosses, observed sex ratios indicated the individual genotypes and an estimation of the driver strength or the responder sensitivities. Allele frequencies of the driver and responder were determined and compared among all seven Trinidad locations.

MATERIALS AND METHODS

Collection of samples. Seven geographically distinct locations in Trinidad were selected for sampling *Ae. aegypti* (Figure 1). During February to April 2004, 10–20 ovitraps[24] were

FIGURE 1. Map of Trinidad showing locations selected for sampling *Ae. aegypti* populations.

distributed at each location. Two ovitraps were set at each house: one inside and the other outside. Ovitraps consisted of a black plastic cup (400-mL volume) with ≈250 mL of water, into which a 12.5 × 2.5-cm hardboard paddle was placed in an upright manner. Female *Ae. aegypti* mosquitoes will readily oviposit on the paddle near the water interface.[25] After 7 days, the paddles were collected and dried for transport to the laboratory at the University of Notre Dame. Ovitraps were maintained for a period sufficient to ensure that eggs were obtained from at least 10 paddles per location.

Rearing and genetic crosses. Each egg paddle was placed into a separate container for hatching and rearing into adults. Ten paddles per study site were selected and hatched. Rearing was conducted in an environmental chamber held at 26°C, 84% relative humidity, and a 16-hour light/8-hour dark cycle with a 1-hour crepuscular period at the beginning and end of each cycle. Larvae were reared on a bovine liver powder suspension, and adults were supplied with cotton balls soaked in 5% sugar solution. Female mosquitoes were blood fed on anesthetized rats. 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. Animals are maintained and cared for in the Freimann Life Science Center, an AAALAC accredited facility.

Crosses to identify genotypes at the meiotic driver locus and its responder were prepared generally following the mat-

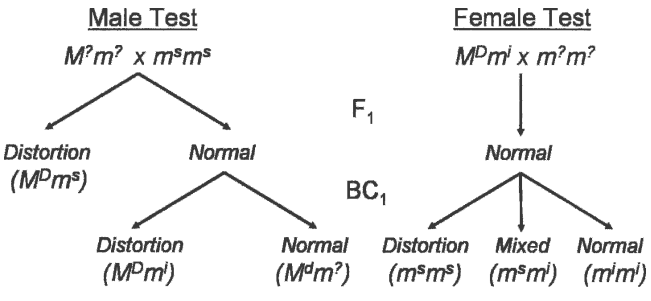

FIGURE 2. Test cross strategy for identifying males carrying the meiotic driver and sensitivity to drive of females. M^D = driver allele; M^d = non-driver allele; M^s = sensitive responder allele; m^i = insensitive responder allele. Modified after Hickey and Craig.[7]

TABLE 1
Observed sex ratios and inferred genotypes at the drive gene locus in male test crosses

Location	Male no.	F_1		BC_1 families (% female)						Genotype
		n	Percent female	1	2	3	4	5	6	
Port of Spain	1	114	51.75	49.14	46.72	49.81	50.00	56.06	48.16	$M^dm^?$
	2	202	48.02	53.02	47.74	55.49	44.50	52.67	61.39	$M^dm^?$
	3	318	48.74	41.89	44.12	40.48	44.59	44.26	43.75	$M^dm^?$
	4	336	45.83	56.94	52.46	46.64	50.39	47.80	47.68	$M^dm^?$
	5	148	51.35	22.99‡	19.76‡	13.73‡	15.5‡	15.67‡	24.32‡	M^Dm^i
	6	186	40.32	51.30	53.33	56.54	43.98	46.64	45.95	$M^dm^?$
	7	67	46.27	44.95	56.31	45.26	48.60	56.25	55.47	$M^dm^?$
	8	184	46.20	57.89	44.59	41.47	54.20	41.76	54.71	$M^dm^?$
	9	181	44.20	15.71‡	6.52‡	10.00‡	14.58‡	6.19‡	16.67‡	M^Dm^i
	10	235	40.85	43.68	47.71	57.30	41.71	54.93	60.61	$M^dm^?$
Curepe	1	408	43.63	30.11‡	35.94†	19.05‡	16.84‡	37.35†	36.22‡	$M^dm^?$
	2	342	50.29	54.42	47.96	49.48	44.94	45.97	52.12	$M^dm^?$
	3	128	55.47	38.89	47.78	42.96	50.00	48.30	44.03	$M^dm^?$
	4	440	46.59	40.65*	50.65	46.87	44.14	50.49	47.95	$M^dm^?$
	5	509	48.13	7.46‡	8.50‡	29.63‡	11.63‡	9.52‡	42.51	M^Dm^i
	6	387	45.74	54.46	47.76	54.35	45.06	52.31	51.02	$M^dm^?$
	7	152	57.24	12.24‡	15.09‡	5.76‡	17.76‡	51.07	17.22‡	M^Dm^i
	8	294	39.80*	15.09‡	31.00†	36.09*	37.21	40.32	34.67†	M^Dm^s
	9	193	49.74	55.56	56.59	51.59	54.95	52.38	44.62	$M^dm^?$
	10	253	46.64	42.70	11.76‡	40.00	16.42‡	30.77*	10.64‡	M^Dm^i
Valencia	1	549	49.54	43.05	48.87	45.74	46.44	51.20	47.20	$M^dm^?$
	2	346	49.42	21.81‡	26.06‡	34.04	26.20‡	30.48‡	16.74‡	M^Dm^i
	3	309	51.46	56.11	47.57	50.00	48.42	45.00	43.81	$M^dm^?$
	4	296	51.35	44.92	47.30	44.58	38.58	48.39	56.18	$M^dm^?$
	5	132	52.27	53.06	54.93	52.10	49.15	67.74	54.62	$M^dm^?$
	6	110	45.45	48.33	49.25	46.32	44.09	51.45	48.29	$M^dm^?$
	7	179	47.49	49.25	47.06	54.48	40.24	48.84	59.42	$M^dm^?$
	8	241	54.36	48.25	47.22	54.17	51.30	44.30	57.14	$M^dm^?$
	9	322	50.93	44.09	49.54	49.04	47.17	51.02	47.2	$M^dm^?$
	10	171	40.94	28.89*	21.15‡	18.37‡	25.15‡	14.29‡	30.85†	M^Dm^i
Matura	1	60	61.67	12.21‡	27.94‡	16.72‡	20.18‡	25.57‡	26.33‡	M^Dm^i
	2	24	45.83	47.26	47.83	40.00	42.04	47.45	42.86	$M^dm^?$
	3	210	49.05	45.92	43.24	55.87	40.70	49.40	46.81	$M^dm^?$
	4	493	48.98	36.21†	29.50‡	47.01	36.67†	41.96	25.68‡	M^Dm^i
	5	120	42.50	53.54	47.37	46.35	42.50	48.41	41.43	$M^dm^?$
	6	124	42.74	50.33	50.00	48.47	43.68	44.91	46.05	$M^dm^?$
	7	215	46.98	44.51	47.83	46.67	47.18	48.15	49.47	$M^dm^?$
	8	342	45.32	34.52†	40.00	31.40†	24.55‡	34.62†	39.47	M^Dm^i
	9	301	47.51	22.92‡	38.46	29.76†	38.06*	45.57	29.41‡	M^Dm^i
	10	122	52.46	43.02	50.61	35.71*	44.63	48.84	46.30	$M^dm^?$
San Fernando	1	165	40.00	6.36‡	40.46*	11.76‡	27.37‡	9.21‡	30.94‡	M^Dm^i
	2	189	48.15	33.33	48.00	41.59	44.57	41.67	44.63	$M^dm^?$
	3	95	33.68	38.62	23.79‡	30.67‡	26‡	27.86‡	15.65‡	M^Dm^s
	4	106	43.40	48.89	49.28‡	44.24	50.32	50.00	53.61	$M^dm^?$
	5	90	45.56	41.30	51.72	50.94	62.50	45.19	47.25	$M^dm^?$
	6	152	44.08	47.37	56.19	57.89	50.00	54.04	41.34	$M^dm^?$
	7	51	33.33	52.50	47.62	41.38	48.86	43.97	45.05	$M^dm^?$
	8	281	52.31	49.25	51.76	43.24	45.31	51.72	51.72	$M^dm^?$
	9	36	44.44	53.30	40.32	50.00	45.61	46.15	54.72	$M^dm^?$
	10	121	47.93	40.00	40.91	38.71	44.55	49.16	35.48	$M^dm^?$
Fyzabad	1	120	55.00	48.11	35.48*	52.00	46.84	41.67	50.00	$M^dm^?$
	2	139	48.92	36.36	45.45	37.21	48.39	50.00	63.64	$M^dm^?$
	3	97	55.67	27.54†	34.74*	41.82	50.00	44.20	50.91	$M^dm^?$
	4	107	51.40	37.82*	43.02	40.63	33.33	61.90	38.98	$M^dm^?$
	5	130	36.92*	36.09*	50.30	45.10	49.52	51.16	45.15	$M^dm^?$
	6	166	38.55*	9.40‡	13.33‡	2.06‡	17.14‡	6.73‡	7.06‡	M^Dm^s
	7	278	48.92	41.53	50.36	46.46	49.24	49.25	48.04	$M^dm^?$
	8	136	50.74	47.92	38.02	22.15‡	41.82	39.13	46.71	$M^dm^?$
	9	155	51.61	50.00	50.00	41.76	34.78	48.84	50.00	$M^dm^?$
	10	177	46.89	12.79‡	18.28‡	12.50‡	14.86‡	4.35‡	31.40‡	M^Dm^i

ing scheme outlined by Hickey and Craig.[7] Genetic stocks for this experiment were the *Ae. aegypti* T37 and RED strains. The T37 strain carries a strong meiotic driver and insensitive responder.[19] T37 strain males (genotype M^Dm^i) were used to test field-collected females for their responder genotype. The RED strain carries a highly sensitive responder.[8,19] RED strain females (genotype m^sm^s) were used to test field-collected males for their driver genotype. With progeny from each egg paddle, two test crosses were set up: 1) a field male and five RED strain females and 2) a T37 strain male and five

TABLE 1
Continued

Location	Male no.	F_1		BC_1 families (% female)						Genotype
		n	Percent female	1	2	3	4	5	6	
Mayaro	1	90	42.22	55.00	40.00	46.15	45.83	45.71	44.44	$M^dm^?$
	2	164	51.22	45.83	57.98	49.62	55.36	39.34	58.39	$M^dm^?$
	3	176	53.98	52.63	53.85	43.71	50.56	41.67	43.23	$M^dm^?$
	4	69	44.93	12.00‡	33.93	24.56‡	15.29‡	32.14	20.69‡	M^Dm^i
	5	59	38.98	37.50	37.74	38.61	34.78*	32.35†	36.11	$M^dm^?$
	6	119	47.90	39.22	34.38	33.83†	32.04†	41.46	39.47	M^dm^i
	7	138	51.45	46.77	58.00	59.09	55.21	43.90	57.69	$M^dm^?$
	8	114	54.39	55.66	48.94	52.53	49.30	51.68	50.78	$M^dm^?$
	9	243	49.38	51.64	42.31	45.12	46.94	43.90	55.26	$M^dm^?$
	10	131	50.38	37.29	34.72*	39.47	54.17	44.63	40.82	$M^dm^?$

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ for 0.535:0.465 expected sex ratio. Significance reported only for male bias.

field females. Each test cross was replicated five times to ensure successful mating and oviposition by at least one pair for the male and female test crosses, respectively, for each paddle. From these, one F_1 family was randomly selected to prepare seven BC_1 families by pairwise mating of one male and three RED females. After mating and blood feeding, females from all crosses were transferred individually to small glass vials containing ≈ 1 mL H_2O and a paper towel strip for oviposition. After oviposition, the egg papers were dried and stored at 16°C until hatched.

Sex ratio and genotyping. Sex ratios observed among progeny from the male test and female test crosses were used to assess genotypes at the driver locus as outlined in Figure 2. Departures from the expected male to female ratio were determined using the χ^2 test.[26] Because *Ae. aegypti* crosses generally reflect a slight bias for male progeny under non-driving conditions and could show significance at the $P = 0.05$ level if tested for an expected 1:1 sex ratio,[8] we elected to test for an expected 0.535:0.465 male to female ratio based on the previously observed sex ratios among progeny from matings within the RED strain.[19] For each field-collected male, sex ratios were determined for five F_1 and six BC_1 families. A male-biased sex ratio in the F_1 indicated that the male was M^Dm^s genotype. A normal sex ratio in the F_1 and male biased sex ratio in the BC_1 indicated that the male was M^Dm^i genotype. No sex ratio distortion in the F_1 or BC_1 indicated the male carried a non-driving allele (M^d). The number of males and females from the five F_1 families were summed to determine the sex ratio. If less than four of six BC_1 families showed a significant departure from a 1:1 sex ratio, the male was classified as a non-driver. Field-collected females were genotyped at the responder locus by examining sex ratios in the BC_1 generation. Females with the m^im^i genotype reflect 1:1 sex ratios in the BC_1 generation. Females with the m^sm^s genotype have male biased BC_1 families, whereas females with the m^sm^i genotype have a mixture of male biased and normal sex ratio BC_1 families.

Genotype data were converted into allele frequencies at the driver and responder loci for each of the seven sample locations. The mean sex ratios of the six BC_1 families were determined to show the strength of the drive alleles for each male test and the responder sensitivities for the female test, respectively. For heterozygous females, the mean sex ratio was determined from male-biased BC_1 families for the sensitive responder and the mean sex ratio from the 1:1 families for the insensitive responder, respectively.

RESULTS

Sex ratio and genotyping. Sex ratios and inferred genotypes at the meiotic driver locus, based on testing males reared from eggs collected at seven locations in Trinidad, are shown in Table 1. Genotypes were determined based on the assumption that male-biased sex ratios were caused by the meiotic drive gene products affecting random segregation of the gametes. Frequencies of the M^D allele ranged from 0.1 to 0.5 (Table 2), with the highest frequency observed in samples collected at Curepe. The mean allele frequencies of the meiotic driver and non-driver were 0.26 ± 0.14 and 0.74 ± 0.14, respectively.

Sex ratios and inferred genotypes at the responder locus are shown in Table 3. Frequencies of the m^s allele ranged from 0.3 to 0.6 (Table 2). The highest frequency was observed in samples collected at Fyzabad. Frequencies of the m^i allele ranged from 0.40 to 0.70. The highest frequency was observed in samples from Valencia. Heterozygous females were predominant at all the locations. The mean allele frequencies of the sensitive responder and insensitive responder were 0.42 ± 0.10 and 0.58 ± 0.10, respectively.

Observed frequencies of the M^D allele did not show significant correlation with observed frequencies of the m^i allele (r = 0.17), suggesting that the two loci are not at equilibrium within sites and, therefore, likely reflect the results of con-

TABLE 2
Observed allele frequencies of the meiotic drive system at each sample location in Trinidad

Location	Allele frequencies			
	Male		Female	
	M^D	M^d	m^s	m^i
Port of Spain	0.20	0.80	0.45	0.55
Curepe	0.50	0.50	0.40	0.60
Valencia	0.20	0.80	0.30	0.70
Matura	0.40	0.60	0.40	0.60
San Fernando	0.20	0.80	0.35	0.65
Fyzabad	0.20	0.80	0.60	0.40
Mayaro	0.10	0.90	0.45	0.55
Mean	0.26	0.74	0.42	0.58

TABLE 3
Observed sex ratios and inferred genotypes at the responder locus in female test crosses

Location	Female no.	BC_1 families (% female)						Genotype
		1	2	3	4	5	6	
Port of Spain	1	41.50	51.47	41.51	14.29‡	34.04	47.14	m^sm^i
	2	51.02	48.85	50.18	49.03	39.34	41.55	m^im^i
	3	55.71	6.90‡	53.06	42.86	36.67	53.93	m^sm^i
	4	33.93	38.62	34.41*	39.44	43.90	37.14	m^sm^i
	5	39.02	53.05	33.83‡	47.04	49.59	47.95	m^sm^i
	6	64.78	41.04	39.82*	43.68†	30.15‡	48.58	m^sm^i
	7	37.68*	50.59	41.72	33.59†	52.12	27.07‡	m^sm^i
	8	23.33‡	46.15	34.95‡	24.73‡	60.18	49.76	m^sm^i
	9	34.18*	47.06	44.90	50.39	27.27†	56.80	m^sm^i
	10	19.51‡	45.70	26.28†	46.43	47.06	41.83	m^sm^i
Curepe	1	50	41.18	46.15	38.1	44.44	44.44	m^im^i
	2	46.67	50.00	25.76‡	53.85	43.9	41.84	m^sm^i
	3	37.76	47.41	37.63*	44.62	50.72	47.50	m^sm^i
	4	43.40	43.36	41.91	51.11	36.51*	39.89	m^sm^i
	5	35.48	51.98	45.33	49.49	42.11†	37.50	m^im^i
	6	26.19†	50.82	44.15	11.11‡	35.42†	24.75*‡	m^sm^i
	7	45.83	41.73	37.86	53.47	51.72	29.63	m^sm^i
	8	50.00	43.08	36.76*	54.55	55.74	40.48	m^sm^i
	9	47.83	51.08	33.95†	43.46	43.32	44.23	m^sm^i
	10	25.71*	36.36	41.43	44.12	43.96	34.78	m^sm^i
Valencia	1	54.88	52.10	43.66	45.83	44.37	51.53	m^im^i
	2	38.79*	50.84	51.20	46.58	52.55	44.17	m^sm^i
	3	32.14*	45.95	13.73‡	9.52‡	44.44	21.15‡	m^sm^i
	4	45.86	13.13‡	48.28	40.43	46.06	51.58	m^sm^i
	5	53.41	48.67	33.75**	43.81	48.15	46.13	m^sm^i
	6	41.09	43.93	52.00	38.24*	54.17	45.62	m^sm^i
	7	43.33	36.00	48.87	50.11	51.85	46.80	m^im^i
	8	51.46	51.15	47.54	48.91	51.03	52.90	m^im^i
	9	45.71	42.47	43.48	45.33	46.26	37.63	m^im^i
	10	45.11	46.92	38.89	35.71*	31.71*	35.04*	m^sm^i
Matura	1	40.59	49.32	48.46	44.57	45.53	45.24	m^im^i
	2	46.67	40.54	34.92	44.12	36.59	40.00	m^im^i
	3	43.46	47.87	59.01	50.37	50.66	43.84	m^im^i
	4	36.11	49.69	43.55	35.96*	35.36†	40.64	m^sm^i
	5	36.76†	46.84	50.00	45.99	36.1‡	45.49	m^sm^i
	6	20.43‡	43.66	51.39	46.15	21.28‡	37.82	m^sm^i
	7	17.41‡	31.67*	39.42*	23.87‡	22.73‡	26.59‡	m^sm^s
	8	28.57‡	42.02	38.46	42.86	48.39	36.13*	m^sm^i
	9	45.90	48.66	33.97†	45.49	44.36	41.29	m^sm^i
	10	55.10	41.53	28.00†	50.00	32.76*	12.50‡	m^sm^i
San Fernando	1	28.57	37.04	46.61	45.45	44.29	37.21	m^im^i
	2	3.45‡	39.47	31.25	29.12‡	42.11	53.33	m^sm^i
	3	33.33	51.28	62.96	50.00	47.93	46.67	m^im^i
	4	48.53	49.61	48.08	40.00	40.74	30.91*	m^sm^i
	5	50.00	40.58	41.49	38.95	51.39	30.43‡	m^sm^i
	6	37.69*	44.10	49.15	53.21	54.00	56.03	m^sm^i
	7	8.33‡	19.44	43.64	25.81*	22.81‡	39.01	m^sm^i
	8	39.86	30.13‡	45.64	19.90‡	15.06‡	41.03	m^sm^i
	9	29.41*	48.48	16.67*	20.83‡	46.67	57.89	m^sm^i
	10	53.10	34.85	44.74	45.07	39.13	32.50	m^im^i
Fyzabad	1	31.18‡	30.30‡	10.67‡	15.63‡	20.59‡	24.10‡	m^sm^s
	2	33.33*	26.25‡	36.76	37.35	36.54*	33.33*	m^sm^i
	3	35*	28.57*	22.11‡	20.97‡	30.48‡	23.08‡	m^sm^s
	4	43.02	44.32	31.40‡	32.91‡	47.60‡	51.28	m^sm^i
	5	37.40*	44.44	40.37	44.44	43.53	45.00	m^sm^i
	6	30.23†	25.61‡	32‡	53.66	49.06	12.12‡	m^sm^i
	7	35.23†	28.98‡	34.72†	34.67‡	32.97‡	29.2‡	m^sm^s
	8	47.35	45.93	45.98	48.76	41.84	45.36	m^im^i
	9	22.31‡	45.37	43.04	20.83‡	32.23†	37.85*	m^sm^i
	10	28.36‡	25.49‡	46.15	32.08‡	40.72	40.58	m^sm^i

tinual migration within the island and immigration from outside sources caused largely by human activities.

Sex ratio distortion. The observed variations in sex ratio distortion by driver alleles carried by field-derived males are shown in Figure 3A. The frequency of a strong driver allele (< 12.5% female) was < 3% across the island. The mean BC_1 sex ratio associated with the M^D alleles was 25.5% female. The observed sex ratio distribution from testing field-derived females is shown in Figure 3B. Previously, Wood[17] classified responder loci into six groups according to their sensitivities

TABLE 3
Continued

Location	Female no.	BC_1 families (% female) 1	2	3	4	5	6	Genotype
Mayaro	1	44.20	39.84	0.00‡	39.50	34.84†	33.73*	$m^s m^i$
	2	46.32	46.02	50.35	56.95	46.70	22.86‡	$m^s m^i$
	3	35.00†	45.57	39.72	45.19	44.00	51.04	$m^s m^i$
	4	31.74‡	33.17‡	34.38†	32.47‡	25.68‡	36.32†	$m^s m^s$
	5	49.11	44.25	41.38	44.55	42.31	44.14	$m^i m^i$
	6	46.43	42.02	48.15	50.85	46.53	47.74	$m^i m^i$
	7	34.43†	39.06	46.61	46.99	50.52	41.01	$m^s m^i$
	8	51.7	32.79†	42.86	42.98	53.33	37.99†	$m^s m^i$
	9	36.72†	32.23†	25.6‡	37.37	42.06	40.88	$m^s m^i$
	10	44.87	39.16	40.68	25.90‡	18.18‡	4.76‡	$m^s m^i$

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ for 0.535:0.465 expected sex ratio. Significance reported only for male bias.

to the driver. Although our data do not fit this discrete classification very well, the observed frequency distribution of sex ratios is similar to results observed with the T30 strain. The frequency of a highly sensitive responder (< 12.5% female) was < 2.5% across the island. The mean BC_1 sex ratio associated with the m^s allele was 29.1% female.

DISCUSSION

The best-characterized meiotic drive system is the segregation distorter (*SD*) in *Drosophila melanogaster*.[27] With *D. melanogaster*, *SD* is found at frequencies of 1–5% in most natural populations.[28–31] Temin and Marthas examined the population dynamics of *SD* for 3 years in Madison, WI, and Sonoma County, CA,[30] and compared their data with a study conducted 25 years previously.[31] Their estimates for *SD* frequency across 3 years were consistently less than that observed in the earlier study 3%, indicating that a selective sweep of the *SD* gene through these populations had not occurred. Two hypotheses have been proposed to explain this phenomena including: 1) that populations challenged by *SD* also contain suppressors or insensitive responders in high frequencies, which prevent *SD* sweeps,[32–35] and 2) that the insensitive responders have significant fitness costs under nondriving conditions and that the low frequency of *SD* is caused by its short evolutionary history.[36,37] Although population frequencies of the insensitive responder (Rsp^i) are highly polymorphic, ranging from 3% to 86%, with mean values of 45%, Rsp^i frequencies are usually higher than the *SD* allele frequency in natural populations,[32,34,38–40] thus providing support for the first hypothesis.

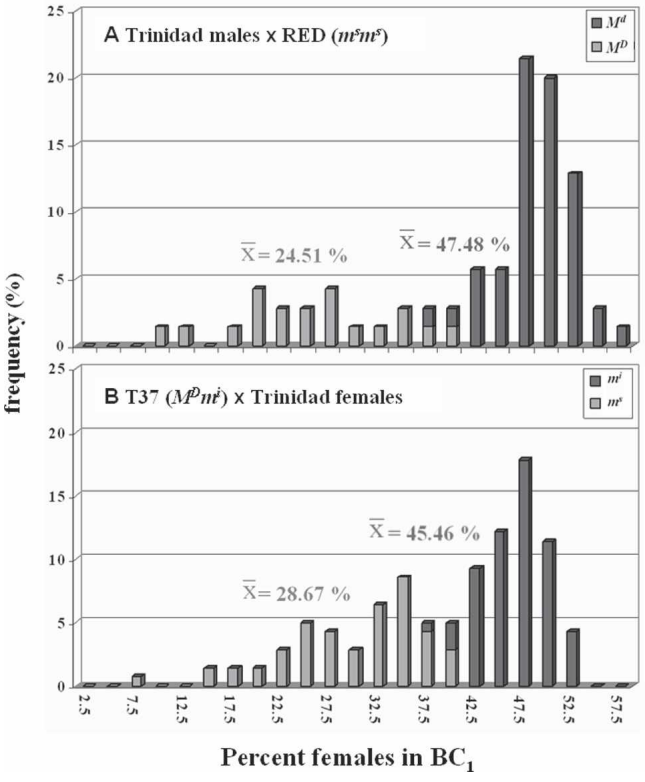

FIGURE 3. Distribution of percentage of females among the BC, progeny of single F_1 males derived from two test crosses. **A,** Trinidad males × RED females ($m^i m^i$), where sex ratios indicate the drive strength. **B,** T37 ($M^D m^i$) males × Trinidad females, where sex ratios indicate the responder sensitivities. Samples from all locations were combined. Mean sex ratios were determined and are shown for each allele.

Our studies of *Ae. aegypti* populations in Trinidad show similarity to that observed in *D. melanogaster* populations as the frequencies of insensitive responders were consistently higher than the frequencies of driver alleles. In addition, Suguna and Wood[41] reported the presence of insensitive responders after testing strong drive strains against drive-free natural populations in India. It also is evident that the degree of distortion observed in Trinidad field populations is lower than in test crosses using the sensitive RED strain. That is, the mean F_1 sex ratio of field-collected driving males carrying a sensitive responder ($M^D m^s$) was 37.34 ± 3.23% female, whereas their BC_1 mean sex ratio that reflects the RED strain responder sensitivity was 21.34 ± 12.08 female. This corresponds well with our previous studies.[19]

It has been suggested that the polymorphism observed in driver strength and responder sensitivities in natural populations is caused by balancing selection. Wood[17] reported between-family variation in sex ratios within the Trinidad-derived *Ae. aegypti* T30 strain and suggested that there were at least six different alleles with varying sensitivity at the responder locus. Our data also reflect considerable polymorphism in sex ratio between sib-families. For example, the Curepe no. 5 male in Table 1 showed highly polymorphic sex ratios ranging from 7.46% to 42.51% female in the BC_1 generation. In some cases, a number of our BC_1 families showed a mixture of distorted and 1:1 sex ratios, indicating the loss of

or suppression of driver effects. Because the observed within-male sex ratio polymorphisms were derived from single responder and driver alleles, our data suggest that there are other factors influencing the observed sex ratios, in addition to the responder sensitivities or driver strength in the Trinidad population. Wood and Ouda[14] also reported the existence of two driver suppressors and one enhancer within *A. aegypti* laboratory strains. Another factor maintaining balanced polymorphism in the Trinidad population could be recombination. Recombination between the male determining locus (*M*) and driver gene (*D*) would result in the loss of the drive allele in male-determining gametes. Indeed, a recombination frequency of 1.2% between *M* and *D* was reported for one of the strong meiotic driver strains (ACCRA).[17]

Our data show that both the strength of drivers and the sensitivity of responders in the Trinidad field populations are highly polymorphic (Figure 3). The distribution of the responder sensitivities in Trinidad populations (Figure 3B) is in contrast with previous data for an *Ae. aegypti* population in India, in which no driver allele was found.[41] The frequency of a highly sensitive responder (< 12.5% female) was much lower in the Trinidad population than the Indian population, which implies that the driver males are selecting against the sensitive responders. Our data confirmed previous studies of the meiotic drive system in the Trinidad *Ae. aegypti* population, which indicated relatively high frequencies of driver males[19] and varying degrees of responder sensitivities.[7,19] The mean frequency of driving males and insensitive responders was 0.26 and 0.58, respectively. The highest driver frequency was observed in the Curepe population. Similar, although slightly lower (0.43) frequencies in the Curepe population were reported previously.[17] Furthermore, our previous cage trials designed to test the impact of releasing males carrying a strong driver and insensitive responder into populations carrying sensitive responders showed strong selection for insensitive responder alleles.[16]

There was no obvious pattern to the observed distribution in the M^D system among all seven sampling locations. However, given the relatively small sample sizes at each location, our statistical power to evaluate differences among locations is limited. Furthermore, other studies have verified that considerable gene flow occurs among *Ae. aegypti* populations between Caribbean islands and the mainland and within Trinidad.[42–44] This implies that continuous migration through human activities likely impacts the population dynamics of the M^D system in Trinidad. However, while population genotypes at neutral marker loci were not determined for this study, we noted that an earlier study showed small, but significant, pairwise F_{ST} estimates for three locations in Trinidad; these included two locations from this study (Curepe and San Fernando), indicating that some population substructure exists within Trinidad populations, and therefore the observed inter-population differences in M^D frequency may be valid.[43] Interestingly, driver allele frequencies at the two major shipping port areas (Port of Spain and San Fernando) were among the lowest we observed, suggesting the likelihood that non-driving alleles may frequently be imported. In addition, repeated pesticide applications that result in periodic population bottlenecks may also be affecting the dynamics of the M^D system in Trinidad.[43]

Finally, the distribution of the M^D system among *Ae. aegypti* populations is not worldwide, and in some regions, the M^D allele has been reported as completely absent.[9,19] The high M^D allele frequencies that have consistently been observed in Trinidad have not been observed elsewhere. For example, previous examinations of five strains/populations including Trinidad indicated that, whereas four of the five carried the M^D allele, the frequency was low (0.13 maximum) in all but the Trinidad population (0.43).[19] The mean frequency across Trinidad in this study was lower (0.26), but still high compared with populations in the earlier study. Therefore, the meiotic drive approach does have potential for development as a novel population replacement strategy in drive sensitive populations. This is dependent on continued studies of the population dynamics of the drive system and its complete molecular characterization.

Received October 27, 2005. Accepted for publication March 13, 2006.

Financial support: This work was supported by NIH NIAID Grant PO1-AI45123.

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