# Polymorphism for *Y*-Linked Suppressors of *sex-ratio* in Two Natural Populations of *Drosophila mediopunctata*

**Antonio Bernardo Carvalho,* Suzana Casaccia Vaz* and Louis Bernard Klaczko†**

**Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil and †Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Caixa Postal 6109, CEP 13081/970, Campinas, Brazil*

Manuscript received August 9, 1996
Accepted for publication March 19, 1997

ABSTRACT

In several Drosophila species there is a trait known as "*sex-ratio*": males carrying certain *X* chromosomes (called "*SR*") produce female biased progenies due to *X-Y* meiotic drive. In *Drosophila mediopunctata* this trait has a variable expression due to *Y*-linked suppressors of *sex-ratio* expression, among other factors. There are two types of *Y* chromosomes (suppressor and nonsuppressor) and two types of *SR* chromosomes (suppressible and unsuppressible). *Sex-ratio* expression is suppressed in males with the $SR^{suppressible}/Y^{suppressor}$ genotype, whereas the remaining three genotypes produce female biased progenies. Now we have found that ~10–20% of the *Y* chromosomes from two natural populations 1500 km apart are suppressors of *sex-ratio* expression. Preliminary estimates indicate that $Y^{suppressor}$ has a meiotic drive advantage of 6% over $Y^{nonsuppressor}$. This *Y* polymorphism for a nonneutral trait is unexpected under current population genetics theory. We propose that this polymorphism is stabilized by an equilibrium between meiotic drive and natural selection, resulting from interactions in the population dynamics of *X* and *Y* alleles. Numerical simulations showed that this mechanism may stabilize nonneutral *Y* polymorphisms such as we have found in *D. mediopunctata*.

MEIOTIC drive is a violation of Mendel's first law in which a heterozygote produces unequal proportions of the two gametic types (SANDLER and NOVITSKY 1957; LYTTLE 1991). In Drosophila, meiotic drive of the *X* chromosome against the *Y* (yielding female-biased progenies) is known in several species and is called "*sex-ratio*" (GERSHENSON 1928; JAENIKE 1996). In most of these species the driver *X* chromosome (usually called "*SR*") carries specific chromosomal inversions.

Normal *X* chromosomes ("*ST*") are transmitted by males to half of their progenies (the daughters), whereas *SR* may achieve 100% transmission. Due to this meiotic drive advantage, *SR* chromosomes are expected to spread and become fixed, which might cause population extinction due to the lack of males (GERSHENSON 1928; HAMILTON 1967). However, stable *SR* polymorphisms are found in natural populations of several species (DOBZHANSKY 1958). For example, the frequency of *SR* chromosomes in the Itatiaia population of *D. mediopunctata* remained ~13% between 1987 and 1996 (CARVALHO *et al.* 1989; A. B. CARVALHO, S. C. VAZ and M. C. SAMPAIO, unpublished data), and molecular data indicate that the *SR* inversion of *D. pseudoobscura* is at least 700,000 years old (BABCOCK and ANDERSON 1996). This stability implies some form of natural selection against *SR*, which has been found in *D. pseudoobscura*, *D. recens* and *D. quinaria*, the sole species investigated in this respect (WALLACE 1948; CURTSINGER and FELDMAN 1980; BECKENBACH 1996; JAENIKE 1996). Furthermore, theoretical studies have shown that the conditions for the stabilization of *SR* polymorphisms by counteracting selection are not unduly restrictive (EDWARDS 1961; CURTSINGER and FELDMAN 1980; JAENIKE 1996). Thus, these polymorphisms probably result from an equilibrium between meiotic drive and natural selection (WALLACE 1948; CURTSINGER 1991).

Another stabilizing mechanism may be provided by autosomal or *Y*-linked modifier genes that suppress the meiotic drive (STALKER 1961; CARVALHO and KLACZKO 1994; VARANDAS *et al.* 1997). Autosomal suppressors of *sex-ratio* are expected to evolve in response to the spread of *SR* because they direct the reproduction to the rarer (and hence more fertile) sex, the males (HAMILTON 1967; WU 1983; VARANDAS *et al.* 1997). This mechanism of natural selection, known as Fisher's principle, is the most accepted explanation for the commonness of the 1:1 sexual proportion in nature (FISHER 1930; BULL and CHARNOV 1988; CONOVER and VOORHEES 1990; BASOLO 1994). Autosomal suppressors of *sex-ratio* compatible with Fisher's principle have been described in *D. mediopunctata* (CARVALHO and KLACZKO 1993; VARANDAS *et al.* 1997) and their occurrence has also been suggested in *D. paramelanica* (STALKER 1961).

The spread of *Y*-linked suppressors of *sex-ratio* in *SR*-bearing populations can be explained by meiotic drive

*Corresponding author:* Antonio Bernardo Carvalho, Departamento de Genética Instituto de Biologia, Universidade Federal do Rio de Janeiro, Caixa Postal 68011 CEP 21944-970, Rio de Janeiro, Brazil. E-mail: bernardo@acd.ufrj.br

theory: any *Y*-linked gene that increases the transmission rate of the *Y* chromosome is expected to spread (THOMSON and FELDMAN 1975). In the presence of a *SR* chromosome, a *Y*-linked suppressor of *sex-ratio* acts in this way because it is transmitted to half of the progeny (the males), whereas a normal *Y* is not transmitted at all (all progeny being female). Powerful *Y*-linked suppressors of *sex-ratio* have been described in *D. paramelanica* (STALKER 1961), *D. affinis* (VOELKER 1972) and *D. mediopunctata* (CARVALHO and KLACZKO 1994), but not in natural populations of *D. pseudoobscura,* despite specific search (POLICANSKY and DEMPSEY 1978; BECKENBACH *et al.* 1982). COBBS (1986, 1987) described in some strains of *D. pseudoobscura Y*-linked and autosomal genes that increase the frequency of sterile *X*/O males produced by *SR*/*Y* fathers but, due to this sterility, these genes have no evolutionary significance to the point discussed here.

An important finding from theoretical studies on the population genetics of the *Y* chromosome is that the conditions for stable selective polymorphisms in this chromosome are particularly restrictive (CLARK 1987a, 1990). This restrictiveness is due to the characteristics of the *Y* chromosome (haploid, patriclinous transmission), which exclude several "stabilizing" mechanisms (*e.g.,* heterosis and differential selection between males and females). Using constant fitness models, CLARK (1987a, 1990) showed that stable *Y* polymorphisms can be obtained only in models including both *X-Y* meiotic drive and natural selection (viability), and even then, only in 2% of the parameter space. The stabilization of *Y* polymorphisms also requires *X* polymorphism. As the stabilization of *Y* polymorphisms requires simultaneously many conditions, new *Y* mutants are expected to be lost or become fixed, unless they are neutral. Thus, these theoretical studies strongly suggest that extant *Y* polymorphisms are neutral, although frequency-dependent selection and more complex mechanisms such as equilibrium between selection and migration could stabilize nonneutral *Y* polymorphisms (CLARK 1987a, 1990).

Several cases of *Y* polymorphism in Drosophila, such as variation in chromosome size (DOBZHANSKY 1935), in the copy number of rDNA genes above a certain level (LYCKEGAARD and CLARK 1989) and in the sequence of rDNA introns, can be explained by neutrality (*i.e.,* equilibrium between mutation and random drift), a hypothesis that has been supported by experimental evidence for the case of rDNA introns (CLARK and LYCKEGAARD 1990). However, at least two Drosophila species are polymorphic for *Y*-linked suppressors of *sex-ratio: D. paramelanica* (STALKER 1961) and *D. mediopunctata* (CARVALHO and KLACZKO 1994). As these polymorphisms could hardly be neutral (because they strongly affect the transmission rate of *Y* chromosomes), they may be an interesting experimental model to study the population genetics of the *Y* chromosome. Two other cases of nonneutral *Y* polymorphisms are known: CLARK (1987b) found variation in the segregation ratios of *Y* chromosomes in *D. melanogaster,* and HOLLOCHER and TEMPLETON (1994) reported that the number of rDNA copies is related to the "*abnormal abdomen*" syndrome of *D. mercatorum* and affects several fitness components. A possible fifth case was described in the plant *Silene alba.* In this species there are strong evidences that *Y*-linked genes influence the sexual proportion, though it is not certain whether this is caused by deleterious viability effects or by meiotic drive (TAYLOR 1994).

In addition to their bearing on meiotic drive evolution and on the population genetics of *Y* chromosomes, *Y*-linked suppressors of *sex-ratio* may also be involved in the speciation process. The theory of *X-Y* meiotic drive predicts a rapid turnover of *X* chromosomes (driver *X* replacing nondriver *X*) and *Y* chromosomes (suppressor *Y* replacing nonsuppressor *Y;* HAMILTON 1967). This predicted fast evolution may explain the large role of sex chromosomes in the post-mating isolation between closely related species (COYNE and ORR 1989): isolated populations may evolve different and incompatible *X*-driver/*Y*-suppressors systems, leading to hybrid sterility/inviability (WU and HAMMER 1990; FRANK 1991; HURST and POMIANKOWSKI 1991; see also JOHNSON and WU 1992 and COYNE and ORR 1993 for contrary evidence).

*D. mediopunctata* belongs to the tripunctata group (FROTA-PESSOA 1954). In this species, *X* chromosomes with the *X:21* (or the rare *X:2*) gene arrangement are *SR* chromosomes; males carrying them usually produce female-biased progenies. However, several *SR*/*Y* males produce progenies containing roughly 50% of males in *D. mediopunctata* (CARVALHO *et al.* 1989). In previous investigations of the causes of this variability we have found a male age effect (CARVALHO and KLACZKO 1992), autosomal suppressors of *sex-ratio* expression (CARVALHO and KLACZKO 1993; VARANDAS *et al.* 1997) and *Y*-linked suppressors (CARVALHO and KLACZKO 1994). Since the sons of *SR*/*Y* males are fertile in this species, whether they come from unsuppressed fathers or from fathers suppressed by autosomal or *Y*-linked genes (CARVALHO 1989; and references cited above), these genes can have evolutionary significance.

The *Y*-linked suppression is quite complex: there are at least two types of *Y* chromosomes (suppressor and nonsuppressor) and two types of *SR* chromosomes (suppressible and unsuppressible). *Sex-ratio* expression is suppressed in males with the $SR^{suppressible}/Y^{suppressor}$ genotype, whereas the remaining three genotypes produce female-biased progenies (CARVALHO and KLACZKO 1994). The frequencies of the *Y* chromosome types in natural populations are unknown.

The purposes of the present study were as follows: (1) to estimate the frequencies of the two types of *Y* chromosomes in two natural populations of *D. mediopunctata,* (2) to search for an explanation for this non-

neutral *Y* polymorphism, and (3) to verify whether the two types of *Y* chromosomes previously detected are homogeneous or, alternatively, if there is a continuum between suppressor and nonsuppressor *Y* chromosomes.

## MATERIALS AND METHODS

***D. mediopunctata* strains:** All strains (except the "M" strains; see below) descend from flies collected at the Parque Nacional do Itatiaia (State of Rio de Janeiro, Brazil).

*ITA-24-P:* This is a strain homokaryotypic for the *Standard* (*ST*) gene arrangement of the *X* chromosome and with good productivity. Its *Y* chromosome ($Y^{ITA\text{-}24\text{-}P}$) is a reference nonsuppressor (CARVALHO and KLACZKO 1994).

*ITF-446, ITF-543 and ITE-407:* These strains were isolated in a previous study (CARVALHO and KLACZKO 1994) and carry reference suppressor *Y* chromosomes.

*NA:* Homokaryotypic for the *X:ST* gene arrangement and carrying visible markers on all autosomes, except the dot (chromosome VI): *Delta* (*Dl*, dominant, chromosome II), *Impar* (*Im*, dominant, III), *coral* (*cr*, recessive, IV) and *alfinete* (*al*, recessive, V; MARQUES *et al.* 1991; H. V. S. MARQUES and L. B. KLACZKO, unpublished results). The strain is heterozygous for *Dl* and *Im* due to their recessive lethality.

*NB:* This strain is homokaryotypic for a reference-suppressible *SR* isolated from the ITF-446 strain (CARVALHO and KLACZKO 1994) and homozygous for *coral* and *alfinete*. Its *Y* chromosome is suppressor. Crosses between NB and NA showed that these strains are free from strong autosomal suppressors of *sex-ratio* (CARVALHO and KLACZKO 1993).

*I strains:* Flies were collected in November 1988 at Parque Nacional do Itatiaia. Ten isofemale strains were randomly chosen and a single male from each one was crossed to ITA-24-P females to ensure "single *Y*" strains. The $F_1$ flies were used in the experiments.

*M strains:* Flies were collected in July 1990 at Morro Santana, a forest area near Porto Alegre (State of Rio Grande do Sul, Brazil) that is ~1500 km South-West to Itatiaia. Ten M strains were obtained through pair mating of the $F_1$ of 10 wild-caught females. The M strains and the I strains are a random sample of *Y* chromosomes from the two natural populations.

**Typing *Y* chromosomes from natural populations:** The general plan of the experiment is to type each *Y* chromosome from our samples by measuring *sex-ratio* expression in males carrying this unknown *Y* ($Y^?$) and a reference-suppressible *SR*. If these males produce female-biased progenies, then $Y^?$ is nonsuppressor; if they produce normal progenies (containing ~50% males), then $Y^?$ is suppressor. The difference between the two types of *Y* chromosomes is clear-cut (CARVALHO and KLACZKO 1994) but can be obscured by autosomal suppressors; an analogous problem interfered with STALKER's (1961) study of *D. paramelanica*. To avoid this problem and also to be able to detect more subtle differences *within* types we measured *sex-ratio* expression individually in many males (25–40) for each *Y* chromosome, controlling the autosomal background, cytoplasm, age (due to the male age effect) and environmental effects (see below). This approach limited the number of *Y* chromosomes sampled from each population.

Figure 1 presents the complete experimental design for a single $Y^?$. These procedures were repeated for each *Y* chromosome tested. From the cross between males carrying $Y^?$ and NA females we collected $F_1$ males with the Delta Impar phenotype. These males were then crossed to NB females in three replicas (named series 1, 2 and 3). The function of these replicas is to avoid common environment effect, which might mimic differences among *Y* chromosomes. These flies were transferred to fresh medium every 5 days. Up to this point all crosses were made in mass, with at least 18 pairs of flies per cross. Every 4 days we sorted and collected the tetra-mutant $F_2$ males produced by the later crosses. All of these $F_2$ males were $SR^{suppressible}/Y^?$ and had the same autosomal background derived from the NA and NB strains, since crossing over does not occur in *D. mediopunctata* males. To measure their level of *sex-ratio* expression, 13-day-old $F_2$ males (25–40 males, distributed among the three series) were each one crossed for 6 days with four or five ITA-24-P females. The flies were then transferred to half-pint bottles to ovoposit and after 15 days they were discarded. Liquid ferment was added to the cultures regularly to reduce competition and the progenies were sexed and counted until bottle exhaustion.

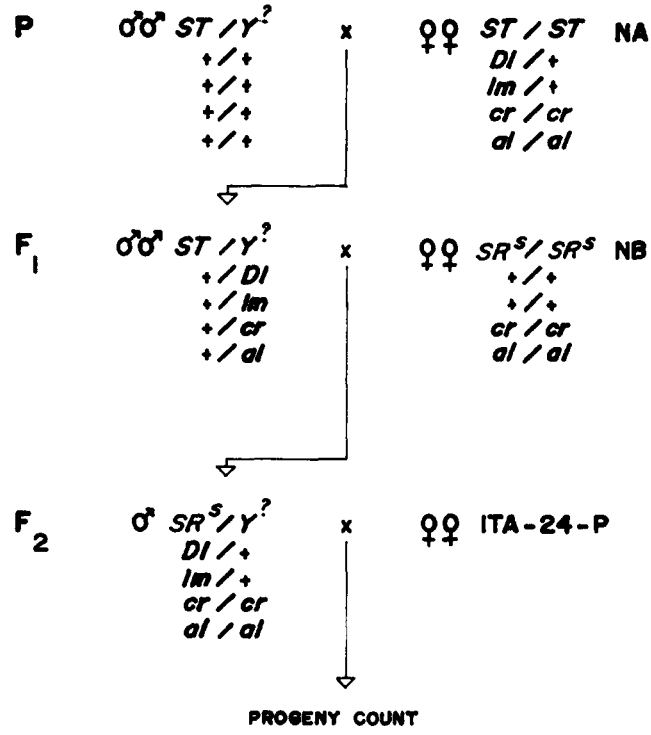


FIGURE 1.—Method of typing *Y* chromosomes. P and $F_1$ crosses were made in mass. $F_2$ males (25–40 per *Y* chromosome) were crossed individually with ITA-24-P females to measure their *sex-ratio* expression. $SR^s$ is the reference suppressible *SR* chromosome.

We made two experiments of this kind, experiment I (with Morro Santana *Y* chromosomes) and II (Itatiaia sample). Flies were reared at 16.5° in trimeveldon medium (CARVALHO *et al.* 1989).

**Statistical analysis:** The raw data, the proportion of males ($p$) in the progeny of each tetra-mutant male, were transformed to arc sin $\sqrt{p}$ before statistical analyses, which were performed with the software SYSTAT 5 for Windows (WILKINSON 1992). We considered only the cultures producing at least 20 flies.

## RESULTS

**Frequency of *Y* chromosome types in natural populations:** *Morro Santana population (experiment I):* We crossed 354 $SR^{suppressible}/Y^?$ tetra-mutant males (representing 11 different *Y* chromosomes) and we obtained 260 valid cultures (73% of success; with a mean of 72

**TABLE 1**

***Sex-ratio* expression in $F_2$ males carrying *Y* chromosomes from Morro Santana (experiment I)**

| *Y* chromosome | Percentage of males |
|---|---|
| M-27 | 6.9 ± 1.8 (25)[a] |
| M-12 | 7.8 ± 2.0 (27)[a] |
| M-252 | 10.0 ± 2.2 (22)[a] |
| ITA-24-P | 10.5 ± 1.8 (27)[a] |
| M-13 | 11.3 ± 2.0 (25)[a] |
| M-150 | 12.7 ± 3.4 (21)[a] |
| M-6 | 14.9 ± 3.9 (26)[a] |
| M-56 | 17.7 ± 3.6 (21)[a] |
| ITF-543 | 35.9 ± 2.5 (25)[b] |
| M-1 | 38.9 ± 2.2 (21)[b] |
| M-111 | 40.5 ± 1.8 (20)[b] |

Values are means ± SE with the number of $F_2$ males tested in parentheses. The superscripts *a* and *b* indicate the means that are not significantly different at the 0.05 level (Tukey-Kramer test, separate ANOVAs for suppressor and nonsuppressor *Y*; see *Variation within types*). The three series were pooled.

**TABLE 2**

***Sex-ratio* expression in $F_2$ males carrying *Y* chromosomes from Itatiaia (experiment II)**

| *Y* chromosome | Percentage of males |
|---|---|
| I-32 | 7.4 ± 2.0 (28)[a] |
| I-37 | 8.0 ± 1.3 (24)[a] |
| ITA-24-P | 8.1 ± 1.7 (36)[a] |
| I-67 | 9.7 ± 2.0 (28)[a] |
| I-51 | 11.2 ± 1.8 (28)[a] |
| I-84 | 12.2 ± 2.5 (23)[a] |
| I-20 | 12.6 ± 3.6 (20)[a] |
| I-23 | 13.4 ± 2.5 (27)[a] |
| I-77 | 16.4 ± 3.1 (30)[a] |
| I-35 | 20.5 ± 3.0 (30)[a] |
| ITF-543 | 35.2 ± 1.4 (39)[b] |
| ITF-446 | 42.7 ± 1.6 (38)[b,c] |
| ITE-407 | 44.2 ± 1.2 (31)[b,c] |
| I-83 | 45.6 ± 1.6 (28)[c] |

Values are means ± SE with the number of $F_2$ males tested in parentheses. The superscripts indicate the means that are not significantly different at the 0.05 level (Tukey-Kramer test, separate ANOVAs for suppressor and nonsuppressor *Y*; see *Variation within types*). The three series were pooled.

flies/culture). Table 1 presents the average percentage of males produced by these $SR^{suppressible}/Y^{?}$ males carrying nine *Y* chromosomes from the Morro Santana sample, plus $Y^{ITF\text{-}543}$ and $Y^{ITA\text{-}24\text{-}P}$ (reference suppressor and nonsuppressor *Y*, respectively). Means and confidence intervals are shown in Figure 2 (left). We analyzed the data with a nested ANOVA, nesting series within *Y* chromosomes. The effect of the *Y* chromosome was very significant ($F_{10,22} = 19.91$; $P < 10^{-6}$), but the series effect was not ($F_{22,227} = 1.04$; $P > 0.40$). The Tukey-Kramer multiple comparison test (SOKAL and ROHLF 1981) identified two internally homogeneous blocks of means (this homogeneity should be seen as a preliminary result; see *Variation within types*), corresponding to suppressor *Y* ($Y^{M\text{-}1}$, $Y^{M\text{-}111}$ and $Y^{ITF\text{-}543}$) and nonsuppressor *Y* (the remaining *Y* chromosomes). It should be noted that the confidence intervals of the means of suppressor and nonsuppressor *Y* did not overlap (Figure 2, left); the difference between types is clear-cut. Since another nonsuppressor *Y* ($Y^{M\text{-}1184}$) was identified in a preliminary experiment, two *Y* chromosomes in a sample of 10 from the Morro Santana population were suppressors of *sex-ratio* expression ($Y^{M\text{-}1}$ and $Y^{M\text{-}111}$) and the remaining eight were nonsuppressors.

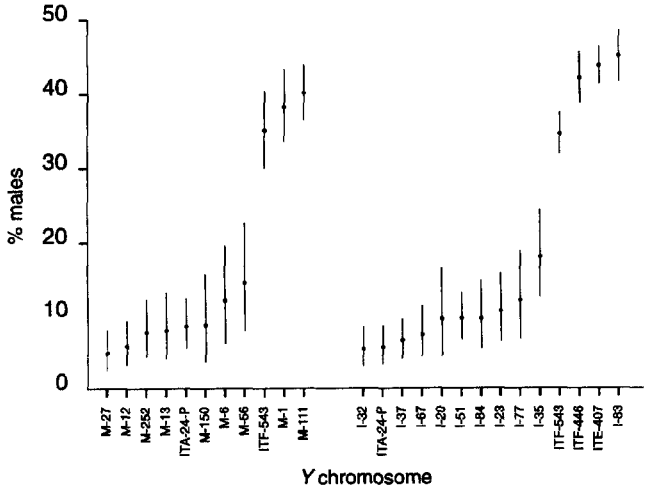


FIGURE 2.—Means and 95% confidence interval (experiment I, left; experiment II, right). Values were obtained from transformed data (arc sin $\sqrt{p}$) and transformed back to "% males."

*Itatiaia population (experiment II):* In this experiment we tested more males for each *Y* chromosome and used more reference genotypes. A total of 431 valid cultures were obtained from 480 crosses (90% of success, with a mean of 92 flies/culture). Table 2 shows the mean sexual proportion of the progenies produced by males carrying the 10 *Y* chromosomes from the Itatiaia sample, plus $Y^{ITF\text{-}543}$, $Y^{ITF\text{-}446}$, $Y^{ITE\text{-}407}$ (reference suppressors) and $Y^{ITA\text{-}24\text{-}P}$ (reference nonsuppressor). Means and confidence intervals are shown in Figure 2 (right). The effect of the *Y* chromosome was very significant ($F_{13,27} = 28.13$, $P < 10^{-6}$) and the effect of series was significant at the 5% level ($F_{27,369} = 1.55$, $P = 0.04$). As in experiment I, the Tukey-Kramer test identified two internally homogeneous blocks of means (but see *Variation within types*), corresponding to suppressor *Y* ($Y^{ITF\text{-}543}$, $Y^{ITF\text{-}446}$, $Y^{ITE\text{-}407}$ and $Y^{I\text{-}83}$) and nonsuppressor *Y* (the remaining *Y* chromosomes) and again there was no overlap in the confidence intervals of the two types of *Y* chromosomes (Figure 2, right). Thus, there were one suppressor *Y* ($Y^{I\text{-}83}$) and nine nonsuppressor *Y* in the Itatiaia sample.

**Variation within types:** To investigate the variation

**TABLE 3**

**ANOVA tables for suppressor and nonsuppressor *Y* chromosomes**

| | Morro Santana | | | | | | Itatiaia | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nonsuppressor | | | Suppressor | | | Nonsuppressor | | | Suppressor | | |
| | d.f. | MS | *F* | d.f. | MS | *F* | d.f. | MS | *F* | d.f. | MS | *F* |
| Among *Y* | 7 | 0.081 | 1.79 | 2 | 0.017 | 3.64 | 9 | 0.051 | 1.07 | 3 | 0.076 | 4.52* |
| Among series, within *Y* | 16 | 0.045 | 1.16 | 6 | 0.005 | 0.33 | 19 | 0.048 | 1.42 | 8 | 0.017 | 2.17* |
| Within series (error) | 170 | 0.039 | | 57 | 0.014 | | 245 | 0.034 | | 124 | 0.008 | |

*$P < 0.05$.

within types of *Y* it is safer to carry separate ANOVAs for the suppressor and nonsuppressor *Y* chromosomes identified above. This caution is necessary because the within-group variances of the nonsuppressor *Y* are always greater than the corresponding values of suppressor *Y* and multiple comparisons tests are not robust to the resulting heterogeneity of variances (SOKAL and ROHLF 1981). Thus we have used the analyses done in the previous section for the coarse separation between suppressor and nonsuppressor *Y* chromosomes and then applied separate ANOVAs to investigate within type variation.

Table 3 shows the ANOVAs for suppressor and nonsuppressor *Y*, for both experiments. Only suppressor *Y* chromosomes from Itatiaia were significantly heterogeneous ($P = 0.039$); the Tukey-Kramer test identified two overlapping blocks of means (Table 2) and inspection of Figure 2 strongly suggests that $Y^{ITE\text{-}543}$ is a weaker suppressor. We had already found in a preliminary experiment that $Y^{ITE\text{-}543}$ was a weaker suppressor than $Y^{ITE\text{-}407}$ (means: 31.5%, $n = 15$; 41.9%, $n = 13$, respectively; $t = 3.8$, $P = 0.001$) so this point is well settled. It should be noted that these reference *Y* chromosomes were also collected from the Itatiaia population; thus, the suppressor *Y* from this population were heterogeneous.

The nonsuppressor *Y* chromosomes from Itatiaia also seemed to be heterogeneous, since the difference between the two extremes ($Y^{I\text{-}32}$, 7.4% and $Y^{I\text{-}35}$, 20.5%; Table 2), thought nonsignificant, was suspiciously large. However, this difference disappeared when we tested again the two chromosomes with a larger sample ($Y^{I\text{-}32}$, 14.9%, $n = 58$ and $Y^{I\text{-}35}$, 15.8%, $n = 59$; $t = 0.46$, $P > 0.6$).

The series differed significantly in the suppressor *Y* chromosomes from Itatiaia ($P = 0.034$; Table 3), which suggests that environmental differences in the rearing conditions of the tetra-mutant *SR/Y* males (*e.g.*, density) may affect *sex-ratio* expression. The nested experimental design controlled for these environmental effects that might otherwise mimic differences among *Y* chromosomes.

## DISCUSSION

**Polymorphism for *Y*-linked suppressors:** There is a polymorphism in the *Y* chromosome of *D. mediopunctata*, ~10-20% of them are suppressors of *sex-ratio* expression in two natural populations 1500 km apart. This result was obtained with a very reliable experimental method that controlled all possible sources of error in *Y* classification (autosomal background, environmental effects and age). The *Y* polymorphism of *D. mediopunctata* raises interesting questions on meiotic drive evolution and on the population genetics of the *Y* chromosome.

The occurrence of *Y*-linked suppressors of *sex-ratio* in *SR*-bearing natural populations is expected on the grounds of meiotic drive theory because they are strongly favored. However, their polymorphic state is not easily explained, given the difficulty of stabilization of nonneutral *Y* polymorphisms (CLARK 1987a, 1990). To examine alternative hypotheses, such as that the polymorphism we found was neutral or transient, it is necessary to estimate the fitness values, which include the meiotic drive component. The meiotic drive advantage of $Y^{suppressor}$ over $Y^{nonsuppressor}$ depends on the frequency of $SR^{suppressible}$; in their absence there is no meiotic drive advantage. The total frequency of *SR* in the Itatiaia population is 13% (CARVALHO *et al.* 1989); if 90% of them are suppressible, the marginal fitness of $Y^{suppressor}$ is 1.08 (relative to $Y^{nonsuppressor}$); if 90% are unsuppressible, it falls to 1.01 (see the APPENDIX). Recent data show that ~60% of the *SR* chromosomes are suppressible in this population (A. B. CARVALHO, G. ROCHA and S. NASCIMENTO, unpublished results), which implies that the marginal fitness of $Y^{suppressor}$ is ~1.06. This value virtually excludes the hypotheses of neutral and transient polymorphisms as explanations for the *Y* polymorphism, since a 6% advantage would raise the frequency of $Y^{suppressor}$ from 10% to 90% in 81 generations, *i.e.*, ~8 years. The polymorphic state of two natural populations 1500 km apart also argues against a transient polymorphism, given its predicted short duration. It is possible that the polymorphism is stabilized by frequency-dependent selection, but this hypothesis is not compelling in the present case because it would mean that the fitness of *Y* chromosome types were intrinsically frequency dependent, and we could not see any reason for it. Polymorphism for *Y*-linked suppressors of *sex-ratio* has also been found in some populations of *D. paramelanica*, the advantage of $Y^{suppressor}$ being 7% in this

species (STALKER 1961). In *D. melanogaster,* CLARK (1987b) found variation in the segregation ratios of *Y* chromosomes that would result in fitness differences of 3%. These cases, and the *D. mediopunctata* one, raise the same theoretical problem: are there stable, nonneutral *Y* polymorphisms?

**Stabilization of the *Y* polymorphism by interactions in the population dynamics of *X* and *Y* alleles:** *Sex-ratio X* chromosomes are deleterious, being maintained in populations due to meiotic drive (WALLACE 1948; CURTSINGER 1991; BECKENBACH 1996; JAENIKE 1996). The meiotic drive advantage of $SR^{suppressible}$ over *X:ST* decreases with higher frequencies of $Y^{suppressor}$, but lower frequencies of $SR^{suppressible}$ lead to decreased advantage of $Y^{suppressor}$ (see the previous section). These interactions may provide a mechanism for the stabilization of the *Y* polymorphism: any increase in the frequency of $Y^{suppressor}$ indirectly diminishes its own advantage because it should lead to a decrease in the frequency of $SR^{suppressible}$. In the same way, an increase in the frequency of $SR^{suppressible}$ will be controlled by the increase of $Y^{suppressor}$, which "robs" the meiotic drive advantage of $SR^{suppressible}$ over *X:ST*. Thus, the frequency of $Y^{suppressor}$ may be "self"-regulated via $SR^{suppressible}$, and vice versa. We just have to assume that $Y^{suppressor}$ is slightly deleterious to explain why it is not fixed. This assumption is quite reasonable: the $Y^{suppressor}$ allele is analogous to the $Rsp^{insensitive}$ allele of *Segregation Distortion* of *D. melanogaster,* which has been shown to be a heterochromatin deletion causing a fitness loss of 13% (WU *et al.* 1988, 1989; PIMPINELLI and DIMITRI 1989). Thus, we propose that the nonneutral *Y* polymorphism of *D. mediopunctata* is stabilized by the special interactions in the population dynamics of *X* and *Y* alleles under meiotic drive; these interactions result in an equilibrium between meiotic drive and natural selection. Without these interactions (for example, if one *X* allele become fixed), any slight imbalance between the meiotic drive advantage of $Y^{suppressor}$ and its presumed deleterious effect would lead to its fixation or loss. It should be noted that the proposed mechanism is a kind of two locus constant fitness selection, and not frequency-dependent selection, for the fitness parameters of each genotype are constant (see the next section); only the marginal fitness of the *X* and *Y* alleles depends on the frequencies of each other.

For the sake of simplicity, we have not considered the $SR^{unsuppressible}$ chromosomes in the above reasoning. They are expected to behave quite similarly to *ST* chromosomes: both have constant transmission rates (insensible to $Y^{suppressor}$ frequency) and both do not contribute for the meiotic drive advantage of $Y^{suppressor}$ over $Y^{nonsuppressor}$. Hence, $SR^{unsuppressible}$ chromosomes do not modify the essence of our argument on the *Y* polymorphism. However, they may indirectly affect the maintenance of the *Y* polymorphism via influences on the $SR^{suppressible}$ frequency. A population containing $SR^{unsuppressible}$ is expected to have a lower frequency of $SR^{suppressible}$ (as compared to a population containing only *ST* and $SR^{suppressible}$), because $SR^{unsuppressible}$ have a meiotic drive advantage over $SR^{suppressible}$ (due to their insensibility to $Y^{suppressor}$). Indeed, they should be more deleterious than $SR^{suppressible}$ otherwise they would increase until the loss of the later. These points are addressed in more detail in the next section.

**Numerical simulations:** We carried out numerical simulations on our model to verify if it really may stabilize $Y^{suppressor}$ polymorphisms, either in presence or in absence of $SR^{unsuppressible}$. The model has three *X* alleles (*ST*, $SR^{suppressible}$ and $SR^{unsuppressible}$; in simulations without $SR^{unsuppressible}$ its initial frequency was set to zero) and two *Y* alleles ($Y^{suppressor}$ and $Y^{nonsuppressor}$), and allows for natural selection (viability) and meiotic drive. The parameter definitions are given in Table 4 and the suitable recursion equations are given in the APPENDIX. The main assumptions of our *sex-ratio* model are shown in Table 4: *SR* was equally deleterious in males and homozygous females, the fitness of *SR* chromosomes had a constant ratio (*e.g.*, $W_{SR^u} = 0.8 \times W_{SR^s}$), and $Y^{suppressor}$ ranged from deleterious to advantageous (for $W_{Y^{sup}}$ ranged from zero to two). Additionally, we assume that fitness was multiplicative ($W_{SR^s/Y^{sup}} = W_{SR^s} \times W_{Y^{sup}}$), that $SR^{unsuppressible}$ is recessive in relation to $SR^{suppressible}$ ($W_{SR^s/SR^u} = W_{SR^s/SR^s}$) and that both heterozygotes involving *ST* have the same fitness ($W_{ST/SR^s} = W_{ST/SR^u}$; Table 4). These "simplifying" assumptions are intentional: they result in a model with only two parameters ($W_{SR^s}$ and $W_{Y^{sup}}$), which allows the direct visualization of the parameter space (Figure 3). More complex models, allowing differential selection in *SR/Y* males and *SR/SR* females, variable meiotic drive, etc., produced essentially the same result (A. B. CARVALHO, G. ROCHA and S. NASCIMENTO, unpublished data).

The simulation procedures were based on CLARK (1987a). Briefly, values for the viabilities of $SR^{suppressible}$ ($W_{SR^s}$) and $Y^{suppressor}$ ($W_{Y^{sup}}$) were drawn from a random uniform distribution and the allele frequencies (randomly initialized) were iterated with the recursion equations for 40,000 generations or until an equilibrium was attained. The type of equilibrium (fixation, *X* polymorphism, *Y* polymorphism, or *X* and *Y* polymorphism) was then determined. If no equilibrium was attained after 40,000 generations, it was checked whether the population had entered a stable cycle. During iterations, allele frequencies below $10^{-5}$ were rounded to zero. The whole procedure was repeated with 100,000 different sets of parameter values ($W_{SR}$ and $W_{Y^{sup}}$). We carried out two types of simulations, assuming either that *SR* is recessive ($W_{SR/ST} = 1$) or overdominant ($W_{SR/ST} = 1.2$). Both cases are compatible with available data for *D. pseudoobscura* (CURTSINGER and FELDMAN 1980; BECKENBACH 1996).

Figure 3 and Table 5 show the results of the numerical simulations: $Y^{suppressor}$ polymorphisms may be stabi-

**TABLE 4**

**Parameter values employed in the numerical simulations**

| | Viability | | | | | | | | | | | | Meiotic drive | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $ST/ST$ | $SR^s/SR^s$ | $SR^u/SR^u$ | $ST/SR^s$ | $ST/SR^u$ | $SR^s/SR^u$ | $ST/Y$ | $ST/Y^{sup}$ | $SR^s/Y$ | $SR^s/Y^{sup}$ | $SR^u/Y$ | $SR^u/Y^{sup}$ | $ST/Y$ | $ST/Y^{sup}$ | $SR^s/Y$ | $SR^s/Y^{sup}$ | $SR^u/Y$ | $SR^u/Y^{sup}$ |
| Parameter name | $v_{11}$ | $v_{22}$ | $v_{33}$ | $v_{12}$ | $v_{13}$ | $v_{23}$ | $v_{14}$ | $v_{15}$ | $v_{24}$ | $v_{25}$ | $v_{34}$ | $v_{35}$ | $m_{14}$ | $m_{15}$ | $m_{24}$ | $m_{25}$ | $m_{34}$ | $m_{35}$ |
| Parameter value | 1 | $W_{SR^s}$ | $W_{SR^u}$ | 1 or 1.2 | 1 or 1.2 | $W_{SR^s}$ | 1 | $W_{Y^{sup}}$ | $W_{SR^s}$ | $W_{SR^s} \times W_{Y^{sup}}$ | $W_{SR^u}$ | $W_{SR^u} \times W_{Y^{sup}}$ | 0.5 | 0.5 | 0.99 | 0.5 | 0.99 | 0.99 |

The fitness of $SR^{suppressible}$ ($W_{SR^s}$) ranged from 0 to 1 and the fitness of $Y^{suppressor}$ ($W_{Y^{sup}}$) ranged from 0 to 2. The fitness of $SR^{unsuppressible}$ ($W_{SR^u}$) was set equal to $0.8 \times W_{SR^s}$. Meiotic drive is defined as the proportion of X-bearing sperm. Abbreviations are as follows: $SR^s$, $SR^{suppressible}$; $Y^{sup}$, $Y^{suppressor}$; $Y$, $Y^{nonsuppressor}$

lized by the mechanism we proposed, whether or not $SR^{unsuppressible}$ is present. This stabilization did not require a rare combination of parameter values, for it occurred in 5–10% of the parameter space (summing up the cases of stable cycles and *X-Y* polymorphisms). In accordance with the verbal model, in all of these cases $Y^{suppressor}$ was deleterious ($W_{Y^{sup}} < 1$) and there was *X* polymorphism involving $SR^{suppressible}$. Figure 3, C and D includes $SR^{unsuppressible}$ chromosomes, assuming that they are more deleterious than $SR^{suppressible}$ ($W_{SR^u} = 0.8 \times W_{SR^s}$). *Y* polymorphism coupled with three *X* alleles polymorphism occurred in 3–8% of the parameter space. This result is important because $SR^{unsuppressible}$ chromosomes are present in the natural populations; our explanation for the *Y* polymorphism would certainly be wrong if it worked only in their absence. As expected, if we assume that $SR^{suppressible}$ and $SR^{unsuppressible}$ have the same fitness ($W_{SR^u} = W_{SR^s}$), then $SR^{suppressible}$ is lost (in the presence of $Y^{suppressor}$) and there is no *Y* polymorphism; the model degenerates to the simple and well known case of *SR-ST* polymorphism (EDWARDS 1961; CURTSINGER and FELDMAN 1980). On the other hand, if $SR^{unsuppressible}$ was excessively deleterious ($W_{SR^u} < 0.6 \times W_{SR^s}$), then it was invariably lost and the numerical simulations produced the same result showed in Figure 3, A and B. Of course the exact values of these boundaries varied, depending on parameter assumptions (*e.g.*, intensity of meiotic drive and suppression; dominance relations between the *X* alleles, etc.). The full analysis of this model will be addressed in another paper (A. B. CARVALHO, G. ROCHA and S. NASCIMENTO, unpublished data). Finally, a very interesting property of models including $SR^{unsuppressible}$ is their tendency to undergo stable cycles: this occurred in roughly half of the parameter space with *Y* polymorphism. Thus, it is quite possible that some natural populations of *D. mediopunctata* are cycling.

These results may be compared with CLARK's (1987a) study. CLARK studied a much more general model that allows meiotic drive in heterozygous females and in all males and all combinations of viabilities (in his model all parameters were variable between 0 and 1); he found that stable *Y* polymorphisms require *X* polymorphism, *X-Y* meiotic drive and natural selection. Our model is tailored for *sex-ratio* polymorphisms (for example, it assumed that *SR* is deleterious and that meiotic drive does not occur in females and *ST* males) and is formally a special case of CLARK's model; its results are in general agreement with those obtained by CLARK (1987a).

The numerical simulations suggest that the $Y^{suppressor}$ polymorphism can be stabilized as we proposed. This hypothesis may be tested in several ways. If it is correct and *D. mediopunctata* populations are in equilibrium, then $Y^{suppressor}$ must be loosing in other fitness components its 6% meiotic drive advantage and, thus, natural populations devoid of $SR^{suppressible}$ should not contain $Y^{suppressor}$ chromosomes. A fitness difference of 6% may

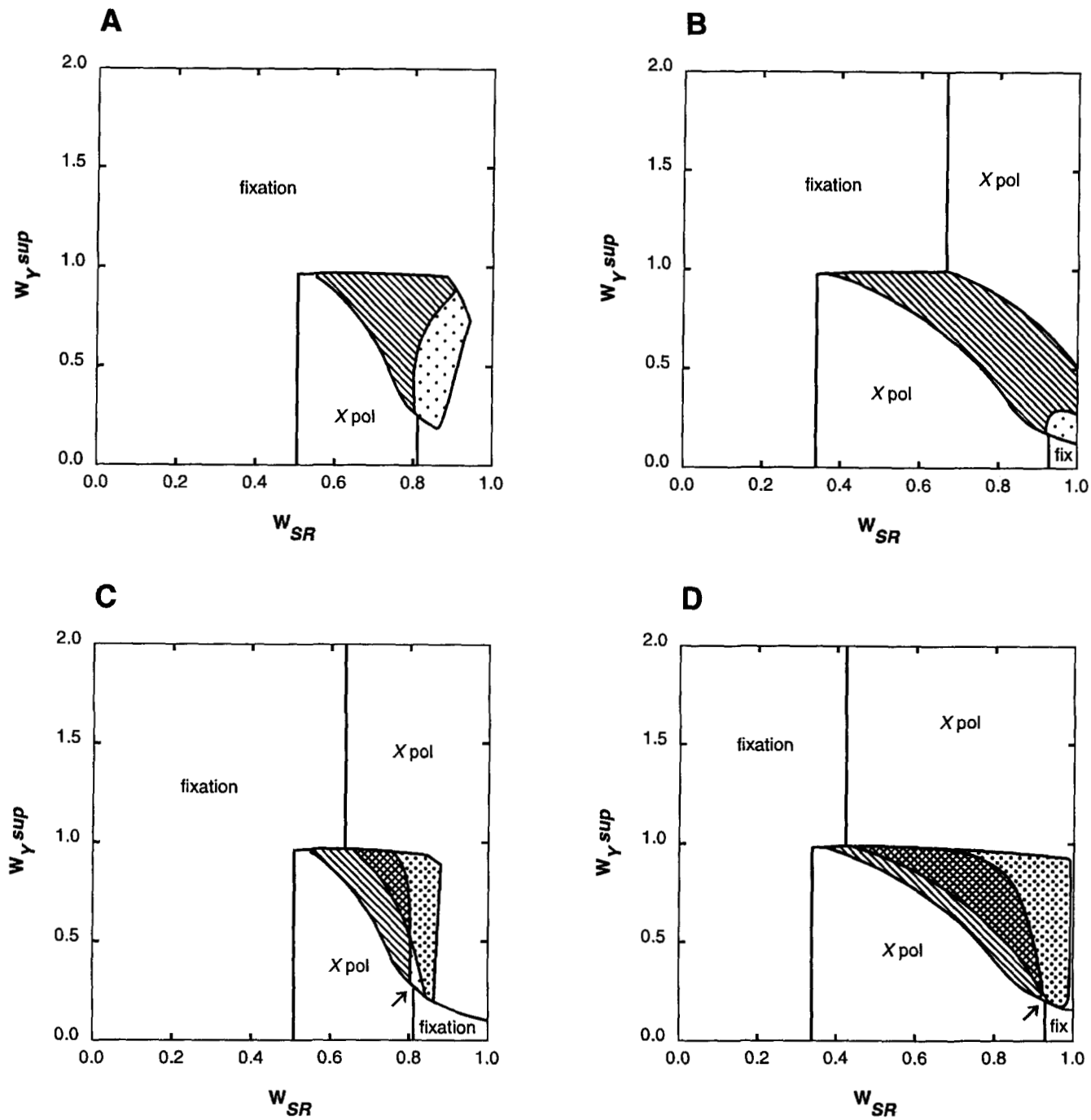


FIGURE 3.—Numerical simulations of the *sex-ratio* model. (A) Two *X* alleles and *SR* recessivity. (B) Two *X* alleles and *SR* overdominance. (C) Three *X* alleles and *SR* recessivity. (C) Three *X* alleles and *SR* overdominance. The portions of the parameter space that maintain polymorphism in the *Y* chromosome are marked as follows: hatched, *X-Y* polymorphisms with two *X* alleles ($ST\text{-}SR^{suppressible}$); cross-hatched, *X-Y* polymorphisms with three *X* alleles ($ST\text{-}SR^{suppressible}\text{-}SR^{unsuppressible}$); small dots, stable cycles with two *X* alleles ($ST\text{-}SR^{suppressible}$); large dots, stable cycles with three *X* alleles ($ST\text{-}SR^{suppressible}\text{-}SR^{unsuppressible}$). Arrows in C and D point to the small regions of two *X* alleles stable cycle. A locus was considered polymorphic when the equilibrium frequency of the rarest allele was $>10^{-3}$.

also be detectable in population cage experiments. Our Itatiaia sample was collected 8 years ago; with another sample we may verify directly whether the *Y* polymorphism is transient. Finally, a study of the frequencies of the types of *SR* in natural populations will lead to a more precise estimate of the meiotic drive advantage of the $Y^{suppressor}$ chromosomes (A. B. CARVALHO, G. ROCHA and S. NASCIMENTO, unpublished data). We are now pursuing some of these approaches.

**May other nonneutral *Y* polymorphisms be stabilized by *X-Y* interactions under meiotic drive?** The proposed mechanism is directly applicable to the *Y* polymorphism of *D. paramelanica* (STALKER 1961), which is very similar to *D. mediopunctata*. It is suggestive that the two other

**TABLE 5**

**Numerical simulations of *sex-ratio* models**

| Model | | Result | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *X* alleles | $W_{ST/SR}$ | Fixation | *X* pol. | *Y* pol. | *X-Y* pol. (*ST-SR*$^s$) | Cycle (*ST-SR*$^s$) | *X-Y* pol. (*ST-SR*$^s$-*SR*$^u$) | Cycle (*ST-SR*$^s$-*SR*$^u$) |
| *ST-SR*$^s$ | 1.0 | 82,600 | 10,623 | 0 | 4,333 | 2,444 | — | — |
| *ST-SR*$^s$ | 1.2 | 51,180 | 39,922 | 0 | 8,421 | 477 | — | — |
| *ST-SR*$^s$-*SR*$^u$ | 1.0 | 59,128 | 35,716 | 0 | 2,042 | 210 | 908 | 1,996 |
| *ST-SR*$^s$-*SR*$^u$ | 1.2 | 38,845 | 51,069 | 0 | 2,269 | 47 | 4,015 | 3,755 |

For each model, 100,000 simulations with random parameters ($W_{Y^{sup}}$ and $W_{SR^s}$) were carried out.

cases of nonneutral *Y* polymorphisms (see below) may also involve *X-Y* segregation distortion (*i.e.*, meiotic drive) and, thus, be stabilized through a similar mechanism. The polymorphism described by CLARK (1987b) in *D. melanogaster* precisely involves variation in the segregation ratios of *Y* chromosomes and there is also a slight *X* chromosome meiotic drive in this species (CURTSINGER 1984, 1991). Hence, *D. melanogaster* is polymorphic for a weak *X-Y* meiotic drive system. It is interesting to note that in this species there is no *Y* polymorphism for male fertility (CLARK 1987b), despite the fact that fertility genes comprise the bulk of the *Y* chromosome genetic material (GATTI and PIMPINELLI 1983). Perhaps this absence of fertility polymorphisms is a consequence of the lack of a simple (*i.e.*, constant fitness) stabilizing mechanism for them, as does exist for segregation distortion. The nonneutral *Y* polymorphism in the number of rDNA copies of *D. mercatorum* (HOLLOCHER and TEMPLETON 1994) may also have a segregation distortion component, for rDNA functions as a *X-Y* pairing site in *D. melanogaster* and very low rDNA copy number leads to sex-chromosome meiotic drive (ZIMMERING 1976; MCKEE and LINDSLEY 1987; MCKEE and KARPEN 1990). It would be interesting to know how the *X* and *Y* alleles of both systems (*X-Y* segregation in *D. melanogaster* and rDNA in *D. mercatorum*) interact, and whether these interactions may result in stable *Y* polymorphisms.

The stringency of conditions led to the rejection of constant fitness models as an explanation for naturally occurring *Y* polymorphisms and, thus, they have been considered either neutral (rDNA introns in *D. melanogaster;* CLARK and LYCKEGAARD 1990), transient (*Y* chromosome segregation in *D. melanogaster;* CLARK 1987b) or stabilized by more complex mechanisms (rDNA in *D. mercatorum;* HOLLOCHER and TEMPLETON 1994). The stabilization of *Y* polymorphisms by an equilibrium between meiotic drive and natural selection (resulting from interactions in the population dynamics of *X* and *Y* alleles) may be an alternative explanation for these phenomena, especially when *X* polymorphism and *X-Y* segregation distortion are likely to be present.

**Variation within types:** Our experiments were also designed to search for variation within types of *Y*, which was found in suppressor *Y* chromosomes from the Itatiaia population, mainly due to the weak suppressor $Y^{ITF\text{-}543}$. This variation, which has not been observed in other *sex-ratio* systems, raises the question of the artificiality of the separation between suppressor and nonsuppressor *Y*. If these types are just part of a continuum, the classification is inadequate and the whole problem will be better approached and described by multiple allele theory. On the other hand, it is possible that the within-type variation is a second order phenomenon, justifying our "two-allele" approach. The distribution of means (Figure 2) strongly suggests that there is not a continuum. To quantify the importance of the within-type variation, we have used a nested ANOVA with the wild-caught *Y* chromosomes from both populations to estimate the "between types" and "within types" variance components (SOKAL and ROHLF 1981, p. 297). As Table 6 shows, within-type variance is proportionally very small, so the classification of *Y* chromosomes in two discrete types (suppressors and nonsuppressors) seems justified. The evolutionary significance of this within-type variation is obscure; perhaps it is caused by nearly neutral mutations. The meiotic drive advantage of $Y^{I\text{-}83}$ over $Y^{ITF\text{-}543}$, the stronger and the weaker suppressor *Y* from Itatiaia, is ~2% in our present experimental conditions but it may be negligible under natural conditions (see below). This variation is analogous to the *Rsp* variation in the *Segregation Distortion* of *D. melanogaster,* which has also required more elaborate genetic experiments to be detected (MARTIN and HIRAIZUMI 1979; LYTTLE *et al.* 1986).

**Residual *sex-ratio* expression in suppressed males:** The means of some suppressed genotypes were slightly female biased (Tables 1 and 2), indicating a weak *sex-ratio* expression, whereas in our previous study (CARVALHO and KLACZKO 1994) these genotypes were fully suppressed. This was especially clear for $SR^{suppressible}/Y^{ITF\text{-}543}$, which produced on average 35.4% sons in experiments I and II, and 47.8% in that previous study; the difference being very significant ($t = 8.0$, 134 d.f., $P < 10^{-9}$). The main difference in experimental conditions between the two studies is the autosomal background. It is possible that the tetra-mutant background used in the present study is more depleted of autosomal suppressors of *sex-ratio* expression than the mixed background used in CARVALHO and KLACZKO (1994) and

**TABLE 6**

**Variance components in *sex-ratio* expression**

| | Morro Santana | | | Itatiaia | | |
|---|---|---|---|---|---|---|
| Variance component | d.f. | Variance | % | d.f. | Variance | % |
| Among types of *Y* | 1 | 393.4 | 70 | 1 | 535.4 | 76 |
| Among *Y*, within type | 7 | 5.9 | 1 | 8 | 12.2 | 2 |
| Among male, within *Y* | 199 | 165.4 | 29 | 256 | 157.9 | 22 |

The significance of the variance components has already been tested in RESULTS with a similar test. We used untransformed data (% males) and excluded the reference *Y* chromosomes from this analysis because we were interested in the naturally occurring variation, but neither their inclusion or the use of arc sin $\sqrt{p}$ changed the result.

this resulted in an enhanced *sex-ratio* expression that overcame the weaker suppression of $Y^{ITF\text{-}543}$. In support for this explanation, nonsuppressed *SR*/*Y* males produced on average 17% males in CARVALHO and KLACZKO (1994) and 12% now. It remains to be determined whether genotypes like $SR^{suppressible}/Y^{ITF\text{-}543}$ express *sex-ratio* weakly in natural conditions. If they do not express it, the weak *sex-ratio* expression of $SR^{suppressible}/Y^{ITF\text{-}543}$ males (and the heterogeneity among suppressor *Y* chromosomes) may be an interesting genetic finding but without evolutionary consequences, all suppressor *Y* having the same advantage over nonsuppressors.

The spread of a suppressor *Y* should lead to a decrease in the frequency of the *X* chromosome allele ($SR^{suppressible}$) that benefits it. This interaction in the population dynamics of *X* and *Y* alleles is unique to meiotic drive (for example, under viability or fertility selection, a *X*-*Y* combination that benefits a *Y* allele will also lead to the spread of the corresponding *X* allele) and may stabilize *Y* polymorphisms. We propose that the $Y^{suppressor}$ polymorphism of *D. mediopunctata* is stabilized this way, by an equilibrium between meiotic drive and natural selection. Numerical simulations showed that this is possible. This hypothesis can be tested experimentally (*e.g.*, another field collection to exclude transient polymorphism; population cages to measure the fitness of $Y^{suppressor}$ in the presence and in the absence of $SR^{suppressible}$) because the alleles involved can be unambiguously identified and probably have rather large fitness differences. Thus, the *sex-ratio* system of *D. mediopunctata* may be a good experimental model, not only for this kind of polymorphism, but also for the more general question of the maintenance of *Y*-linked polymorphisms.

We thank D. DORIGO, J. POWELL, F. FARIA, J. BAPTISTA, A. CLARK, P. OTTO, B. BITNER-MATHÉ, B. MCKEE, A. SOLÉ-CAVA, J. P. VAZ and two anonymous referees for many valuable suggestions during this work and in the manuscript. We also thank Ms. M. SAMPAIO and Ms. F. VARANDAS for help in some experiments, Ms. MÔNICA BAHIA SCHLEE for graphical assistance, Ms. CLÉA KNAUER and Ms. ROSA DA SILVA for technical assistance, and Dr. V. VALENTE's laboratory for the warm hospitality during Morro Santana collection. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento do Pessoal de Ensino Superior, Fundação Universitária José Bonifácio and Sub-Reitoria de Ensino para Graduados/UFRJ.

## LITERATURE CITED

BABCOCK, C. S., and W. W. ANDERSON, 1996 Molecular evolution of the sex-ratio inversion complex in *Drosophila pseudoobscura:* analysis of the *esterase-5* gene region. Mol. Biol. Evol. **13:** 297–308.

BASOLO, A. L., 1994 The dynamics of Fisherian sex-ratio evolution: theoretical and experimental investigations. Am. Nat. **144:** 473–490.

BECKENBACH, A. T., 1996 Selection and the "sex-ratio" polymorphism in natural populations of *Drosophila pseudoobscura.* Evolution **50:** 787–794.

BECKENBACH, A. T., J. W. CURTSINGER and D. POLICANSKY, 1982 Fruitless experiments with fruit flies: the "sex-ratio" chromosomes of *D. pseudoobscura.* Dros. Inf. Serv. **58:** 22.

BULL, J. J., and E. L. CHARNOV, 1988 How fundamental are Fisherian sex ratios?, pp. 96–135 in *Oxford Surveys on Evolutionary Biology,* Vol. 5, edited by P. H. HARVEY and L. PARTRIDGE. Oxford University Press, Oxford.

CARVALHO, A. B., 1989 *Sex-ratio em* Drosophila mediopunctata. M. Sc. Thesis, Universidade Federal do Rio de Janeiro, Brazil.

CARVALHO, A. B., and L. B. KLACZKO, 1992 Age and sex-ratio expression in *Drosophila mediopunctata.* Genetica **87:** 107–111.

CARVALHO, A. B., and L. B. KLACZKO, 1993 Autosomal suppressors of sex-ratio in *Drosophila mediopunctata.* Heredity **71:** 546–551.

CARVALHO, A. B., and L. B. KLACZKO, 1994 Y-linked suppressors of the *sex-ratio* trait in *Drosophila mediopunctata.* Heredity **73:** 573–579.

CARVALHO, A. B., A. A. PEIXOTO and L. B. KLACZKO, 1989 *Sex-ratio* in *Drosophila mediopunctata.* Heredity **62:** 425–428.

CLARK, A. G., 1987a Natural selection and Y-linked polymorphism. Genetics **115:** 569–577.

CLARK, A. G., 1987b Variation in *Y* chromosome segregation in natural populations of *Drosophila melanogaster.* Genetics **115:** 143–151.

CLARK, A. G., 1990 Two tests of *Y* chromosomal variation in male fertility of *Drosophila melanogaster.* Genetics **125:** 527–534.

CLARK, A. G., and E. M. S. LYCKEGAARD 1990 Two neutrality tests of Y-linked rDNA variation in *Drosophila melanogaster.* Evolution **44:** 2106–2112.

COBBS, G., 1986 An investigation of the genetics of the "male sex-ratio" phenotype in *Drosophila pseudoobscura.* Genetics **113:** 355–365.

COBBS, G., 1987 Modifier genes of the sex-ratio trait in *Drosophila pseudoobscura.* Genetics **116:** 275–283.

CONOVER, D. O., and D. A. VOORHEES, 1990 Evolution of a balanced sex ratio by frequency-dependent selection in a fish. Science **250:** 1556–1558.

COYNE, J. A., and H. A. ORR, 1989 Two rules of speciation, pp. 180–207 in *Speciation and Its Consequences,* edited by D. OTTE and J. ENDLER. Sinauer Associates, Sunderland, MA.

COYNE, J. A., and H. A. ORR, 1993 Further evidence against meiotic-drive models of hybrid sterility. Evolution **47:** 685–687.

CURTSINGER, J. W., 1984 Components of selection in X chromosome lines of *Drosophila melanogaster:* sex ratio modification by meiotic drive and viability selection. Genetics **108:** 941–952.

CURTSINGER, J. W., 1991 X chromosome segregation distortion in Drosophila. Am. Nat. **137:** 344–348.

CURTSINGER, J. W., and M. W. FELDMAN, 1980 Experimental and theoretical analysis of the "sex-ratio" polymorphism in *Drosophila pseudoobscura.* Genetics **94:** 445–466.

DOBZHANSKY, T., 1935 The Y chromosome of *Drosophila pseudoobscura.* Genetics **20:** 366–376.

DOBZHANSKY, T., 1958 Genetics of natural populations. XXVII. The genetic changes in populations of *Drosophila pseudoobscura* in American Southwest. Evolution **12:** 385–401.

EDWARDS, A. W. F., 1961 The population genetics of "sex-ratio" in *Drosophila pseudoobscura.* Heredity **16:** 291–304.

FISHER, R. A., 1930 *The Genetical Theory of natural selection.* Claredon Press, Oxford.

FRANK, S. A., 1991 Divergence of meiotic drive suppression systems as an explanation for sex-biased hybrid sterility and inviability. Evolution **45:** 262–267.

FROTA-PESSOA, O., 1954 Revision of the tripunctata group of Drosophila with description of fifteen new species (Drosophilidae, Diptera). Arquivo do Museu Paranaense, Curitiba **10:** 253–330.

GATTI, M., and S. PIMPINELLI, 1983 Cytological and genetic analysis of the Y chromosome of *D. melanogaster* I. Organization of the fertility factors. Chromosoma **88:** 349–373.

GERSHENSON, S., 1928 A new sex-ratio abnormality in *D. obscura.* Genetics **13:** 488–507.

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

HARTL, D. L., and A. G. CLARK, 1989 *principles of Population Genetics.* Sinauer Associates, Sunderland, MA.

HOLLOCHER, H., and A. R. TEMPLETON, 1994 The molecular through ecological genetics of *abnormal abdomen* in *Drosophila mercatorum.* VI. The non-neutrality of the *Y* chromosome rDNA polymorphism. Genetics **136:** 1373–1384.

HURST, L. D., and A. POMIANKOWSKI 1991 Causes of sex-ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. Genetics **128:** 841–858.

JAENIKE, J., 1996 Sex ratio meiotic drive in the *Drosophila quinaria* group. Am. Nat. **148:** 237–254.

JOHNSON, N. A., and C-I WU, 1992 An empirical test of the meiotic drive models of hybrid sterility: sex-ratio data from hybrids between *Drosophila simulans* and *Drosophila sechelia.* Genetics **130:** 507–511.

LYCKEGAARD, E. M. S., and A. G. CLARK, 1989 Ribosomal DNA and *Ste* copy number variation on the *Y* chromosome of *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **86:** 1944–1948.

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

LYTTLE, T. W., J. G. BRITTNACHER and B. GANETZKY, 1986 Detection of Rsp and modifier variation in the meiotic drive system Segregation Distortion of *Drosophila melanogaster.* Genetics **114:** 183–202.

MARQUES, H. V. S., A. B. CARVALHO, C. A. ELIAS and L. B. KLACZKO, 1991 Mutants of *D. mediopunctata.* Dros. Inf. Serv. **70:** 280.

MARTIN, D. W., and Y. HIRAIZUMI, 1979 On the models of segregation distortion in *Drosophila melanogaster.* Genetics **93:** 423–435.

MCKEE, B. D., and G. H. KARPEN, 1990 Drosophila ribosomal RNA gene function as a X-Y pairing site during meiosis. Cell **61:** 61–72.

MCKEE, B. D., and D. L. LINDSLEY, 1987 Inseparability of X-heterochromatic functions responsible for X:Y pairing, meiotic drive, and male fertility in *Drosophila melanogaster.* Genetics **116:** 399–407.

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.

POLICANSKY, D., and B. DEMPSEY, 1978 Modifiers and "sex-ratio" in *Drosophila pseudoobscura.* Evolution **32:** 922–924.

SANDLER, L., and E. NOVITSKY, 1957 Meiotic drive as an evolutionary force. Am. Nat. **91:** 105–110.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry.* W. H. Freeman and Company, New York.

STALKER, H. D., 1961 The genetic systems modifying meiotic drive in *Drosophila paramelanica.* Genetics **46:** 177–202.

TAYLOR, D. R., 1994 The genetic basis of sex ratio in *Silene alba* (= *S. latifolia*). Genetics **136:** 641–651.

THOMSOM, G. J., and M. W. FELDMAN, 1975 Population genetics of modifiers of meiotic drive: IV. On the evolution of sex-ratio distortion. Theor. Pop. Biol. **8:** 202–211.

VARANDAS, F. R., M. C. SAMPAIO and A. B. CARVALHO, 1997 Heritability of sexual proportion in experimental *sex-ratio* populations of *Drosophila mediopunctata.* Heredity (in press).

VOELKER, R. A., 1972 Preliminary characterization of "sex-ratio" and rediscovery and reinterpretation of "male sex-ratio" in *Drosophila affinis.* Genetics **71:** 597–606.

WALLACE, B., 1948 Studies on "sex-ratio" in *Drosophila pseudoobscura.* I. Selection and "sex-ratio." Evolution **2:** 189–217.

WILKINSON, L., 1992 *SYSTAT for Windows.* Version 5. SYSTAT Inc., Evanston, IL.

WU, C-I., 1983 The fate of autosomal modifiers of the sex-ratio trait in *Drosophila* and other sex-linked meiotic drive systems. Theor. Popul. Biol. **24:** 121–135.

WU, C-I., and M. F. HAMMER, 1990 Molecular evolution of ultraselfish genes of meiotic drive systems, pp. 177–203 in *Evolution at the Molecular Level,* edited by R. K. SELANDER, A. G. CLARK and T. S. WHITTAM. Sinauer Press, Sunderland, MA.

WU, C-I., T. W. LYTTLE, M-L. WU and G-F. LIN, 1988 Association between a satellite DNA sequence and the *Responder* of *Segregation Distortion* in *D. melanogaster.* Cell **54:** 179–189.

WU, C-I., J. R. TRUE and N. A. JOHNSON, 1989 Fitness reduction associated with the deletion of a satellite DNA array. Nature **341:** 248–251.

ZIMMERING, S., 1976 Genetic and cytogenetic aspects of altered segregation phenomena in *Drosophila,* pp. 569–613 in *The Genetics and the Biology of Drosophila,* edited by M. ASHBURNER and E. NOVITSKY. Academic Press, London.

Communicating editor: A. G. CLARK

## APPENDIX

**Marginal fitness of $Y^{suppressor}$:** The meiotic drive marginal fitness of $Y^{suppressor}$ relative to $Y^{nonsuppressor}$ is given by the ratio of their transmission rates. The transmission rate of *Y* chromosomes is equal to the average male proportion of the corresponding progenies. In the *D. mediopunctata* case, there are six types of males, which produce the male proportion showed below (data from CARVALHO and KLACZKO 1994).

| | $Y^{suppressor}$ | $Y^{non\text{-}suppressor}$ |
|---|---|---|
| $ST$ $(p)$ | 0.47 | 0.47 |
| $SR^{suppressible}$ $(q)$ | 0.47 | 0.17 |
| $SR^{unsuppressible}$ $(r)$ | 0.17 | 0.17 |
| transmission rate | $0.47(p + q) + 0.17r$ | $0.47p + 0.17(q + r)$ |

where $p$, $q$ and $r$ are the frequencies of $ST$, $SR^{suppressible}$ and $SR^{unsuppressible}$ among males. Then, the marginal fitness of $Y^{suppressor}$ is

$$\frac{0.47(p + q) + 0.17r}{0.47\,p + 0.17\,(q + r)}.$$

The total frequency of $SR$ $(q + r)$ is 13% in the Itatiaia population (CARVALHO *et al.* 1989); ~60% of them are suppressible ($p$ = 87%; $q$ = 8%; $r$ = 5%; A. B. CARVALHO, G. ROCHA and S. NASCIMENTO, unpublished data). Hence, the marginal fitness of $Y^{suppressor}$ is 1.056. The expected frequency of $Y^{suppressor}$ on generation $t$ may then be calculated by standard haploid selection formula (*e.g.,* HARTL and CLARK 1989, p. 182):

$$\frac{y_t}{1 - y_t} = \frac{y_0}{1 - y_0}\,1.056^t,$$

where $y_t$ and $y_0$ are the frequency of $Y^{suppressor}$ on generations

0 and $t$, respectively. The same formula may be used to calculate the expected number of generations necessary for a given increase (say, from 10 to 90%) of the $Y^{suppressor}$ frequency.

**Sex-ratio model recursion equations:** Parameter definitions are given in Table 4. Let $p_f$, $q_f$ and $r_f$ represent the female frequency of *ST*, $SR^{suppressible}$ and $SR^{unsuppressible}$, respectively ($p_m$, $q_m$ and $r_m$ are the corresponding male values) and $y$ represent the frequency of $Y^{suppressor}$. The frequencies in the next generation are

$$p'_f = \frac{p_m p_f v_{11} + 0.5v_{12}(p_m q_f + p_f q_m) + 0.5v_{13}(p_m r_f + p_f r_m)}{\bar{w}_f}$$

$$q'_f = \frac{q_m q_f v_{22} + 0.5v_{12}(p_m q_f + p_f q_m) + 0.5v_{23}(q_m r_f + q_f r_m)}{\bar{w}_f}$$

$$r'_f = \frac{r_m r_f v_{33} + 0.5v_{13}(p_m r_f + p_f r_m) + 0.5v_{23}(q_m r_f + q_f r_m)}{\bar{w}_f}$$

$$p'_m = \frac{p_f(1-y)m_{14}v_{14} + p_f y m_{15}v_{15}}{\bar{w}_{xm}}$$

$$q'_m = \frac{q_f(1-y)m_{24}v_{24} + q_f y m_{25}v_{25}}{\bar{w}_{xm}}$$

$$r'_m = \frac{r_f(1-y)m_{34}v_{34} + r_f y m_{35}v_{35}}{\bar{w}_{xm}}$$

$$y' = \frac{p_f y(1-m_{15})v_{15} + q_f y(1-m_{25})v_{25} + r_f y(1-m_{35})v_{35}}{\bar{w}_{ym}},$$

where

$$\bar{w}_f = p_m p_f v_{11} + v_{12}(p_m q_f + p_f q_m) + v_{13}(p_m r_f + p_f r_m) + q_m q_f v_{22} + v_{23}(q_m r_f + q_f r_m) + r_m r_f v_{33}$$

$$\bar{w}_{mx} = p_f(1-y)m_{14}v_{14} + p_f y m_{15}v_{15} + q_f(1-y)m_{24}v_{24} + q_f y m_{25}v_{25} + r_f(1-y)m_{34}v_{34} + r_f y m_{35}v_{35}$$

$$\bar{w}_{my} = p_f y(1-m_{15})v_{15} + q_f y(1-m_{25})v_{25} + r_f y(1-m_{35})v_{35} + p_f(1-y)(1-m_{14})v_{14} + q_f(1-y)(1-m_{24})v_{24} + r_f(1-y)(1-m_{34})v_{34}.$$

The above model is based on CLARK's (1987a) "*X*- and *Y*-linked meiotic drive and viability" model, the main difference being that the later allows for two (instead of three) *X* alleles.