Zoological Studies **43**(3): 622-628 (2004)

Zoological Studies

Sex Ratio Distortion in Hybrids of *Drosophila albomicans* and *D. nasuta*

Yung-Yu Yang[1], Fei-Jann Lin[2] and Hwei-yu Chang[1,2,*]

[1]*Department of Entomology, National Taiwan University, Taipei, Taiwan 106, R.O.C.*

[2]*Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan 115, R.O.C.*

(Accepted June 5, 2004)

Yung-Yu Yang, Fei-Jann Lin and Hwei-yu Chang (2004) Sex ratio distortion in hybrids of *Drosophila albomicans* and *D. nasuta*. *Zoological Studies* **43**(3): 622-628. A sex-ratio distorter in *Drosophila albomicans* was uncovered by the hybridization between Japanese Okinawa *D. albomicans* females and Indian *D. nasuta* males. The F_1 male from this cross produces female-biased offspring. The genic nature demonstrated in the present study suggests that meiotic drive instead of non-disjunction of the sex chromosomes during meiosis is the major cause for this. The meiotic driver was found to be located on the neo-X chromosome of *D. albomicans*, whose genome also contains drive suppressors, while that of *D. nasuta* is suppressor-free. In addition, hybrid F_1 and F_2 males were found to be semisterile probably due to an interaction between the 3rd and Y chromosomes of *D. nasuta* and the autosomes of *D. albomicans*. http://www.sinica.edu.tw/zool/zoolstud/43.3/622.pdf

Key words: *Drosophila albomicans*, *D. nasuta*, Meiotic drive, Sex-ratio distortion.

Most bisexual populations with an XY sex determination system have a sex ratio of around 1:1 (Bull 1983). However, some of these show distortions in the sex ratio. An X-linked meiotic driving mechanism was found to explain the sex-ratio distortion in natural populations of several *Drosophila* species, such as for *D. affinis* (Morgan et al. 1925), *D. obscura* (Gershenson 1928), *D. pseudoobscura*, *D. persimilis*, *D. athabasca*, *D. azteca* (Sturtevant and Dobzhansky 1936), and *D. subobscura* (Jungen 1968) in the subgenus *Sophophora*, and for *D. paramelanica* (Stalker 1961), *D. mediopunctata* (Carvalho et al. 1989), *D. quinaria*, and *D. testacea* (James and Jaenike 1990) in the subgenus *Drosophila*. Males bearing a driver X chromosome predominantly transmit X-bearing sperm, which results in only or mostly female progeny.

Several studies have theoretically demonstrated the possible existence of sex-ratio distorters, which are usually masked by fixed suppressors within populations (Frank 1991, Hurst and Pomiankowski, 1991). Hiraizumi et al. (1960) considered that if the preferential segregation of a meiotic driver is sufficiently strong, such an allele may become fixed in a population even if that is disadvantageous. Hence, they suggested that meiotic drive might be seen in hybrids between geographically distant populations of the same species, but they provided no real example from natural populations. Their prediction was supported by Mercot et al. (1995). A sex-ratio distorter was found with a high frequency in *D. simulans* strains from the Seychelles and New Caledonia. Its presence was indeed detected by crossing flies from different geographic regions. In those *D. simulans* strains, a driver on the X chromosome and a resistance factor on the Y chromosome that inhibits sex-ratio distortion were found (Mercot et al. 1995). In addition, in the process of studying the effects of interspecific introgressions from *D. sechellia* to *D. simulans*, Dermitzakis et al. (2000) found non-Mendelian segregation of sex chromosomes in hybrid males, suggesting that these introgression lines fail to suppress a normally hidden meiotic drive system.

When investigating the origin of reproductive isolation between *D. nasuta* and *D. albomicans*, Chang and Ayala (1989) discovered that the F_2 progeny of a hybrid cross between Japanese

*To whom correspondence and reprint requests should be addressed. E-mail: hwei@gate.sinica.edu.tw

Okinawa *D. albomicans* females and Indian *D. nasuta* males showed a low percentage (17%) of males. Yu et al. (1997) further demonstrated that 12 of 22 such hybrid strains retained obvious sex ratio distortions up to the 45th generation. The experiments described in this report were run to eliminate alternative possibilities, and results strongly suggested that a sex-ratio distorter caused the sex ratio distortion in hybrids of Japanese Okinawa *D. albomicans* females and Indian *D. nasuta* males. Since the distorter is suppressed in *D. albomicans*, the genetic components of this meiotic-drive system were explored by specifically designed crosses. In addition, the sterility of hybrids F_1 and F_2 supports the 3rd and Y chromosomes of *D. nasuta*, which comprise about 60% of the genome, possibly playing a major role in this sterility.

MATERIALS AND METHODS

Drosophila strains

Four *D. nasuta* strains from India (#193.7, #252.2, #252.7, and #252.8), 3 *D. albomicans* strains from Okinawa, Japan (#162.2, #162.4, and #163.5), and a tychoparthenogenetic *D. albomicans* strain from Kyushu, Japan (#300.62) were used in this study. The parthenogenetic strain had been maintained for 6 yr at Tokyo Metropolitan University and was still able to cross with *D. albomicans* males. All of these are isofemale strains, and they were reared in glass vials (3 cm in diameter, 10 cm in height) containing 2.5 cm (depth) of standard corn culture media, placed in growth chambers maintained at 22 ± 1°C and 75% relative humidity. Flies were sexed within 8 h after emergence and cultured for 3 d before use.

Karyotype assay

Karyotype assays were performed as previously described (Yu et al. 1997). In brief, 3rd-instar larvae were fed 0.02% colchicine in yeast paste for 1.5 to 2 h, and the brain ganglia were removed and soaked in a hypotonic solution (1% sodium citrate aqueous solution) for 6 m. Afterward, they were fixed in a methanol-acetic acid (3:1) solution for 1 h, and then transferred to 50 (l of 60% acetic acid. Cells were dispersed by pipetting the brain tissue up and down several times, and then dropping them onto a warm slide (50°C) for approximately 15 s. The solution was aspirated off. The air-dried slides were then stained with 5% Giemsa in phosphate buffer (pH 6.8) for 1 h, and were observed using an Olympus (Japan) BH2 microscope.

Esterase analysis

Electrophoresis of esterase was performed as previously described (Chang and Lin 1990). In brief, each individual fly was homogenized in 20 µl distilled water in a 1.5 ml Eppendorf tube. After centrifugation at 12 000 rpm for 5 min, 10 µl of the supernatant was mixed with 2 µl of a bromophenol blue-glycerol solution, and loaded into a well of a 7.5% polyacrylamide slab gel. The gel was run with Tris-glycine buffer (pH 8.3) at 4°C with a voltage of 150 V for about 2 h until the dye front reached the gel bottom. Esterase patterns were then visualized by the specific staining method described by Ayala et al. (1972).

EXPERIMENTAL DESIGN

Sex-ratio distortion

Crosses were performed between Okinawa *Drosophila albomicans* females and Indian *D. nasuta* males using the 9 possible combinations of 3 *nasuta* strains (#193.7, #252.2, and #252.7) with 3 *albomicans* strains (#162.2, #162.4, and #163.5). F_1 females were mated to their male siblings. For each of the 9 combinations, 3 replicas of 5 pairs of F_1 sib-matings were used, and the sex ratios of F_2 flies were then obtained. Only crosses that produced more than 40 F_2 offspring were taken into account. As controls, the sex ratios of *D. nasuta*, *D. albomicans*, and of hybrids from reciprocal crosses were also obtained with the same criterion described above. To clarify which F_1 sex is responsible for the sex-ratio distortion in F_2 progeny, F_1 females and males were backcrossed to both maternal and paternal strains.

Non-disjunction in F_1

Drosophila albomicans (#163.5) females were crossed with *D. nasuta* (#252.7) males, and the F_1 males and females were individually backcrossed to *D. nasuta* (the paternal strain). *D. nasuta* (#252.7) was used as a control. In order to determine whether the sex-ratio distortion was caused by non-disjunction of the sex chromosomes during meiosis of F_1, 3rd instar larvae of the F_2 progeny

were subjected to karyotype examination. *D. nasuta* (2n=8) and *D. albomicans* (2n=6) are morphologically indistinguishable, but with different fixed karyotypes. *D. nasuta* has separate 3rd autosomes and sex chromosomes, but these are fused in *D. albomicans*, forming 3-X and 3-Y sex chromosomes. This chromosome evolution was discussed in Yu et al. (1999).

Suppressor in *D. albomicans*

F_1 hybrid males from *Drosophila albomicans* females crossed with *D. nasuta* males were able to produce offspring with sex-ratio distortion, but no sex-ratio distortion was found in either *D. nasuta* or *D. albomicans* strains. It follows that *D. albomicans* may carry a 3-X chromosome with a meiotic driver allele termed *SR* and a non-sensitive 3-Y chromosome (3-X^{SR}/3-X^{SR} in females or 3-X^{SR}/3-Y in males). In addition, *D. albomicans* may carry a suppressor on the 2nd autosome.

To test this hypothetical autosomal suppressor, *D. albomicans* females were crossed with *D. nasuta* males. Their F_1 hybrid males were obtained and backcrossed with *D. albomicans* females, and then F_2 males were individually backcrossed with D. albomicans females. Electrophoretic analysis of esterase (Yu et al. 1997) was used to determine the type of 2nd chromosome pair in each male. Two kinds of F_2 males were expected: homozygous A/A ("A" indicates the 2nd autosome of *D. albomicans*) and heterozygous A/N ("N" is that of *D. nasuta*). If *D. albomicans* carries a recessive suppressor on its 2nd autosomes, A/A males should produce offspring with a normal sex ratio, while that of A/N males should be distorted. In contrast, if no suppressor is carried on the 2nd autosome, both A/A and A/N males will produce offspring with similarly distorted sex ratios. This experiment was conducted with 2 Japanese *D. albomicans* strains (#163.5 and #362.62) and 2 Indian *D. nasuta* strains (#193.7 and #252.8).

Sterility in F_1 and F_2 males

F_1 hybrid males were obtained from a cross between Okinawa *D. albomicans* (#163.5) females and Indian *D. nasuta* (#193.7) males, and each male was backcrossed with 3 *D. albomicans* (#163.5) virgin females. Each F_2 male was also backcrossed with 3 *D. albomicans* (#163.5) virgin females. The male was removed from the vial after 7 d, and the females were transferred to a new vial every 2 d until they died. The F_1 males were discarded after removal, but the F_2 males were checked electrophoretically to determine their esterase genotype (A/A or A/N). All offspring emerging from those vials were examined, and a male with less than 10 offspring was regarded as sterile. A reciprocal cross was also performed to determine the effect of the Y chromosome on the fertility of F_1 hybrid males. The fertility of both *D. nasuta* (#193.7) and *D. albomicans* (#163.5) was used as a control.

RESULTS

Sex-ratio distortion in F_2 hybrids

All 6 isofemale strains (i.e., 3 *Drosophila nasuta* and 3 *D. albomicans*) individually showed no differences by Chi-square homogeneity test, so the data were combined to reveal a consistent sex ratio of 1:1 (χ^2 = 0.36 and 0.45, *df* = 1, $p > 0.05$) (Table 1). The 9 data sets of sex ratios of F_2 obtained from sib-matings of F_1 from *D. nasuta* females and *D. albomicans* males were also homogeneous, and when combined, revealed a 1:1 ratio (χ^2 = 0.67, *df* = 1, $p > 0.05$), whereas a significant sex-ratio distortion was found in F_2 hybrids from sib-matings of F_1 from reciprocal crosses. Although the 9 data sets showed no difference by the Chi-square homogeneity test and thus they were combined as the previous 2 sets of data, the combination significantly deviated from a 1:1 ratio (χ^2 = 10.71, *df* = 1, $p < 0.01$) (Table 1). As shown in table 2, a considerable sex-ratio distortion was observed by backcrossing F_1 males with either the paternal or maternal strain. Data on the 18 F_1 male backcrosses were combined because of the homogeneity regardless of which strains of the original cross or the female mate were used; the F_1 female backcross data were likewise combined. Backcrossed F_1 males showed a significant sex-ratio distortion (χ^2 = 8.92, *df* = 1, $p < 0.01$), whereas F_1 females did not (χ^2 = 0.63, *df* = 1, $p > 0.05$) (Table 2). These results indicate several points: (1) the sex-ratio distortion was consistently observed in the F_2 offspring produced from crosses between any Indian and any Okinawa strain used in this study, and (2) the sex-ratio distortion caused by interspecific hybridization was unidirectional between an Okinawa *D. albomicans* female and an Indian *D. nasuta* male; and F_1 males, but not females, caused the sex-ratio distortion observed in the F_2

hybrids. The parthenogenetic *D. albomicans* (#300.62) also showed a significant F_2 sex-ratio distortion (41/153 = 0.27, χ^2 = 32.9, *df* = 1, p < 0.01) when crossed with *D. nasuta* strains.

Two cases (XXY) of sex chromosome non-disjunction were observed among the 57 F_2 larvae from backcrossing F_1 males with *D. nasuta* females, but not in progeny of F_1 females. The 2 cases of sex chromosome non-disjunction could only theoretically change the male/total value from 0.50 to 0.48, which was not statistically significant. Therefore, non-disjunction is unlikely to play an important role in the sex-ratio distortion in the present situation.

Meiotic drive suppression

Table 3 indicates that *D. albomicans* strains do not carry the suppressor of the sex-ratio distorter on their 2nd autosomes, because the offspring of all homozygous A/A males were significantly female-biased the same as were A/N males. Since the data obtained with the 300.62 and 252.8 strains were not normally distributed, the Mann-Whitney rank sum test was adopted and confirmed that the difference between A/A and A/N was not significant ($Z = 1.83 < Z_{0.05/2} = 1.96$).

Hybrid male sterility

The high sterility (8/17 = 0.471 for A/N and 10/13 = 0.769 for A/A) of F_2 males from the #163.5 and #193.7 cross suggests the existence of an interaction between the 2nd autosome of *D. albomicans* and the 3rd and Y chromosomes of *D. nasuta*. The sterility (26/118 = 0.22) of F_1 males from this cross was significantly higher than that of #193.7 *D. nasuta* males (5/89 = 0.056) (χ^2 = 10.81, *df* = 1, p < 0.01), about the same as that of #163.5 *D. albomicans* males (16/101= 0.158) (χ^2 = 1.35, *df* = 1, p > 0.05), but much lower than that of A/N F_2 males (χ^2 = 4.88, *df* = 1, p < 0.01). In addition, their sterility was also significantly higher than that of F_1 males from the reciprocal cross (a #193.7 female *D. nasuta* crossed with a #163.5 *D. albomicans* male: 2/90 = 0.022) (χ^2 = 17.21, *df* = 1, p < 0.001).

DISCUSSION

Sex-ratio distortion in interspecific F_2

Chang and Ayala (1989) showed that when an Okinawa *D. albomicans* female was crossed with an Indian *D. nasuta* male, the sex ratio of the F_2 hybrid offspring significantly deviated from the expected 1:1. This was confirmed in the present study by using different strains of Okinawa *D. albomicans* and Indian *D. nasuta* (Table 1). The "female-biased sex ratio in F_2 hybrids" should be a rule instead of an exception due to the particular strains used. Data in table 2 clearly show that F_1 males instead of females caused this distortion.

Table 1. Sex ratio (no. of males/total) of Okinawa *Drosophila albomicans*, Indian *D. nasuta strains*, and their hybrid F_2 from F_1 sib-mating[1]

	D. albomicans			*D. nasuta*		
♀ / ♂	162.2	162.4	163.5	193.7	252.2	252.7
162.2	0.50 ± 0.01 (389)	-	-	0.33 ± 0.02 (741)	0.34 ± 0.03 (638)	0.28 ± 0.01 (630)
162.4	-	0.47 ± 0.01 (609)	-	0.26 ± 0.03 (440)	0.29 ± 0.04 (595)	0.22 ± 0.04 (520)
163.5	-	-	0.50 ± 0.01 (588)	0.23 ± 0.04 (604)	0.24 ± 0.03 (598)	0.22 ± 0.05 (426)
193.7	0.45 ± 0.01 (613)	0.51 ± 0.03 (737)	0.45 ± 0.01 (466)	0.50 ± 0.03 (407)	-	-
252.2	0.47 ± 0.03 (365)	0.53 ± 0.04 (451)	0.50 ± 0.02 (287)	-	0.50 ± 0.01 (599)	-
252.7	0.48 ± 0.02 (728)	0.50 ± 0.02 (582)	0.50 ± 0.02 (350)	-	-	0.51 ± 0.02 (622)

[1]Data are presented as the average of 3 replicates ± SE, and total sample sizes are shown in parentheses.

There are several possibilities for the sex-ratio distortion of interspecific hybrids in *Drosophila*: (1) cytoplasmic incompatibility (Rousset et al. 1992), (2) differences in viability between sexes, (3) non-disjunction of the sex chromosome in meiosis, and (4) meiotic drive (Frank 1991, Hurst and Pomiankowski 1991). Cytoplasmic incompatibility can occur in crosses between populations or closely related species, and there is usually an incompatibility between the sperm and egg induced by endosymbiotic microorganisms such as *Wolbachia* that causes unisexual zygotic death (Jiggins et al. 2001). This possibility was rejected because the distortion should be observed in F_1 hybrids, but the F_1 sex ratio was normal in this case. Chang and Ayala (1989) ruled out the possibility of differential fitness due to abortion or poor viability of zygotes, as neither F_1 nor F_2 showed any differences in offspring production. Non-disjunction of the sex chromosome of F_1 during meiosis produces aneuploids (XXY and XO) in the F_2 generation, and if XXY functional gametes outnumber XO ones, more female offspring will be produced. Table 1 shows that on average, 27% of males in F_2, and 46% more females could not be explained by only about a 2% non-disjunction incidence. Therefore, the only plausible explanation left is meiotic drive.

Theoretically, a meiotic driver on the X chromosome creates the possibility for a female-biased sex ratio by the formation of more X gametes. Table 2 shows that F_1 males instead of females produced female-biased offspring, and this further indicates the possibility of an X-linked meiotic driver. Dermitzakis et al. (2000) provided the 1st example of a cryptic meiotic driver which was revealed in interspecific introgression. However, their experiments could not directly unmask a meiotic drive system in F_1 hybrid males, and the female-biased sex ratio was observed in recombinant inbred lines. Mercot et al. (1995) showed that de-suppression occurred in the F_1 from crosses between local populations of the same species. Here, a cryptic meiotic driver can be revealed by interspecific hybridization.

The spread of an X-linked driver is expected to elicit the evolution of suppressors. Since the meiotic driver works in F_1 males, the X-linked driver allele may be suppressed within a population either via a Y-linked dominant suppressor or by an autosomal recessive suppressor (Frank 1991, Hurst and Pomiankowski 1991). Y chromosomes

Table 2. F_2 sex ratios (no. of males/total) of F_1 backcrosses[1]

Origin of F_1	Sex ratio of the backcross offspring			
maternal x paternal	♂ F_1 backcross		♀ F_1 backcross	
	maternal	paternal	maternal	paternal
#162.2 x #193.7	0.34 ± 0.01 (285)	0.30 ± 0.04 (430)	0.49 ± 0.06 (557)	0.45 ± 0.02 (671)
x #252.2	0.33 ± 0.03 (479)	0.35 ± 0.03 (660)	0.49 ± 0.02 (459)	0.49 ± 0.05 (908)
x #252.7	0.35 ± 0.04 (340)	0.30 ± 0.05 (311)	0.48 ± 0.06 (592)	0.48 ± 0.05 (597)
#162.4 x #193.7	0.20 ± 0.06 (277)	0.21 ± 0.02 (307)	0.48 ± 0.05 (783)	0.50 ± 0.04 (685)
x #252.2	0.21 ± 0.05 (346)	0.26 ± 0.03 (480)	0.48 ± 0.03 (618)	0.49 ± 0.02 (473)
x #252.7	0.29 ± 0.10 (410)	0.24 ± 0.03 (461)	0.50 ± 0.01 (938)	0.45 ± 0.02 (741)
#163.5 x #193.7	0.22 ± 0.02 (227)	0.19 ± 0.05 (423)	0.50 ± 0.06 (652)	0.52 ± 0.02 (623)
x #252.2	0.26 ± 0.02 (272)	0.22 ± 0.04 (596)	0.48 ± 0.06 (483)	0.49 ± 0.05 (820)
x #252.7	0.27 ± 0.06 (266)	0.26 ± 0.04 (145)	0.46 ± 0.04 (433)	0.47 ± 0.02 (800)

[1]Data are presented as the average of 3 replicates ± SE, and total sample sizes are shown in parentheses.

bearing suppressors are selected at the individual level, because they are transmitted better than sensitive Y chromosomes by males that carry an X-linked driver. The existence of an autosomal suppressor is compatible with the operation of "Fisher's principle" in a species with an SR chromosome (Fisher 1930), i.e., when such a driver produces a distorted sex ratio within a species, natural selection will favor its suppression through the accumulation of autosomal modifier alleles that restore the sex ratio to normal. In fact, both autosomal and Y-linked suppressors for X-linked drivers have been found in natural populations of many species (*D. paramelanica*, *D. affinis*, *D. mediopunctata*, *D. simulans*, and *D. quinaria*) (Stalker 1961, Atlan et al. 1997). A meiotic-drive system which cannot be detected by sex-ratio distortion if a suppressor is present in the population, however, can be uncovered by interspecific hybridization.

In this study, after F_1 hybrid males were crossed with *D. albomicans* females, 1/2 of their F_2 male progeny were homozygous for the *D. albomicans* 2nd chromosome (A/A), and the other 1/2 were heterozygous (A/N). The possibility of a recessive suppressor on the 2nd autosome can be ruled out, because no difference was detected between those A/A and A/N males. Jaenike (1999) developed a model for maintenance of a Y chromosome polymorphism (both sensitive and resistant) in species polymorphic for an X-linked driver. Atlan et al. (1997) suggested that an isolated ecosystem might cause a high frequency of a distorter and complete Y-linked resistance (e.g., *D. simulans* populations in the Seychelles and New Caledonia). A sex-ratio distorter is common in Okinawa *D. albomicans*, but it is not expressed, due to the co-occurrence of suppressors at a high frequency. In *D. albomicans* strain #163.5, suppression of the driver may result from a Y-linked suppressor. In fact, all 7 *D. albomicans* strains collected from Okinawa showed the same sex-ratio distortion in F_2 as did #163.5 when crossed with *D. nasuta* strain #193.7 (Yang 2001). The high frequency of the distorter and suppressor in Okinawa *D. albomicans* populations may have been caused by drift in its initial founder population.

Table 3. Sex-ratio distribution and mean sex ratio of F_3 produced by F_2 males with specific 2nd chromosome compositions

F_3 sex-ratio rank	#163.5 (A♀) x #193.7 (N♂)		#300.62 (A♀) x #252.8(N♂)	
	A/A	A/N	A/A	A/N
0.0~0.2	0.77	0.59	0.29	0.5
0.2~0.4	0.23	0.35	0.21	0.05
0.4~0.6	0	0.06	0.41	0.33
0.6~0.8	0	0	0.09	0.10
0.8~1.0	0	0	0	0.02
Mean sex ratio	0.18	0.38	0.36	0.32
Std. error	0.068	0.086	0.058	0.102
Sample size	13	17	34	42

Hybrid male sterility

About 1/2 of the F_2 hybrid males were sterile. A tested male was given 3 virgin females, and was regarded as sterile if fewer than 10 offspring were produced. These males all carried the 3,Y chromosomes of *D. nasuta* and the 3-X chromosome of *D. albomicans*, but part of them had a combination of 2nd autosomes from both *D. nasuta* and *D. albomicans* (A/N), and the rest had two 2nd autosomes from *D. albomicans* (A/A). The sterility of the heterozygous (A/N) and homozygous (A/A) groups showed no significant difference ($\chi^2 = 2.7$, $df = 1$, $p > 0.05$). We suggest that the interaction of the 3,Y chromosomes of *D. nasuta* with the foreign genome may play a major role in this sterility, because F_1 males from a cross between *D. albomicans* females and *D. nasuta* males were also semisterile (0.221) compared to the low sterility of the 2 parental strains (0.056 for *D. nasuta* #193.7, and 0.158 for *D. albomicans* #163.5) and that of F_1 males from the reciprocal cross (0.022). The significantly higher sterility of F_2 males compared to F_1 males indicates some recessive element in the *D. albomicans* genome may be enhancing the sterility when homozygous.

The meiotic-drive models of hybrid sterility were criticized by Coyne and Orr (1993). Their main point was based on a prediction from Hurst and Pomiankowski (1991): if the sterility of heterogametic hybrids is produced by meiotic-drive alleles suppressed within 1 species but re-expressed in hybrids, then the meiotic drive may reappear in semisterile hybrids that should produce progeny with distorted sex ratios. But the absence of sex-ratio distortion goes against the meiotic-drive hypothesis for hybrid sterility (Coyne and Orr 1993). However, Tao et al. (2001) provided evidence for the sex-ratio distortion associated with a reduction in hybrid male fertility. They introgressed homozygous segments from the 3rd chromosome of *D. mauritiana* into the genome of its

sibling species *D. simulans*, and reported a conspicuous sex-ratio distortion as well as associated hybrid male sterility. Similarly, Orr and Presgraves (2000) suggested that genes causing hybrid sex-ratio distortion can be mapped to the same chromosomal intervals as those causing hybrid male sterility in subspecies of *D. pseudoobscura*. In other words, Orr and Presgraves also indirectly admitted the possibility of the meiotic-drive theory. Male sterility and sex-ratio distortion were simultaneously observed in our study, but they might not have the same genetic basis. The hybrid sterility resulted from genetic incompatibility rather than sex-ratio distortion.

Acknowledgments: We would like to thank Dr. Yoshiaki Fuyama (Tokyo Metropolitan Univ., Tokyo, Japan) for kindly sending us the parthenogenetic *Drosophila albomicans* strain (#300.62 in this paper). We thank Ms. Ling-Gin Liu and Jia-Chi Liu for preparing the experimental material. This work was supported by a grant (NSC89 -2313-B002-152) from the National Science Council of the R.O.C.

REFERENCES

Atlan A, H Mercot, C Landre, C Montchamp-Moreau. 1997. The Sex-Ratio trait in *Drosophila simulans*: geographical distribution of distortion and resistance. Evolution **51:** 1886-1895.

Ayala FJ, JR Powell, ML Tracey, CA Mourao, S Perez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. Genetics **70:** 113-139.

Bull JJ. 1983. Evolution of sex determining mechanisms. Menlo Park, CA: Benjamin/Cummings.

Carvalho AB, AA Peixoto, LB Klaczko. 1989. Sex-ratio in *Drosophila mediopunctata*. Heredity **62:** 425-428.

Chang H, FJ Ayala. 1989. On the origin of incipient reproductive isolation: the case of *Drosophila albomicans* and *D. nasuta*. Evolution **43:** 1614-1624.

Chang H, FJ Lin. 1990. On the polymorphic *Esterase-F* locus in *Drosophila albomicans*. Bull. Inst. Zool. Acad. Sinica **29:** 81-88.

Coyne JA, HA Orr. 1993. Further evidence against meiotic-drive models of hybrid sterility. Evolution **47:** 685-687.

Dermitzakis ET, JP Masly, HM Waldrip, AG Clark. 2000. Non-Mendelian segregation of sex chromosomes in interspecific *Drosophila* males. Genetics **154:** 687-694.

Fisher RA. 1930. The genetic theory of natural selection. Oxford, UK: Clarendon Press.

Frank SA. 1991. Divergence of meiotic drive suppression systems as an explanation for sex-biased hybrid sterility and inviability. Evolution **45:** 262-267.

Gershenson S. 1928. A new sex-ratio abnormality in *Drosophila obscura*. Genetics **13:** 488-507.

Hiraizumi Y, L Sandler, JF Crow. 1960. Meiotic drive in natural populations of *Drosophila melanogaster*. III. Population implications of the segregation distorter locus. Evolution **14:** 433-444.

Hurst LD, A Pomiankowski. 1991. Causes of sex ratio bias may account for unisexuality in hybrids: a new explanation of Haldane's rule and related phenomena. Genetics **128:** 841-858.

Jaenike J. 1999. Suppression of Sex-Ratio meiotic drive and the maintenance of Y-chromosome polymorphism in *Drosophila*. Evolution **53:** 164-174.

James AC, J Jaenike. 1990. "Sex Ratio" meiotic drive in *Drosophila testacea*. Genetics **126:** 651-656.

Jiggins FM, JK Bentley, MEN Majerus, GDD Hurst. 2001. How many species are infected with *Wolbachia*? Cryptic sex ratio distorters revealed to be common by intensive sampling. Proc. R. Society of London B Biol. Sci. **268:** 1123-1126.

Jungen H. 1968. *Sex-ratio* in naturlichen populationen von *Drosophila subobscura*. Arch. Jul Klaus-Stift. **43:** 52-57.

Mercot H, A Atlan, M Jacques, C Montcham-Moreau. 1995. Sex-ratio distortion in *Drosophila simulans*: co-occurrence of a meiotic drive and a suppressor of drive. J. Evol. Biol. **8:** 283-300.

Morgan TH, CB Bridges, T Sturtevant. 1925. The genetics of *Drosophila*. Bibliogr. Genet. **2:** 1-262.

Orr HA, D Presgraves. 2000. Speciation by postzygotic isolation: forces, genes and molecules. Bioessays **22:** 1085-1094.

Rousset F, D Bouchon, B Pintureau, P Juchault, M Solignac. 1922. Wolbachia endosymbionts responsible for various alternations of sexuality in arthropods. Proc. R. Soc. London **B250:** 91-98.

Stalker HD. 1961. The genetic systems modifying meiotic drive in *Drosophila paramelanica*. Genetics **46:** 177-202.

Sturtevant AH, TH Dobzhansky. 1936. Geographical distribution and cytology of *sex-ratio* in *Drosophila pseudoobscura* and related species. Genetics **21:** 473-490.

Tao Y, DL Hartl, CC Laurie. 2001. Sex ratio segregation distortion associated with reproductive isolation in *Drosophila*. PNAS **98:** 13183-13188.

Yang YY. 2001. The evolutionary genetics of *Drosophila albomicans*. PhD dissertation, Graduate Institute of Entomology, National Taiwan University, Taipei, Taiwan.

Yu YC, FJ Lin, HY Chang. 1997. Karyotype polymorphism in hybrid populations of *Drosophila nasuta* and *Drosophila albomicans*. Zool. Stud. **36:** 251-259.

Yu YC, FJ Lin, HY Chang. 1999. Stepwise chromosome evolution in *Drosophila albomicans*. Heredity **83:** 39-45.