An Experimental Demonstration of Fisher's Principle: Evolution of Sexual Proportion by Natural Selection

Antonio Bernardo Carvalho,* Michelle Cristina Sampaio,* Flavia Roque Varandas* and Louis Bernard Klaczko†

**Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, CEP 21944-970, and †Departamento de Genética e Evolução, Universidade Estadual de Campinas, CEP 13081-970, Campinas, Brazil*

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ABSTRACT

Most sexually reproducing species have sexual proportions around 1:1. This major biological phenomenon remained unexplained until 1930, when Fisher proposed that it results from a mechanism of natural selection. Here we report the first experimental test of his model that obeys all its assumptions. We used a naturally occurring *X-Y* meiotic drive system—the *sex-ratio* trait of *Drosophila mediopunctata*—to generate female-biased experimental populations. As predicted by Fisher, these populations evolved toward equal sex proportions due to natural selection, by accumulation of autosomal alleles that direct the parental reproductive effort toward the rare sex. Classical Fisherian evolution is a rather slow mechanism: despite a very large amount of genetic variability, the experimental populations evolved from 16% of males to 32% of males in 49 generations and would take 330 generations (29 years) to reach 49%. This slowness has important implications for species potentially endangered by skewed sexual proportions, such as reptiles with temperature sex determination.

THE evolution of sexual proportion is a major biological question. It has been known for a long time that the majority of sexually reproducing species have sexual proportions around 1:1; the explanation of this phenomenon eluded Darwin himself, who concluded that "the whole problem is so intricate that it is safer to leave its solution for the future" (Darwin 1871). In 1930, Fisher proposed an explanation that is notably simple, robust, and general (reviewed in Bull and Charnov 1988). His argument can be put as follows: in any sexually reproducing population, half of the genes come from each sex, irrespective of its rarity. If the sex determining system generates unequal sex proportions, the rare sex will be effectively more fertile as a result of a greater *per capita* contribution to the next generation. Consequently, individuals investing their reproductive effort on the rare sex will be more represented in the gene pool of the next generations. If this investment is a hereditary trait, the alleles causing it will spread in the population until the attainment of equal number of males and females. At this point it makes no difference to invest in sons or daughters. [The preceding argument assumes equal cost of daughters and sons. If this does not hold, it is only necessary to substitute "equal number of males and females" for "equal investment in males and females." For example, if males cost twice as much as the females, the predicted equilibrium is 2 females : 1 male. For the sake of simplicity, we will assume (unlike Fisher) equal cost of males and females.] This mechanism of natural selection, known as "Fisher's Principle," predicts the evolution towards the 1:1 proportion irrespective of the sex-determining system. Indeed, essentially the same equation (Equation 1, below) predicts the Fisherian evolution under temperature sex-determination (in which females control the offspring sexual proportion by choosing hot or cold nest sites), haplo-diploidy (control by choosing to fertilize or not an egg), and chromosomal sex-determination (control by the regulation of *X-Y* segregation; Bulmer and Bull 1982). Because of this generality and robustness, Fisher's Principle became the most accepted explanation for the commonness of the 1:1 sexual proportion. Furthermore, it is also very important on theoretical grounds: sex allocation theory, which is a whole field in evolutionary biology, can be viewed as a set of alternative models violating one or more assumptions (see below) of a "Fisherian core" (Charnov 1982; Bull and Charnov 1988).

At the heart of Fisher's argument lies the assumption that males and females contribute equally for the gene pool (". . . each sex must supply half the ancestry of all future generations of the species"; Fisher 1930). This assumption, known as "biparentalism," is valid only for autosomal genes (Shaw 1958; Hamilton 1967; Godfrey and Werren 1996). Fisher's Principle

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

also assumes that the alleles controlling the sexual proportion have Mendelian segregation. Violation of these critical assumptions frequently lead to the evolution of unequal sex proportions (Bull and Charnov 1988). For example, *X*-linked genes do not have biparental inheritance because the sons do not receive the father's *X*. So the number of sons is irrelevant to the fitness of an *X*-linked gene in males and it is expected that, if this gene can control the sexual proportion, female bias will evolve (Shaw 1958; Hamilton 1967). This situation is known in natural populations of several Drosophila species and is called "*sex-ratio*": males carrying certain *X* chromosomes (called "*SR*" and usually associated with chromosome inversions) produce female biased progenies due to *X-Y* meiotic drive (Gershenson 1928; Jaenike 1996). The frequency of *SR* in natural populations may reach 40%, the remaining *X* being normal ("*ST*") *X* chromosomes (James and Jaenike 1990). *Y*-linked genes behave analogously to *X*-linked ones, except that male bias is favored (Hamilton 1967). Thus, genes that are localized in the sex-chromosomes and that are under meiotic drive lie outside Fisher's model because, in this case, each sex does not supply half the ancestry of future generations, and the segregation is not Mendelian.

Fisher's Principle has several additional, fundamental assumptions (Bull and Charnov 1988). Not all violations of these assumptions lead to non-Fisherian sexual proportions, but one should pay attention to rather subtle details that may have important consequences, as exemplified by parental control. This assumption means that the genetic variation for sexual proportion is expressed in the parents (*e.g.*, an allele that increases the proportion of *Y*-bearing sperm). It was studied in polygenic models by Bulmer and Bull (1982), who found that its violation (zygotic control, meaning that the genetic variation is expressed in the zygote, *e.g.*, alleles that increase the tendency of developing as a male) leads only to an increase on the expected evolutionary rate (all else being equal, zygotic control is twice as fast as parental control). However, if we move from polygenic to major gene models (which probably occurred in real cases; see discussion), the situation changes entirely: a major gene coupled with zygotic control will behave exactly as a sex-chromosome (*e.g.*, *Aa* males and *aa* females); it violates the assumption of biparentalism in the same way as *X* and *Y* chromosomes and will lead invariably to 1:1 ratio in one generation. Of course there is a continuum between a major gene and the infinitesimal polygenic model; under zygotic control, intermediate (oligogenic) cases are expected to evolve with intermediate velocity. On the other hand, under parental control the genetic basis makes little (if any) difference: single-gene model trajectories (Nur 1974) fit nicely on Bulmer and Bull's (1982) polygenic model (see appendix). Fisher's argument clearly assumes parental control (Fisher 1930) and this assumption avoids inadvertent introduction of sex-chromosome effects. The applicability of Fisher's Principle under zygotic control seems to depend on the genetic basis: polygenic models are Fisherian, whereas major gene models, though still Fisherian, are better described by sex-chromosome segregation theory (*e.g.*, Karlin and Lessard 1986, p. 82; see also discussion).

Fisher's Principle is a mechanism of natural selection in the strict sense. Besides it, several other evolutionary mechanisms and forces—such as sex-chromosome meiotic drive, for example—can change the sexual proportion, even towards the 1:1 equilibrium. For example, powerful *Y*-linked suppressors of *SR* have been found in some Drosophila species bearing *SR* chromosomes (Stalker 1961; Voelker 1972; Carvalho *et al.* 1997); these genes are expected to evolve because any *Y*-linked gene that increases the transmission rate of the *Y* chromosome is expected to spread in the population (Hamilton 1967; Thomson and Feldman 1975). In the presence of a *SR* chromosome, a suppressor *Y* acts in this way: it is transmitted to half of the progeny (the males), whereas a normal *Y* is not transmitted at all (only females in the progeny). The spread of a suppressor *Y* is expected to be very fast (much faster than autosomal ones) and will cause a quick return to the 1:1 sexual proportion (Wu 1983). However, this is a pure meiotic drive mechanism that is not directly related to Fisher's Principle. The difference between them can be clarified if we consider a *Y*-linked "suppressor" that yields 90% (instead of 50%) of sons in progeny. It will be fixed quickly, irrespective of the sexual proportion of the population and will result in a population with a non-Fisherian sexual proportion. A similar outcome was experimentally observed (Lyttle 1977). On the other hand, an autosomal suppressor of *SR* that yields 90% sons will spread until the attainment of the 1:1 sexual proportion. This illustrates the greater generality, robustness, and importance of Fisher's Principle in relation to other evolutionary mechanisms: it is not necessary to assume any specific effect of the genes on sexual proportion, nor a precise genomic localization; they only have to be autosomal, as the majority of the genes are.

Despite its theoretical and empirical importance to a major biological phenomenon, Fisher's Principle has suffered very few direct tests (Conover and Van Voorhees 1990; Basolo 1994), and none of them have been in a system with parental control and clear autosomal inheritance of the genes that controlled the sexual proportion. The recognition of the assumptions of Fisher's Principle and of evolutionary mechanisms that "mimic" its effects is important because it provides a framework to test it. An ideal system to carry out this test may have the following characteristics: (1) The cost of males and females should be set equal by the biology of the species, because it is very difficult to precisely determine them (and hence, the equilibrium sexual proportion)

when costs are unequal (Bull and Charnov 1988); (2) The sex-proportions should be unequal, which is suitable to observe a "return" to 1:1. This dynamic test is necessary because static 1:1 ratios may be a mere consequence of Mendelian segregation of the sex-chromosomes (Williams 1979; Toro and Charlesworth 1982). (3) There must be genetic variation for sexual proportion. Fisher's Principle is a mechanism of natural selection and, thus, can only operate if there is genetic variation for the selected trait. (4) This variation should be autosomal. Autosomal inheritance is the genetic translation for biparentalism. (5) The control of sexual proportion should be parental. This is a fundamental assumption of Fisher's Principle (Fisher 1930; Bull and Charnov 1988), and also avoids inadvertent introduction of sex-chromosome effects.

The *sex-ratio* system of *D. mediopunctata* has these characteristics. The biology of Drosophila assures equal cost of males and females because both zygotes cost the same and there is no parental care, whereas the *sex-ratio* trait provides the unequal sexual proportion. The expression of *sex-ratio* is variable in *D. mediopunctata*: several *SR*/*Y* males produce normal (instead of female-biased) progenies, due to at least four autosomal suppressors of *SR* expression (Carvalho and Klaczko 1993) among other factors (Carvalho and Klaczko 1992, 1994). Thus, there is autosomal genetic variation for the sexual proportion, and the control is parental. The autosomal suppressors should be favored by Fisher's Principle, since they induce in *SR*/*Y* males the production of progenies with a greater proportion of the rare sex (the males; Hamilton 1967; Wu 1983; Varandas *et al.* 1997). The increase of autosomal suppressor frequency in *SR*-bearing populations may provide an experimental test of Fisher's Principle that obeys its fundamental assumptions of biparentalism and parental control. Such a test should avoid sex-chromosome effects, as *D. mediopunctata* also has *Y*-linked suppressors of *SR* (Carvalho and Klaczko 1994).

The experiments with the *sex-ratio* system of *D. mediopunctata* reported in this paper lasted six years and involved the counting of more than 250,000 flies. They provide a simple, general, and robust experimental demonstration of Fisher's Principle. Our aim was to answer two questions: Will a population with unequal sex proportions really evolve toward 1:1 due to the mechanism proposed by Fisher? And how many generations will it take to reach the equilibrium?

MATERIALS AND METHODS

The experimental design was straightforward: we founded four *SR* populations fixed for the same *SR* and *Y* chromosomes and segregating for autosomal suppressors. The *SR* chromosome causes a strong female bias. If Fisher's Principle really works, it will select for the autosomal suppressors and gradually restore equal sex proportions. Four other populations were used to measure the accumulation of autosomal suppressors and to control for pleiotropic effects and mutation.

***SR* populations (populations 1, 2, 3, and 4):** These populations were described in Varandas *et al.* (1997). Briefly, we founded four experimental populations that contained autosomes from 24 wild-caught strains and are fixed for the same *SR* and *Y* chromosomes. Autosomal suppressors of *sex-ratio* seem to be very common in *D. mediopunctata* strains (as indicated by widespread suppression among wild-caught *SR*/*Y* males; Carvalho *et al.* 1989), and thus the *SR* populations are polymorphic for them. The *SR* chromosome employed ($SR^{ITC\text{-}221}$) was unsuppressible by *Y* chromosomes and the *Y* chromosome ($Y^{ITA\text{-}24\text{-}P}$) was a nonsuppressor one (Carvalho and Klaczko 1994). *SR* populations (and also populations 5–8; see below) were maintained under continuous generations (generation time was around five weeks). Population sizes were around 800. Sexual proportions were estimated from samples of eggs collected every two weeks, cultured under optimal conditions in half-pint bottles, and produced around 200 flies per population.

***ST* populations (populations 5 and 6):** These populations were founded at the same time and bearing the same autosomal background of the *SR* populations, but they carried a normal *X* chromosome (*ST*) that always produces 1:1 progenies, irrespective of autosomal suppressors (see Varandas *et al.* 1997). *ST* populations were used as a negative control as they could not suffer the Fisherian selection (see rationale).

Populations 7 and 8: These populations were used to control for *Y* chromosome mutation (see results). Population 7 was generated by repeated backcross of males from population 6 (*ST*) to females from population 1 (*SR*). On generation 29, we collected 16 males from population 6 and crossed them individually with females from population 1. A single son from each cross was again individually crossed with females from population 1. We repeated the backcross four times and then we founded population 7 with 30 pairs from each of the 16 backcross lines. Thus, population 7 was founded with 480 pairs, representing 16 different *Y* chromosomes from the *ST* population 6 and containing the autosomes (and the *X* chromosome) from the *SR* population 1. Population 8 has the opposite constitution [*Y* chromosomes from the *SR* population 1 and the autosomes (and the *X* chromosome) from the *ST* population 6] and was founded in an analogous way, by backcrossing males from population 1 (*SR*) to females from population 6 (*ST*).

Theoretical Fisherian trajectory: The theoretical Fisherian rate of change suitable for the *D. mediopunctata* case (polygenic and parental-male control of sexual proportion) was calculated by Bulmer and Bull (1982). We modified their equation (19) by assuming $P(w) = w$ in their equation (11), instead of the cumulative normal distribution and we obtained

$$\Delta M = \tfrac{1}{2} V_p \; h^2 \; \frac{(0.5 - M)}{M(1 - M)} \qquad (1)$$

where ΔM is the change in the population sexual proportion in one generation, h^2 and V_p are the heritability and the phenotypic variance of the sexual proportion and the last term is the Fisherian selection coefficient. An independent derivation of Equation 1, starting from a Mendelian model, is given in the appendix. The original version of this equation is suitable for theoretical comparisons between parental and zygotic control of the sexual proportion (Bulmer and Bull 1982), whereas the above version is much more adequate for experimental data.

Estimation of the realized heritability of the sexual proportion: Approximating ΔM (the change in one generation) for dM/dt in Equation 1 and solving the differential equation, we obtained:

$$M^2 - M - \tfrac{1}{2}\ln|0.5 - M| = V_p\ h^2\ t + constant \qquad (2a)$$

The left-hand term is a linear function of t (the number of generations) and, thus, may be used as a linearizing transformation. We made this transformation to our experimental M values (see Figure 2B) and fit a linear regression to estimate $V_p\ h^2$. (This procedure is analogous to the use of a linear regression on log-transformed values to fit an exponential function.) Then we measured V_p by crossing 390 males collected in the *SR* populations and counting their progenies (Varandas *et al.* 1997). Division of $V_p\ h^2$ by the experimental value of V_p yielded h^2, the realized heritability of sexual proportion. The confidence interval of h^2 was calculated using Fillier's theorem (Finney 1971). The standard error of V_p was calculated as

$$V_p \sqrt{\frac{2}{d.f.}},$$

where *d.f.* is the degrees of freedom of V_p (Kendall and Stuart 1969, p. 258). As described in the appendix, we tested the accuracy of Equation 1 by comparison with a well known Fisherian system and of Equation 2a by estimation of $V_p\ h^2$ on fictitious data.

Test of autosomes: The accumulation of autosomal suppressors was measured by introgressing the autosomes from each experimental population in a reference *SR* strain and comparing the *SR* expression in the resulting males (Figure 1). The parental and F_1 crosses were made with at least 15 pairs. In the F_2 cross (eight replicas for populations 1–6; thirteen replicas for populations 7 and 8), four SR^{am}/Y males aged for seven days were crossed in mass for six days with four females from a laboratory strain (ITA-24-P; Carvalho and Klaczko 1993). Flies were allowed to ovoposit for 15 days and then were discarded. Progenies were counted until bottle exhaustion. The whole procedure was repeated in six independent batches for each experimental population, to avoid a common environment effect that might simulate differences among populations (Carvalho *et al.* 1997). For example, if rearing density affects *sex-ratio* expression (which is quite possible) and we had carried out a single batch, then F_2 SR^{am}/Y males from an overcrowded rearing bottle (representing one population) would have appeared genetically different from F_2 SR^{am}/Y males reared on a less crowded bottle (representing another population). The test of each population in six independent batches avoided this error. The raw data (p, the proportion of males produced by each cross) was transformed to arc sin $\sqrt{p}$ and analyzed with a nested ANOVA, nesting batches within populations. Populations 1–6 were tested at generation 32, and populations 7 and 8 were tested at generation 37. The two experiments were analyzed separately because they were not carried out at the same time.

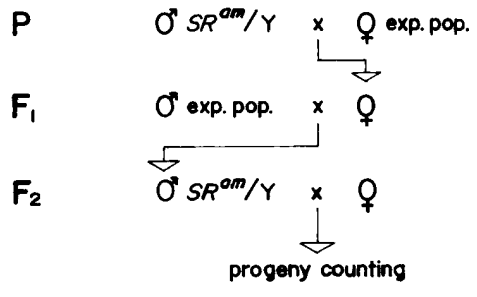

Figure 1.—Test of the experimental population autosomes. SR^{am} is the reference *SR* marked with the visible mutant *amarelo* (yellow body). This mutant originated spontaneously in a *SR* population and was introgressed in the autosomal background of a *ST* population, before being used in this experiment. Only the relevant genotypes are shown.

Statistical analyses: Unless otherwise stated, analyses were carried with the untransformed proportion of males, which is an adequate scale for studies on Fisher's Principle (Carvalho and Klaczko 1993) and were performed with the software SYSTAT 5 for Windows (Wilkinson 1992). We considered only the cultures that produced at least 20 flies. Sexual proportion was always expressed as the proportion (or the percentage) of males.

RATIONALE

As explained before, the four *SR* populations have a strong female bias. If Fisher's Principle really works, it will select for the autosomal suppressors and gradually restore equal sex proportions. Only the autosomes are genetically variable so, barring mutation, sex chromosomes could not cause changes in the sexual proportion. The *SR* populations carry a *X-Y* meiotic drive system but this does not violate the Fisher's Principle assumption of Mendelian segregation of the alleles controlling the sexual proportion: the control was effectively autosomal, for the *SR* and *Y* chromosomes were fixed. The control was also parental (and not zygotic): the autosomal suppressors are expressed in the parental males.

Now suppose that we do observe an increase in the male proportion. A general and robust demonstration of Fisher's Principle would require testing three critical predictions: (i) there must be enough autosomal genetic variation in the sexual proportion to account for its observed rate of change; (ii) this change should have been caused by the increase in the frequency of the autosomal alleles that direct the reproduction to the rare sex, the males; (iii) female excess, rather than pleiotropic fitness effects, should have caused the spread of these alleles. These tests guard us against "mimic" evolutionary forces, such as sex chromosome effects and natural selection unrelated to Fisher's Principle. Prediction (i) was tested by estimating the realized heritability of the sexual proportion. If Fisher's Principle was the cause of the increase of male proportion, then a direct measurement of h^2 in the same populations (*e.g.*, by father-offspring regression; Varandas *et al.* 1997) should produce a compatible value.

The *ST* populations allowed the test of predictions (ii) and (iii): as these populations could not have suffered the Fisherian selection (because they lack the female excess), their autosomes were nearly equivalent to a "sample" of the autosomes from the *SR* populations at generation 0. By comparison between the autosomes from the *ST* and *SR* populations at the end of the experiment, we could verify whether *SR* populations accumulated autosomal suppressors (prediction ii). At the same time, possible pleiotropic fitness effects (of the autosomal suppressors) unrelated to Fisher's Principle were automatically discounted (prediction iii) because

they should affect *SR* and *ST* populations equally. Thus, if these two Fisher's Principle predictions hold true for our experimental system, then the autosomes of the *SR* populations should have more suppressor alleles than the autosomes from the *ST* populations. This comparison was carried out as described before (Figure 1): the autosomes from the four *SR* and the two *ST* populations were introgressed into a reference *SR* strain; the resulting *SR*/*Y* males were crossed and their progenies were counted.

Finally, mutation might have introduced *Y* variation and caused artifacts. This possibility was tested with populations 7 and 8 (see results).

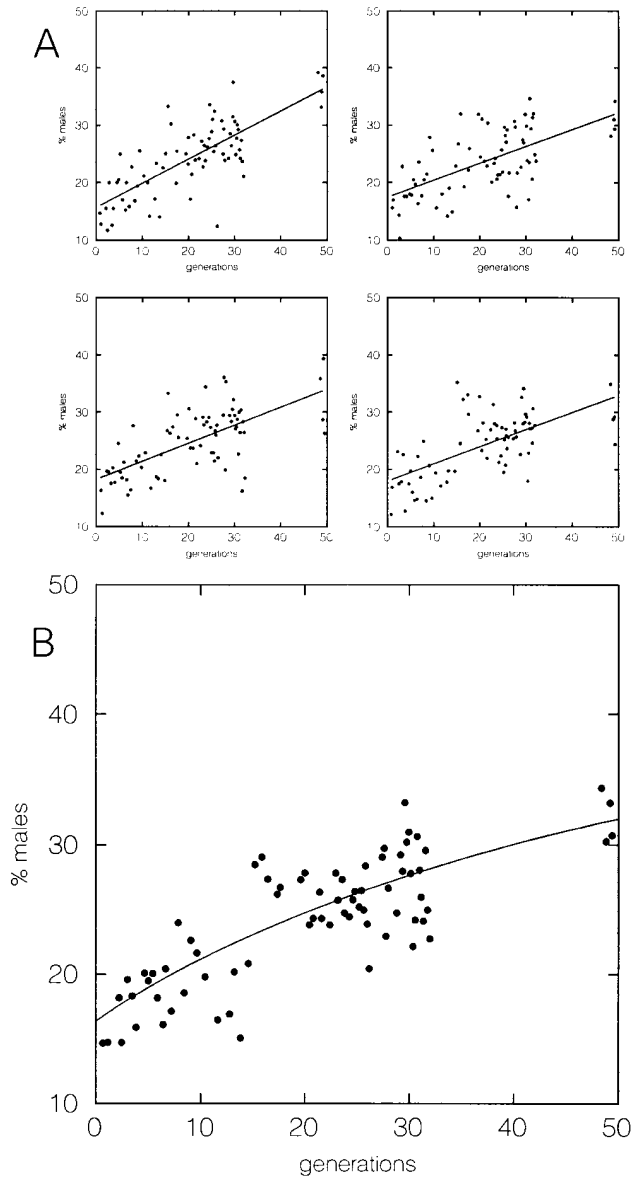

Figure 2.—Evolution of the sexual proportion in the four *SR* populations. (A) Populations 1–4. The linear regression is shown. (B) Each point is the average of the four populations. The line is the best fit Fisherian trajectory (Equation 1 parameters: $M_0 = 0.164$; $V_p\,h^2 = 0.00466$).

RESULTS

***SR* populations and the realized heritability:** Figure 2A shows that in the four independent populations the proportion of males (the rare sex) rose from 16% to 32% in 49 generations (approximately five years), as qualitatively predicted by Fisher's Principle. The increase of male proportion was significant at the 10^{-3} level (linear regression) for each population. An analysis of covariance did not detect any significant differences among them ($P > 0.6$ for the slope and y-intercept). Hence, the data from the four populations may be averaged as shown in Figure 2B.

We applied the linearizing transformation $M^2 - M - \frac{1}{2} \ln |0.5 - M|$ to each data point from Figure 2B and carried out a linear regression on transformed values. We obtained $V_p\,h^2 \times 10^4 = 46.6 \pm 5.3$ and *constant* $= 0.408 \pm 0.011$ (estimate $\pm$ SE; see Equation 2a). As expected, the linear regression of transformed values was also very significant ($P < 10^{-6}$). Then we measured V_p as described in materials and methods (see also Varandas *et al.* 1997) and we obtained $V_p \times 10^4 = 238.7 \pm 17.5$. Division of $V_p\,h^2$ by this quantity yielded h^2, the realized heritability of sexual proportion. We found $h^2 = 20\%$ (95% confidence interval: 15–25%), which is a surprisingly high value for this trait; sexual proportion is not heritable in most species, particularly under chromosomal sex-determination (Falconer 1954; Toro and Charlesworth 1982; Bull and Charnov 1988). If Fisher's Principle (rather than an artifact) had caused the increase of male proportion, then the sexual proportion should be highly heritable in *D. mediopunctata*. We reported in another paper a direct estimation of h^2 by father-offspring regression in the same *SR* populations: it was 41% (95% confidence interval: 22–60%; Varandas *et al.* 1997). Thus, there was enough autosomal genetic variation in the sexual proportion to explain (by Fisher's Principle) its observed rate of change. It should be noted that this father-offspring h^2 was due to autosomal genes, for the experimental design of Varandas *et al.* (1997) excluded sex-chromosome effects. The difference between the realized and the directly estimated heritabilities was significant at the 0.05 level (Welch's *t*-test for unequal variances; Sokal and Rohlf 1995) and might have been caused by several factors (see discussion).

The parameters $V_p\,h^2$ and *constant* were also used to calculate expected M values at any generation (M_t), by solving the equation

$$M_t^2 - M_t - \tfrac{1}{2}\ln|0.5 - M_t| = 0.00466\ t + 0.408 \qquad (2b)$$

Equation 2b can be solved numerically (*e.g.*, by Newton's method), as it does not have an explicit solution. Applying this procedure, we found that the initial sex-

TABLE 1

Comparison between *SR* and *ST* populations (accumulation of autosomal suppressors)

Population tested	Percentage of males
Population 1	28.7 ± 2.6 (47)
Population 2	25.1 ± 1.5 (48)
Population 3	25.5 ± 2.4 (47)
Population 4	25.0 ± 1.8 (46)
Average for *SR* populations	26.1 ± 1.0 (188)
Population 5	10.7 ± 0.8 (95)
Population 6	12.5 ± 1.3 (95)
Average for *ST* populations	11.6 ± 0.8 (190)

Values are means ± SE with the number of $F_2 SR^{am}/Y$ males tested in parentheses. See Figure 1 for details. Standard errors were calculated taking into account the nested design (they have 5 *d.f.* in each population).

ual proportion (M_0) was 16.4% and that its value at the end of the experiment (M_{49}) was 31.9%. Once M_0 and $V_p h^2$ are known, M_t may be more easily obtained by iterating Equation 1. The best fit Fisherian trajectory, shown as a line in Figure 2B, was obtained in this way.

***ST* populations and test of autosomes:** We measured the sexual proportion of *ST* populations in some generations and, as expected, it remained around 50% (46.1 ± 1.3%), with no directional trend ($P = 0.18$; linear regression). Furthermore, autosomal suppressors of *SR* are not expressed in *ST*/*Y* males (Carvalho 1989). Hence, the *ST* populations have not suffered the Fisherian selection. If, as predicted by Fisher's Principle, the increase in male proportion in *SR* populations was caused by an accumulation of autosomal suppressors and if female excess, rather than pleiotropic fitness effects, had caused the spread of these alleles, then the autosomes of the *SR* populations should have more suppressor alleles than the autosomes from the *ST* populations. Table 1 shows this comparison and the result was very clear: the populations with female excess accumulated autosomal alleles that shift the sexual proportion towards 1:1 and this accumulation was due to the female excess itself (and not to pleiotropic effects of these alleles), as predicted by Fisher's Principle. The difference between the six populations was highly significant ($F_{5,30} = 24.54$, $P < 10^{-3}$; nested ANOVA). The Tukey-Kramer test (Sokal and Rohlf 1995) showed that this was due to the differences between *SR* and *ST* populations, which were highly significant ($P < 10^{-3}$), whereas the differences between populations of the same type were not ($P > 0.8$). The difference between experimental batches from the same populations was also significant ($F_{30,342} = 2.08$, $P < 10^{-2}$). Thus, microenvironmental effects (*e.g.*, rearing density of the F_2 SR^{am}/Y males) and/or residual between-batches genetic variation affected *sex-ratio* expression in this experiment (see materials and methods). The nested experimental design controlled for these effects which might otherwise mimic differences among populations. Variance component analysis (Sokal and Rohlf 1995) showed that this "between batches-within population" component was proportionally small (6% of the total variance), the bulk of the variance coming from the differences between populations and from the error variance (39% and 55%, respectively).

There was a good fit between M_{32} (28.2%, the phenotypic level of suppression in *SR* populations at the same generation in which their autosomes were tested) and the amount of suppression in *SR* populations detected by the method shown in Figure 1 (26.1%). This indicates that this method estimates well the accumulation of autosomal suppressors. There was also a rather good fit between M_0 (16.4%, which measures the initial level of suppressors in *SR* populations) and the amount of suppression in *ST* populations (11.6%). This suggests that the autosomes from the *ST* populations were indeed a good "sample" of the autosomes from the *SR* populations before the Fisherian selection.

Populations 7 and 8 and *Y* chromosome mutation: Mutation might have introduced *Y* variation and caused an artifact, for the spread of a mutant suppressor *Y* would simulate Fisher's Principle both in Figure 2 and Table 1 (in Figure 1, experimental design did not separate *Y* and autosomal effects). We excluded this possibility by testing as above the populations 7 and 8. Population 7 carried the autosomes from a *SR* population and the *Y* from a *ST* population; population 8 had the opposite constitution (*i.e.*, autosomes from a *ST* population and *Y* from a *SR* population). As can be seen in Table 2, population 7 behaves in the test like a *SR* population and population 8 as a *ST* population. The difference between them was significant ($F_{1,10} = 9.12$, $P = 0.013$). Thus, the autosomes (rather than a mutant *Y*) caused the increase in sexual proportion. As in the previous experiment, the difference between batches of the same population was significant ($F_{10,130} = 2.57$, $P < 10^{-2}$). The difference between population 8 and the *ST* populations was also significant but we could not be sure that there was a genetic difference between them, because they were not tested together.

Asymptotic approach to the 1:1 equilibrium: The Fisherian selection is frequency-dependent: it weakens as populations approach 1:1 (Bull and Charnov 1988), causing a decrease in the expected rate of change. Mathematically, this is due to the term $(0.5 - M)$ of Equation 1, which approaches zero as M approaches 50%. We searched for this effect in our data, for it could provide an additional evidence that we are dealing with Fisherian evolution. The increase in male proportion was originally tested with a linear regression (Figure 2A), which did not allow for a decrease in ΔM. When we fitted a curvilinear regression by adding a quadratic term to the linear regression, we obtained a significantly better fit ($P = 0.03$ for the quadratic

TABLE 2

Comparison between populations 7, 8, *SR*, and *ST* (verification of possible *Y* chromosome artifacts)

Population tested	Origin of *Y* chromosome	Origin of autosomes	Percentage of males
SR populations	*SR* population	*SR* population	26.1 ± 1.0 (188)
ST populations	*ST* population	*ST* population	11.6 ± 0.8 (190)
Population 7	*ST* population	*SR* population	25.4 ± 2.7 (67)
Population 8	*SR* population	*ST* population	15.4 ± 1.1 (75)

Values are means ± SE with the number of $F_2 SR^{am}/Y$ males tested in parentheses. See Figure 1 for details. Data of *SR* and *ST* populations were obtained from Table 1. Standard errors were calculated taking into account the nested design (they have 5 *d.f.* in populations 7 and 8).

term). The curvilinear regression line was similar to Figure 2B line, showing that ΔM decreased significantly as M approached 1:1. This result might have been caused by exhaustion of genetic variability during the Fisherian selection. However, the heritability experiment was carried out at generation 25 (Varandas *et al.* 1997) and it still detected very significant levels of genetic variability. Furthermore, Varandas *et al.* (1997) found roughly the same value of h^2 in the *ST* populations, which had not been selected; this also argues against exhaustion of genetic variability in the *SR* populations. Thus, as predicted by Fisher's Principle, the rate of change of the sexual proportion decreased as the experimental populations approached 1:1 and this effect was probably due to Fisher's Principle itself.

DISCUSSION

Our results provide a clear experimental demonstration of Fisher's Principle. They show that female biased populations of *D. mediopunctata* consistently evolved toward equal sexual proportions and that both the cause of evolutionary change (female excess) and its genetic basis (accumulation of autosomal suppressors) were exactly those predicted by Fisher's Principle. All reasonable artifacts such as pleiotropy, mutation, and sex-chromosome effects have been controlled for. The experimental system used obeys all assumptions of Fisher's Principle, since it has strict biparentalism (autosomal inheritance) and parental control of sexual proportion (Fisher 1930; Shaw 1958; Hamilton 1967; Bull and Charnov 1988). Hence, our results share the generality and robustness of Fisher's Principle, being directly applicable to other sex-determining systems such as haplo-diploidy and temperature sex determination. To our knowledge, this is the first experimental demonstration of Fisher's Principle of such scope.

The experimental populations certainly evolved toward 1:1 because of the mechanism proposed by Fisher. But how many generations will they take to reach this point? We may answer this question as follows. The populations have been evolving exactly as predicted by Fisher's Principle over many generations. In fact, the line shown in Figure 2B was calculated taking into account only the first 35 generations and yet, there was a nearly perfect fit between the predicted sexual proportion around generation 49 and its observed value (31.9% and 32.2 ± 1.0%, respectively). Thus, we may confidently answer the above question by extrapolating from the Figure 2 line. The populations are expected to reach 40% in 120 generations (nine years) and 49% in 330 generations (29 years). The rate was indeed slow: in the first generations, the rate was +0.5% per generation, whereas around generation 49 it fell to +0.2%. This slowness seems to be characteristic of "classical" Fisherian evolution (autosomal and parental control of sexual proportion); evolution in most species is expected to be even slower, since our experimental populations had an unusually large amount of autosomal genetic variability for sexual proportion (Varandas *et al.* 1997). Sexual proportion has low effective heritabilities (Bull *et al.* 1982; Orzack and Gladstone 1994) or is not heritable at all in most species (Falconer 1954; Williams 1979; Toro and Charlesworth 1982).

The above findings have important biological implications. Skewed sexual proportions are a potential threat to all sexually reproducing species and may occur in all sex-determining systems. Species with chromosomal sex determination are prone to the invasion of meiotically driven sex-chromosomes that may cause strongly biased sexual proportions (Hamilton 1967). The *sex-ratio* trait in Drosophila is a well known example, though in this case natural selection against *SR* chromosomes maintains them in a relatively low frequency (Wallace 1948; Jaenike 1996) and there is only indirect evidence that populations had experienced very skewed sexual proportions (Varandas *et al.* 1997). However, some populations of the butterfly *Acraea encedon* were invaded by a sex-linked meiotic drive gene that caused a drop in the sexual proportion from nearly 50% to 3% between 1910 and 1965 (Owen 1965; Chanter and Owen 1972). Ovoposition of unfertilized eggs was common in these populations, clearly demon-

strating the threat of skewed sexual proportions. Haplo-diploidy is also vulnerable, for cytoplasmic elements (including microorganisms) have an "evolutionary interest" in directing the reproduction toward females (Shaw 1958; Hamilton 1967). A very interesting example occurred in several species of parasitic wasps (*Trichogramma sp.* and *Apoanagyrus diversicornis*). There are strains that became all-female (and parthenogenetic) due to a bacterium; these strains revert to sex after antibiotic treatment (Stouthamer *et al.* 1990; Pijls *et al.* 1996). Even hermaphroditism is not safe: many plant species bear "male sterilizing" cytoplasmic factors, which increase the seed (ovule) output at the expense of pollen production (Lewis 1941; Frank 1989). Finally, global warming may drive species with temperature sex determination (such as turtles) to extinction; it has been demonstrated that the sexual proportion in their nests is strongly correlated with air temperature (Bull 1983; Janzen 1994). Theoretically, Fisher's Principle is the most general evolutionary response in all these cases and our results suggest that it may play this role. On the other hand, Fisher's Principle is slow and a population threatened by skewed sexual proportion may became extinct in very few generations. It may be argued that Fisher's Principle slowness is not harmful, because female excess may even increase the population growth rate. This might be valid only in the case of moderate female bias, as the *Acraea encedon* case warn us. Furthermore, the female bias is only a particularity of our experimental model, but the slowness we observed applies to male bias as well, which could hardly be advantageous in any sense. Thus, it is quite possible that unisexual extinctions are a regular feature in the evolution of populations and even species, due to the slowness of Fisher's Principle and/or lack of genetic variability for sexual proportion. It is probable that in some cases other less general evolutionary mechanisms such as *Y*-linked suppressors of *SR* or sex-chromosome aneuploidy may have taken Fisher's Principle's place and "corrected" the skewed sexual proportions (Stalker 1961; Lyttle 1981; Carvalho *et al.* 1997; below). As skewed sexual proportions seem to be rare (particularly in species with sex-chromosomes), it is possible that they do not represent a serious threat to species survival. However, this rarity may be due to an observational bias: strong sexual proportion skewness is probably short lived in evolutionary time because the affected species or population may become extinct (leaving no sign of the cause) or may become fixed for modifiers of the sexual proportion. In the latter case it would be very difficult to unearth a past of strong oscillations in the sexual proportion, beneath a presently normal proportion. The strength of this observational bias could be estimated by consideration of the *D. simulans* case: despite more than 70 years of research with this species, only recently it became clear that many of its populations have a high frequency of a *SR* chromosome and its suppressors (Sturtevant 1920; Faulhaber 1967; Magalhães *et al.* 1985; Merçot *et al.* 1995; Cazemajor *et al.* 1997).

The results of our experimental populations also have implications to the more restricted field of *sex-ratio* trait evolution in Drosophila. Autosomal suppressors of *sex-ratio* occur in *D. mediopunctata* (Carvalho and Klaczko 1993; Varandas *et al.* 1997), *D. simulans* (Cazemajor *et al.* 1997), and possibly in *D. paramelanica* (Stalker 1961). Their evolutionary explanation relies entirely on Fisher's Principle (Hamilton 1967; Wu 1983; Varandas *et al.* 1997), but up to now there was not a direct evidence. Our results showed that Fisher's Principle indeed causes the spread of these genes in *SR*-bearing populations.

The difference between the realized and the directly estimated heritabilities of the sexual proportion deserves comment. It may be a mere experimental artifact, as the heritabilities were measured under different experimental conditions (it is not possible to measure the direct h^2 inside the populations, where the realized h^2 "took place"). For example, male age is known to affect *sex-ratio* expression (Carvalho and Klaczko 1992) and could be controlled only in the direct h^2. Our direct h^2 estimate (41%) was obtained in controlled crosses "outside" the populations but its experimental design also allows the estimation of the minimum value of h^2 inside the populations (the so-called "natural heritability"; Riska *et al.* 1989): it was 30% (Varandas *et al.* 1997), which was not significantly different from the realized h^2 (20%). Thus, the difference between the heritabilities might well disappear, if it would be possible to measure them under the same experimental conditions. Alternatively, the difference may be real. Indeed, the agreement between "realized" and "directly estimated" heritabilities is an unsolved issue in quantitative genetics (Hill and Caballero 1992; Gimelfarb and Willis 1994). Nonlinearity of parent-offspring regression is one possible cause of discrepancies and it may be specially important under weak selection (Gimelfarb and Willis 1994), which is precisely the case of Fisherian selection. We have not found nonlinearity in our father-son data (reported in Varandas *et al.* 1997) using the procedures described by Gimelfarb and Willis (1994). However, the test has little power in the present case, for we could measure *sex-ratio* expression in only one parent (the father). Exhaustion of genetic variability, though unlikely (see results), may also have contributed to the difference between the heritabilities. In conclusion, several factors may have caused the discrepancy between the heritabilities, and our data do not allow a precise identification. These facts do not change the main conclusion of the comparison between the heritabilities, namely, that there was enough autosomal genetic variation to explain (by Fisherian selection) the observed rate of change in the experimental populations.

Two previous experimental studies, using the fishes *Menidia menidia* (Conover and Van Voorhees 1990; Conover *et al.* 1992) and *Xiphophorus maculatus* (Basolo 1994), clearly demonstrated the convergence to 1:1. Both found a very fast evolution (the 1:1 equilibrium was attained in one to six generations; Figure 3), probably due to sex-chromosome effects. In the Xiphophorus system, genetic variation for sexual proportion was provided by its three sex-chromosomes (*XX,XW,* and *WY* are female; males are *XY* and *YY*). Populations started with different frequencies of these chromosomes contained from 25% to 80% of males and evolved in two or three generations to 1:1, following closely the predicted trajectories for this more special system of sexual determination (Basolo 1994). The nature of this evolution may be made explicit if we consider first a population founded with 10% males, with a standard *XY*/*XX* sex determination (a two-allele system). Mendelian segregation of sex-chromosomes coupled to sexual reproduction (but without biparentalism: *Y*-linked genes are contributed only by the fathers) ensure that a 1:1 sex proportion is attained in one generation. As the number of alleles increases to three, as found in *X. maculatus*, the same forces (segregation of sex-chromosomes and sexual reproduction) may still cause a 1:1 equilibrium, but with a slower, asymptotic approach (in two or three generations the sexual proportion is near 1:1). It should be noted that three allele systems do not evolve automatically to 1:1. The sexual proportions may even move away from this point, before reaching the equilibrium (Basolo 1994). Systems with two to six alleles have been explicitly modeled by Karlin and Lessard (1986, p. 82). In *M. menidia*, the sexual proportion is under zygotic and environmental control: sex is determined by temperature (high temperature induces zygotes to became males and cold has the opposite effect) and genetic factors acting on zygotes (Conover and Heins 1987). Manipulating both factors, Conover and Van Voorhees (1990) generated laboratory populations containing from 30% to 95% of males. These populations converge to equal sexual proportions very quickly (one to six generations). There are evidences of major sex-determining genes acting in zygotes in this species (Conover and Heins 1987; Conover *et al.* 1992). Note that such genes behave like a sex-chromosome. This may explain the very fast evolution of *M. menidia* populations but further genetic characterization is needed for a quantitative approach to this system. The Xiphophorus system (and very probably Menidia) did not have parental control, nor did they have biparental transmission (autosomal inheritance) of the alleles controlling the sexual proportion. Their results showed that zygotic control coupled to major genes is more than a mere violation of formal assumptions: it causes a characteristically fast evolution. As shown in Figure 3, the *X. maculatus* populations (and also *M. menidia*; Conover *et al.* 1992) evolved nearly 100 times faster than the *D. mediopunctata* populations. Furthermore, the Fisherian prediction of equal investment does not apply to systems with zygotic control (Trivers 1974): if males cost twice as much as the females, the equilibrium sexual proportion in a Xiphophorus-type system is 48.1%, whereas the Fisherian optimum is 33% (Bull 1983, p. 83). Although sex-chromosomes and sex-determining genes (with Mendelian segregation) are under the scope of Fisher's Principle (Bull and Charnov 1988; Conover and Van Voorhees 1990; Basolo 1994), we think they are special cases, with particular properties, and are not representative of the general mechanism envisioned by Fisher (1930). The Xiphophorus and Menidia results provided clear examples of convergence to the 1:1 ratio in these special cases. Fisher's Principle has also been tested in a very elegant (and unfortunately unpublished) study by S. W. Skinner, using the haplo-diploid wasp *Nasonia vitripennis.*

The above points are quite related to Lyttle's (1981) discussion of the distinct ways in which skewed sexual proportions may be "corrected." Lyttle's discussion was based on his findings with pseudo-*Y* meiotic drive in *D. melanogaster*, which was generated by translocation of the *Sd* gene (a meiotic driver localized on chromosome *II*) to the *Y* chromosome. Some experimental populations went extinct due to lack of females whereas suppressors evolved in other popula-

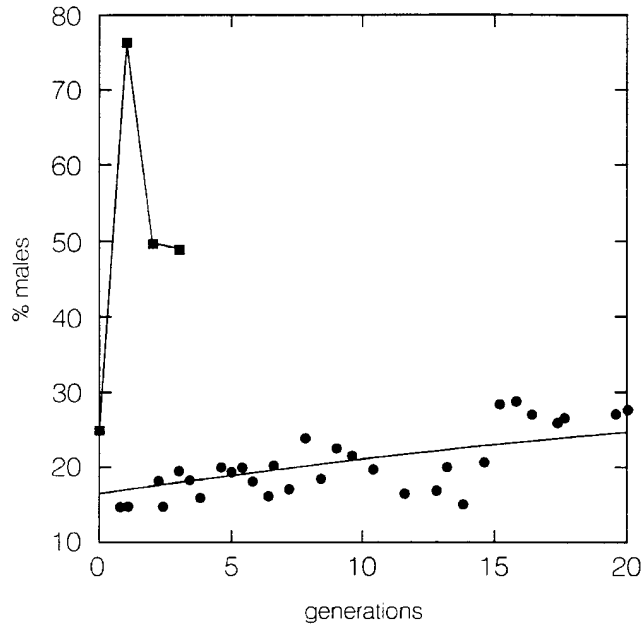

Figure 3.—Comparison of the rate of evolution of *D. mediopunctata* and *X. maculatus* populations. Solid circles: *D. mediopunctata* populations (data from the first 20 generations, average of four populations; see Figure 2B). Solid squares: *X. maculatus* populations (data from Basolo 1994; average of the two female-biased populations).

tions, though it was not possible to separate sex-chromosome effects (suppressors localized either in the *X* or in the normal chromosome II, which are meiotically linked by the translocation) from truly autosomal suppressors (Lyttle 1977, 1979). However, in one population the sexual proportion was "corrected" by the evolution of sex-chromosome aneuploidy (due to its meiotic behavior, aneuploidy induces more even sexual proportions; Lyttle 1981). This very unexpected alternative route may have occurred in the karyotypic evolution of several species, particularly in insects with unusual sex chromosome karyotypes (Lyttle 1982). Also, it is interesting to note that both Menidia and Xiphophorus systems are natural and in both cases the evolutionary response was not the classical Fisherian selection. This suggests that alternative routes to Fisher's Principle may be common.

In conclusion, our results provided an experimental demonstration of Fisher's Principle that obeys all its assumptions. They showed that female biased populations evolved toward equal sex proportions due to natural selection, exactly as predicted by Fisher (1930). They also lend additional support to the field of sex-allocation theory, whose models are all based on Fisher's Principle (Charnov 1982; Bull and Charnov 1988). Given the genetic facilities provided by the *sex-ratio* trait of *D. mediopunctata*, it was possible to exclude sex-chromosome effects, which is not feasible in other systems. We observed a rather slow evolution and the rate of return to equal sexual proportions is an important issue: strong male or female bias caused, for example, by the invasion of a *SR* chromosome or by global warming in species with temperature sex determination, might drive a population or species to extinction before it could respond (Gershenson 1928; Hamilton 1967; Chanter and Owen 1972; Lyttle 1977; Janzen 1994). Classical Fisherian selection (parental and autosomal control of the sexual proportion) is the most general evolutionary response because it applies to many sex-determining systems (Bulmer and Bull 1982; Bull and Charnov 1988) and there is potentially much genetic variation available (as the majority of the genes are autosomal), but it may be slow. Other related evolutionary mechanisms involving three sex-chromosome systems (Karlin and Lessard 1986; Basolo 1994), *Y*-linked suppressors of *SR* (Stalker 1961; Carvalho *et al.* 1997) or sex-chromosome aneuploidy (Lyttle 1981) may cause a very fast return to 1:1 (thus preventing classical Fisherian selection), but they depend critically on the sex-determining system and on the availability of suitable genetic variation. The relative importance of both types of response is unknown. They may occur together: *D. mediopunctata* natural populations contain, as well as *SR* chromosomes, both autosomal suppressors (Carvalho and Klaczko 1993; Varandas *et al.* 1997) and *Y*-linked suppressors of *SR* expression (Carvalho *et al.* 1997), probably the result of classical Fisherian selection and of meiotic drive, respectively.

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APPENDIX

Derivation of Equation 1: Nur (1974; see also Uyenoyama and Bengtsson 1979) studied the expected changes in the frequency of autosomal alleles affecting the sexual proportion, *i.e.*, he studied a Mendelian model for Fisher's Principle. Equation 1 may be obtained by converting this genetic change into phenotypic change, using standard quantitative genetics theory. The following account was based on Nur's (1974) paper, which should be consulted for more details.

Nur assumed that the sexual proportion is controlled by parental females, though the same results would be obtained under male control (as occurs in *D. mediopunctata*). The sexual proportion of the progenies is determined by an autosomal locus *S*, as shown below

Female genotypes	S_1S_1	S_1S_2	S_2S_2
Frequency of genotype	X_f	$2Y_f$	Z_f
Sexual proportion in progeny	e	$e + b - c$	$e + b$

where $e \geq 0$, $b > 0$, $(e + b) < 1$, $b \geq c$, and $c \geq 0$. Thus, the sexual proportions produced by each genotype are $S_1S_1 < S_1S_2 \leq S_2S_2$. Let q_m and q_f be the frequency of S_2 among males and females, respectively. Then, in the next generation (G') the frequency of S_2 among the zygotes (q_t'; throughout this appendix, primes indicate the successive generations) will be $q_t' = \frac{1}{2} q_m + \frac{1}{2} q_f$. According to Nur (1974), "in the previous generation, *G*, the frequency of S_2 among the zygotes, q_t, was $q_t = q_f (1 - M) + q_m M$, with M representing the sexual pro-

portion among the zygotes in *G*. The change in the frequency of S_2 is then

$$\Delta q_t = q_t' - q_t = \tfrac{1}{2}q_m + \tfrac{1}{2}q_f - q_f(1-M) - q_m M = (\tfrac{1}{2} - M)(q_m - q_f). \quad (3)"$$

Nur (1974) showed that Equation 3 may be expressed as follows

$$\Delta q_t = \tfrac{1}{2}\frac{(\tfrac{1}{2} - M')}{M'(1-M)}(X_f Y_f (b-c) + X_f Z_f b + Y_f Z_f c) \quad (4)$$

where $\Delta q_t = q_t'' - q_t'$ and $M' = X_f e + 2Y_f(e + b - c) + Z_f(e + b)$. Assuming that the female frequencies X_f, $2Y_f$ and Z_f are in Hardy-Weinberg proportions (which is an approximation; Nur 1974) and expressing the phenotypic values e, $(e + b - c)$ and $(e + b)$ in the standard scale for quantitative genetics ($+ a$, d, and $- a$, respectively; Falconer 1989), we obtained after some algebraic transformations

$$\Delta q_t = -\tfrac{1}{2}p_f q_f \alpha \frac{\tfrac{1}{2} - M'}{M'(1-M')} \quad (5)$$

where α is the average effect of gene substitution (Falconer 1989).

According to Falconer (1989, p. 202), the expected phenotypic change caused by changes in gene frequency is

$$\Delta M \approx -2\alpha\Delta q \quad (6)$$

where M is the population mean of the quantitative trait. Note that the average sexual proportion produced by the females (M in Falconer's sense) is the same as the sexual proportion of the population (M in Equations 3–5 sense).

Substituting (5) in (6), we obtained

$$\Delta M \approx p_f q_f \alpha \frac{\tfrac{1}{2} - M}{M(1-M)} = \tfrac{1}{2}V_A \frac{\tfrac{1}{2} - M}{M(1-M)} \quad (7)$$

which is equivalent to our Equation 1, for V_A (the additive genetic variance) is equal to $V_p h^2$.

Some approximations were necessary for the passage from (5) and (6) to (7). This is due to the meaning of (6) in the context of parental and female-limited control of the sexual proportion. Thus, in (6) Δq should read Δq_f instead of Δq_t (female-limited expression). Note also that q determines M' and not M (parental control) and that Δq_t in (5) refers to $(q_t'' - q_t')$; thus ΔM in Equation 7 refers to $(M''' - M'')$. The approximations necessary to the obtainment of (7) may be summarized as $(q_t' - q_t) \approx (q_t'' - q_t') \approx (q_f' - q_f)$. We verified the overall validity of these approximations and, hence, the precision of (7), by comparison of its predicted ΔM with the ΔM produced by direct iteration of allelic frequencies in Nur's equations. As shown in Figure 4, the fit was very good. This result is important because it shows that under parental control the genetic basis (polygenes or major gene) is irrelevant to the rate of Fisherian evolution.

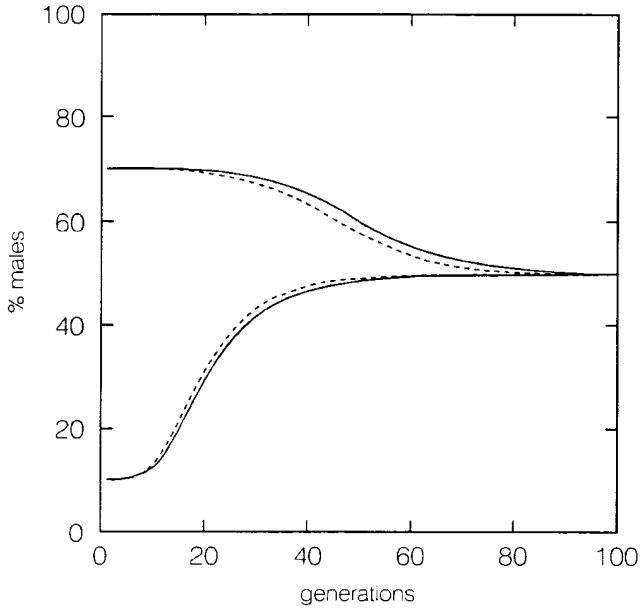

Figure 4.—Comparison of Equation 7 with Nur's Mendelian model. Solid lines: standard Fisherian trajectories, generated by iteration of Nur's equations. Sexual proportions produced by S_1S_1, S_1S_2, and S_2S_2 females were respectively 10%, 30%, and 70%; initial S_2 frequency was 0.999 (upper lines) or 0.001 (lower lines). Broken lines: Equation 7 trajectories, obtained as follows. From generation 4 on (after the "shuffling" of allelic frequencies between males and females), the V_A parameter was obtained each generation from the Nur's model allelic frequencies (solid line). Each iteration of (7) used its previously calculated M.

Equation 7 shows explicitly that the Fisherian rate of evolution depends directly only on the additive variance for the sexual proportion and on the population sexual proportion, and not on the heritability. This is important because the heritability of sexual proportion is severely limited by the binomial variance, unless progeny number is very large (for a general discussion on this topic, see Falconer 1954 and Varandas *et al.* 1997). For example, Varandas *et al.* (1997) found an heritability of 41%, with an average progeny number of 110; this value would fall to 21% if the progeny number was five. The reduction of heritability would be even larger in stable natural populations, in which the average lifetime progeny number is two. Equation 7 shows that the binomial variance (and the associated reduction in the heritability) has no effect on the Fisherian rate of evolution.

Precision of Equation 2a and of the $V_p h^2$ estimation procedure: Equation 2a was obtained from (1) by approximating ΔM (the change in one generation) for dM/dt. To verify the precision of Equation 2a and of the $V_p h^2$ estimation procedure, we simulated with (1) several Fisherian trajectories with arbitrary $V_p h^2$ and M_0 values. Then, we estimate $V_p h^2$ in these fictitious data, applying the linear regression after the linearizing

transformation (Equation 2a). The agreement between the estimated $V_p\ h^2$ and M_0 and the arbitrary values used in the simulation was excellent in the range of *D. mediopunctata*'s values ($V_p\ h^2 \approx 0.005$; and $M_0 \approx 0.15$); when we simulated a Fisherian trajectory using these values, the "Equation 2a, linear regression procedure" estimated them as 0.00504 and 0.151, respectively. The fit was even better for smaller $V_p\ h^2$ (not shown), as probably occurs in most biological systems, since *D. mediopunctata* has an unusually large amount of genetic variability for sexual proportion (Varandas *et al.* 1997). Thus, the "Equation 2a, linear regression procedure" may be applied to most cases of Fisherian evolution.