

Y-linked suppressors of the *sex-ratio* trait in *Drosophila mediopunctata*

ANTONIO BERNARDO DE CARVALHO* & LOUIS BERNARD KLACZKO†

Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Caixa Postal 68011, CEP 21944-970, Rio de Janeiro and †Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, Caixa Postal 6109, CEP 13081-970, Campinas, SP, Brazil

X-linked meiotic drive causing female-biased progenies is known to occur in nine *Drosophila* species and is called '*sex-ratio*'. In *D. mediopunctata* this trait is associated with the X:21 chromosome inversion and has variable expression. We describe here a powerful Y-linked suppressor system of *sex-ratio* expression in this species. There are two types of Y chromosomes (suppressor and nonsuppressor) and two types of X:21 chromosomes (suppressible and unsuppressible). *Sex-ratio* expression is suppressed in males with the 21^{suppressible}/Y^{suppressor} genotype, whereas the remaining three genotypes produce female-biased progenies.

Keywords: *Drosophila mediopunctata*, meiotic drive, *sex-ratio*, Sex ratio evolution, Y chromosome.

Introduction

Meiotic drive is the genetic condition where a heterozygote produces an unequal proportion of the two types of gametes (Sandler & Novitsky, 1957; Lyttle, 1991). In *Drosophila*, meiotic drive of the X chromosome against the Y (giving rise to female-biased progenies) is known in nine species and is called '*sex-ratio*' (Gershenson, 1928; James & Jaenike, 1990). In natural populations this condition is polymorphic, i.e. both driver and nondriver X chromosomes occur. In most cases the driver chromosomes (called hereafter 'SR') are associated with chromosomal inversions. SR chromosomes may represent a threat to a species because their fixation (as a result of the meiotic drive) will lead to its extinction due to lack of males (Gershenson, 1928; Hamilton, 1967). These polymorphisms can be stabilized by natural selection against SR, as occurs in *D. pseudoobscura* (Wallace, 1948; Curt-singer, 1991) and also by autosomal or Y-linked modifier genes which suppress or attenuate the meiotic drive (Stalker, 1961; Carvalho & Klaczko, 1993).

Autosomal suppressors are expected to evolve in populations with SR chromosomes because they direct reproduction to the rarer sex, the males. This is a case of the mechanism known as Fisher's Principle, which is the most accepted evolutionary explanation for the

equivalence of sexual proportions (Fisher, 1930; Bull & Charnov, 1988; Conover & Voorhees, 1990). Autosomal suppressors of *sex-ratio* compatible with the operation of Fisher's Principle have been described in *D. mediopunctata* (Carvalho & Klaczko, 1993); their existence has also been suggested in *D. paramelanica* (Stalker, 1961).

Any Y-linked gene that increases the transmission rate of the Y chromosome is expected to spread in the population (Thomson & Feldman, 1975). This meiotic drive mechanism explains why Y-linked suppressors of *sex-ratio* are expected to evolve: in the presence of a SR chromosome, this suppressor Y is transmitted to half of the progeny (the males) whereas a normal Y is not transmitted at all (only females in the progeny). Stalker (1961) described a powerful suppressor of this kind in *D. paramelanica*; a similar one is present in *D. affinis* (Voelker, 1972) but not in natural populations of *D. pseudoobscura* (Policansky & Dempsey, 1978; Beckenbach *et al.*, 1982).

Several interesting questions are raised by the study of Y-linked suppressors of *sex-ratio*. First of all, they are expected to evolve in natural populations because they are strongly favoured. Second, they are not expected to be polymorphic (except transiently), as theoretical studies have shown that the conditions for stable Y polymorphisms (except neutral) are particularly restrictive, even with meiotic drive (Clark, 1987). Third, the rapid turnover of X chromosomes (driver X

*Correspondence.

replacing nondriver X) and Y chromosomes (suppressor ones replacing nonsuppressor ones) may explain the large role of sex chromosomes in the postzygotic isolation between closely related species (Coyne & Orr, 1989): isolated populations can evolve different and incompatible X driver/Y suppressors systems, leading to hybrid sterility/inviability (Frank, 1991; Hurst & Pomiankowski, 1991). As Y-linked suppressors of *sex-ratio* have been studied in detail in only one species (*D. paramelanica*), more empirical studies are clearly desirable to test current theories and to stimulate new ones.

There are four gene arrangements in the X chromosome of *D. mediopunctata*: Standard and inversions 1, 2 and 3. Inversions 1 and 2 usually occur associated; X chromosomes carrying both are named X:2+1 or simply X:21. The X:2 and X:21 gene arrangements are associated with the *sex-ratio* trait, which has a very variable expression in *D. mediopunctata* (Carvalho *et al.*, 1989; Carvalho, 1989). Previous investigations of the causes of this variability have demonstrated a male age effect (Carvalho & Klaczko, 1992) and autosomal suppressors of *sex-ratio* expression (Carvalho & Klaczko, 1993). Now we report a rather complex Y suppressor system in this species.

Materials and methods

D. mediopunctata strains

All strains are descended from flies collected at Parque Nacional do Itatiaia (State of Rio de Janeiro, Brazil) between 1986 and 1987, either from inseminated females or from males crossed with laboratory females.

ITA-24-P is a reference strain carrying the Standard ('ST') gene arrangement and free of strong autosomal suppressors. Its Y chromosome will be called hereafter Y^{lab} .

ITC-61-A is a strain homokaryotypic for a reference X:21 (called hereafter 21^{lab}) carrying the cytoplasm from ITA-24-P. As males with genotype $21^{\text{lab}}/Y^{\text{lab}}$ can produce *sex-ratio* progenies, Y^{lab} is a nonsuppressor Y and 21^{lab} is a functional SR chromosome.

ITC-229-ET is a derivation of ITA-24-P sharing its cytoplasm, X chromosome and half of the autosomes.

Method of detection of Y-linked suppressors and nonfunctional X:21

The method we have used (Fig. 1) is composed of three steps (see below; more details are given in Carvalho [1989]). Only progenies containing 20 or more flies were considered.

(a) *Identification of suspect wild-caught 21/Y males* Wild-caught *D. mediopunctata* males were crossed in the laboratory with three ITC-229-ET virgin females (ITA-24-P females were used with the ITD-332 male; see the Results). The male X chromosome gene arrangement was determined by the inspection of salivary gland squashes of up to eight F_1 larvae. The emerging adults were sexed and counted until vials were exhausted. The distributions of progenies of these males (and further details) are shown in Carvalho *et al.* (1989). Several of the 21/Y males did not express the *sex-ratio* trait (that is, they did not produce female-biased progenies). We selected seven of them to test their X:21 and Y chromosomes (called hereafter $21^?$ and $Y^?$).

(b) *Synthesis and test of $21^?/Y^?$ males with controlled background* We crossed in mass the F_1 of the selected male and then crossed individually 24 F_2 males with three ITA-24-P virgin females to measure their *sex-ratio* expression. Half of these males were $21^?/Y^?$ (the other half were $ST/Y^?$) and were identified by the polytene chromosomes of one daughter larva. These males carried the same X:21 and Y of the wild-caught parental male, the cytoplasm and half of the autosomes from ITC-229-ET. Note that the ITC-229-ET cytoplasm came from ITA-24-P. We counted the progeny of $21^?/Y^?$ males and also of some $ST/Y^?$, as a control. If at least one F_2 $21^?/Y^?$ male expressed *sex-ratio* (defined as a progeny containing less than 20 per cent males), we stopped at this point, concluding that in principle $21^?$ was functional and $Y^?$ was not a *sex-ratio* suppressor.

(c) *Separation of X:21 from Y effects* If none of the F_2 $21^?/Y^?$ males expressed *sex-ratio*, we concluded that $Y^?$ was a suppressor, or $21^?$ was nonfunctional or that there was some specific interaction between their sex chromosomes. To discriminate among these three hypotheses we produced males with the four possible combinations between $21^?$, 21^{lab} , $Y^?$ and Y^{lab} (Fig. 1, F_4 and F_6 generations) and measured their *sex-ratio* expression as before.

Rationale

This method has been designed to detect genetic variation for *sex-ratio* expression caused by the sex chromosomes. When a wild caught $21^?/Y^?$ male does not express *sex-ratio* (step a), several other factors may also be the cause: it may carry autosomal or cytoplasmic suppressors or might have been exposed in nature to some inhibitory environmental condition (temperature, food, etc.). We first separated these factors from X-Y effects by generating several $21^?/Y^?$ males in suit-

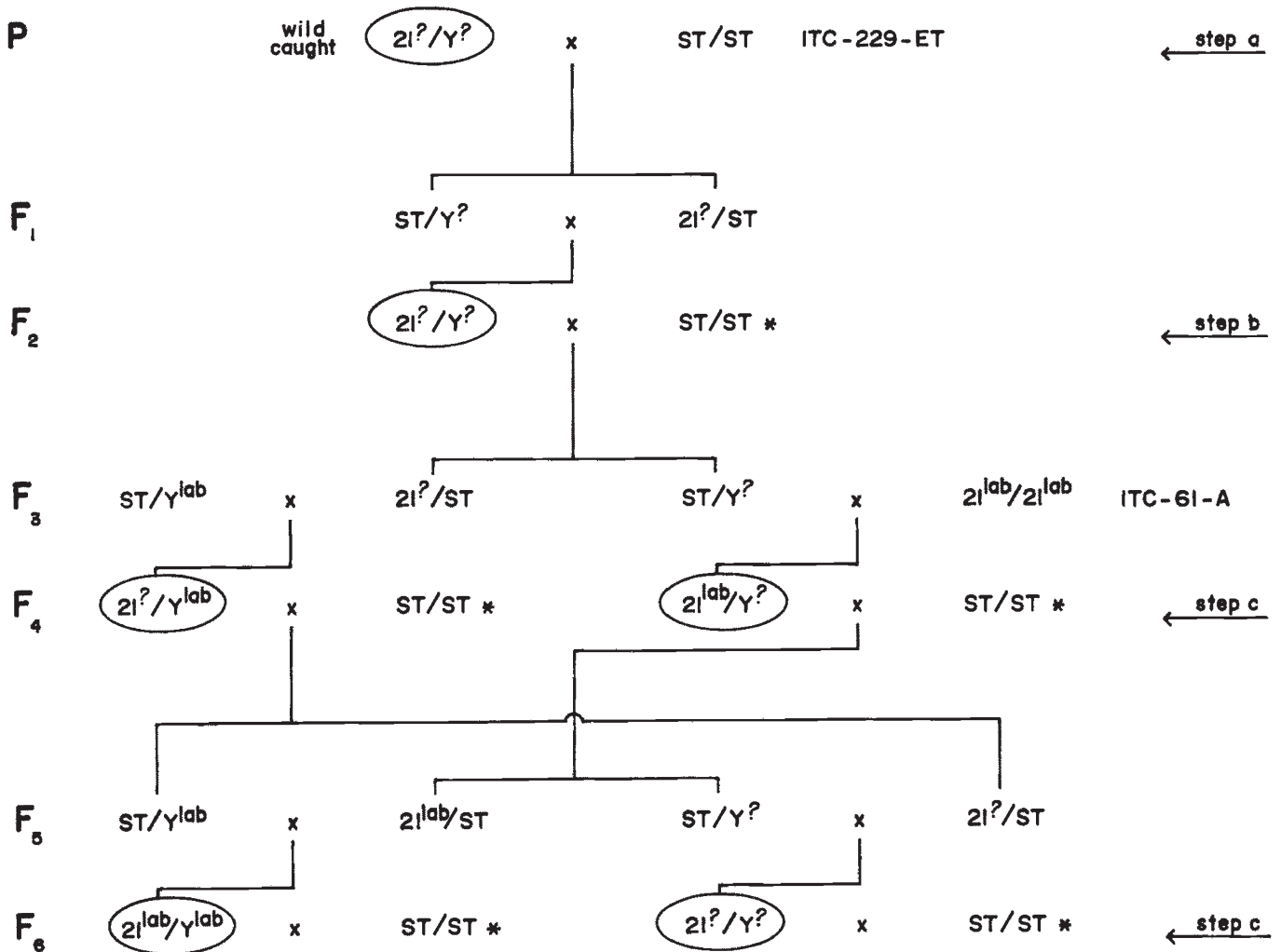


Fig. 1 Method of detection of Y-linked suppressors and nonfunctional X:21. Steps are indicated with arrows. Only the relevant genotypes are included in the scheme. Males tested for *sex-ratio* expression are encircled. The flies marked with an asterisk belong to the ITA-24-P strain.

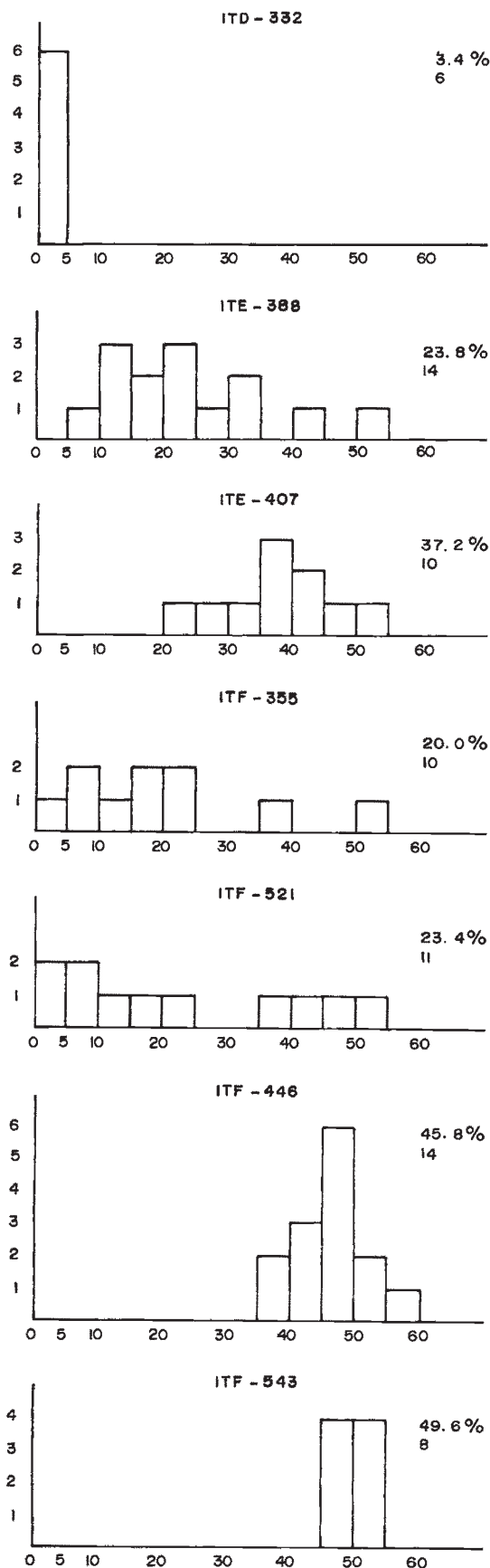
able conditions for *sex-ratio* expression, that is, with ITA-24-P cytoplasm, autosomes segregating for ITC-229-ET alleles and reared at 16.5°C on laboratory medium (step *b*). If none of them expressed *sex-ratio*, their sex-chromosomes were probably the cause. We tested this hypothesis and disentangled X from Y effects producing the four possible combinations between 21[?], 21^{lab}, Y[?] and Y^{lab} (step *c*). For example, compare the F₆ males 21[?]/Y[?] and 21^{lab}/Y^{lab} (Fig. 1): they have exactly the same cytoplasm and autosomes so any differences between them must be caused by the X or Y chromosomes.

Results

The seven selected strains that did not express *sex-ratio* were: ITD-332 (31.7 per cent males in the

progeny of the wild-caught male), ITE-388 (45.0 per cent), ITE-407 (28.9 per cent), ITF-355 (39.8 per cent), ITF-521 (64.5 per cent), ITF-446 (56.2 per cent) and ITF-543 (46.5 per cent). The distributions of progenies of their F₂ 21[?]/Y[?] males are shown in Fig. 2.

In four strains—ITD-332, ITE-388, ITF-355 and ITF-521—the F₂ 21[?]/Y[?] males clearly expressed *sex-ratio*, corresponding to the situation described in step *b* in Materials and methods. Their mean *sex-ratio* expression compares well with the value obtained from wild-caught 21/Y males (20.5 per cent; Carvalho *et al.*, 1989). These four strains were supposed to carry a nonsuppressor Y and a functional X:21 at the beginning. The case of ITE-407 was not clear: although none of the progenies produced by its F₂ males contained less than 20 per cent males, their distribution and mean were skewed to an excess of females.



The $21^2/Y^2$ males of ITF-446 and ITF-543 were clearly non-*sex-ratio*, corresponding exactly to step *c*: they must carry a suppressor Y or a nonfunctional X:21 or there was some specific interaction between their sex-chromosomes. We followed step *c* to clarify this point. Figure 3 shows all the results produced by the application of our method to strains ITF-446 and ITF-543. F_4 $21^{ITF-466}/Y^{lab}$ and $21^{lab}/Y^{ITF-446}$ males expressed *sex-ratio* and the same result was obtained for the ITF-543 chromosomes. Moreover, none of the F_6 $21^{ITF-446}/Y^{ITF-446}$ and $21^{ITF-543}/Y^{ITF-543}$ males were *sex-ratio* whereas F_6 $21^{lab}/Y^{lab}$ males (the positive control, tested only for ITF-543) again produced female-biased progenies.

To summarize, $Y^{ITF-446}$ suppressed the *sex-ratio* expression of $21^{ITF-446}$ but not of 21^{lab} ; $21^{ITF-446}$ was suppressed by $Y^{ITF-446}$ but not by Y^{lab} . The same occurred with $21^{ITF-543}$ and $Y^{ITF-543}$. The conclusion is unambiguous: there are at least two types of Y chromosomes (suppressor [$Y^{ITF-446}$, $Y^{ITF-543}$] and nonsuppressor [Y^{lab}]) and two types of X:21 chromosomes (suppressible [$21^{ITF-446}$, $21^{ITF-543}$] and unsuppressible [21^{lab}]). $21^{suppressible}/Y^{suppressor}$ males did not express *sex-ratio*; all other genotypes expressed it, producing female-biased progenies.

We also tested $21^{ITF-446}/Y^{ITF-543}$ and $21^{ITF-543}/Y^{ITF-446}$ males and found that none of them expressed *sex-ratio* (means: 59.7 per cent, $n = 10$ and 48.3 per cent, $n = 10$, respectively). Thus, the X:21 and Y chromosomes from the ITF-446 and ITF-543 strains belong to the same *sex-ratio* expression type.

Y^{lab} appears to be consistently nonsuppressor, as ten different X:21 strains can express *sex-ratio* with it. We also tested another unsuppressible X:21 (derived from an independent strain, ITC-221) against eight different Y chromosomes (three of them suppressors): none could suppress it (data not shown).

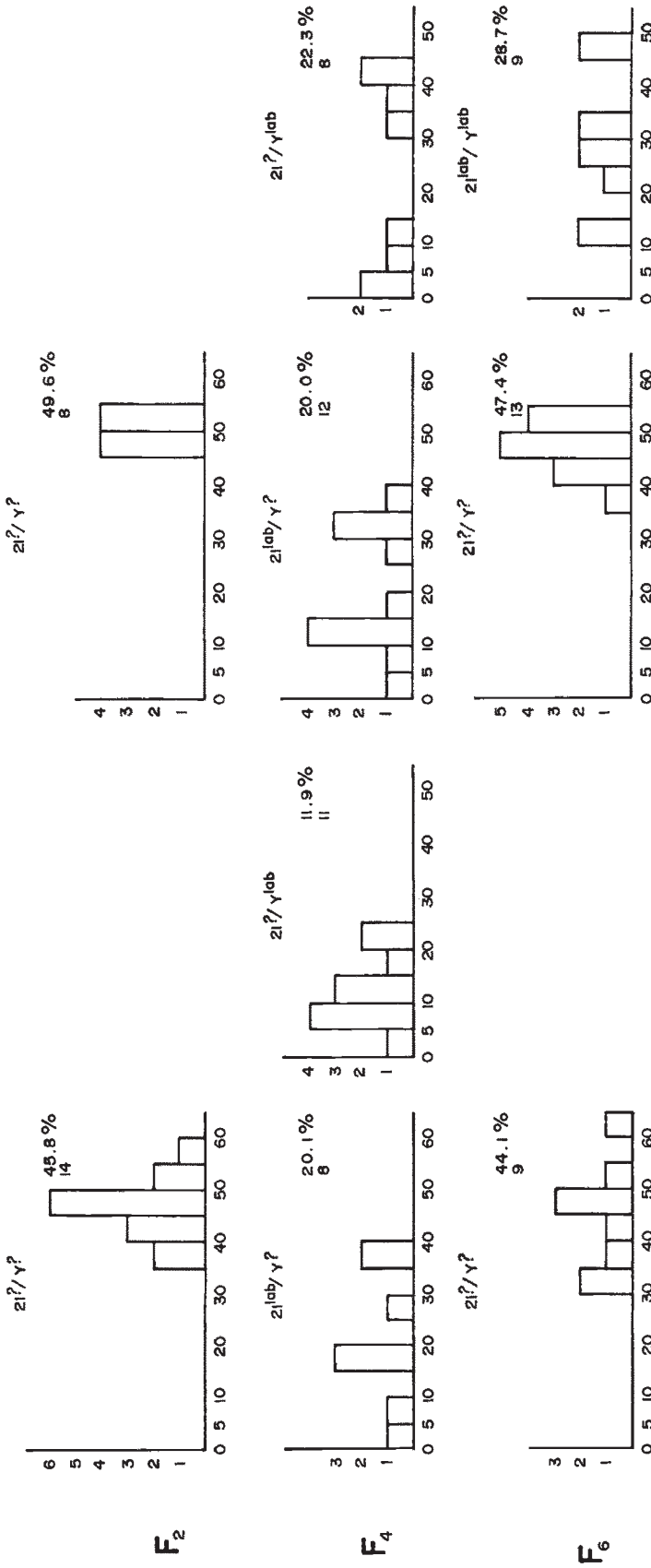
Figure 4 shows the distribution of progenies of all ST/Y, suppressed 21/Y and nonsuppressed 21/Y males tested until now. As it can be seen, in our present experimental conditions the Y-suppression is very strong, the suppressed 21/Y males being identical to ST/Y males in the mean sex ratios of their progenies.

The suppressed 21/Y males and their sons showed normal fertility throughout the experiment.

Fig. 2 Progenies of F_2 $21^2/Y^2$ males. The strain name, the mean sex ratio and the number of progenies are indicated in each histogram. Abscissae—per cent males; ordinates—absolute frequency of progenies.

ITF - 543

46.5% (66:76)



ITF - 446

56.2% (41:32)

Fig. 3 Application of the method of detection of Y-linked suppressors and/or nonfunctional X:21 to the ITF-446 and ITF-543 strains. The male genotype is indicated in each histogram. Axes are the same as in Fig. 2.

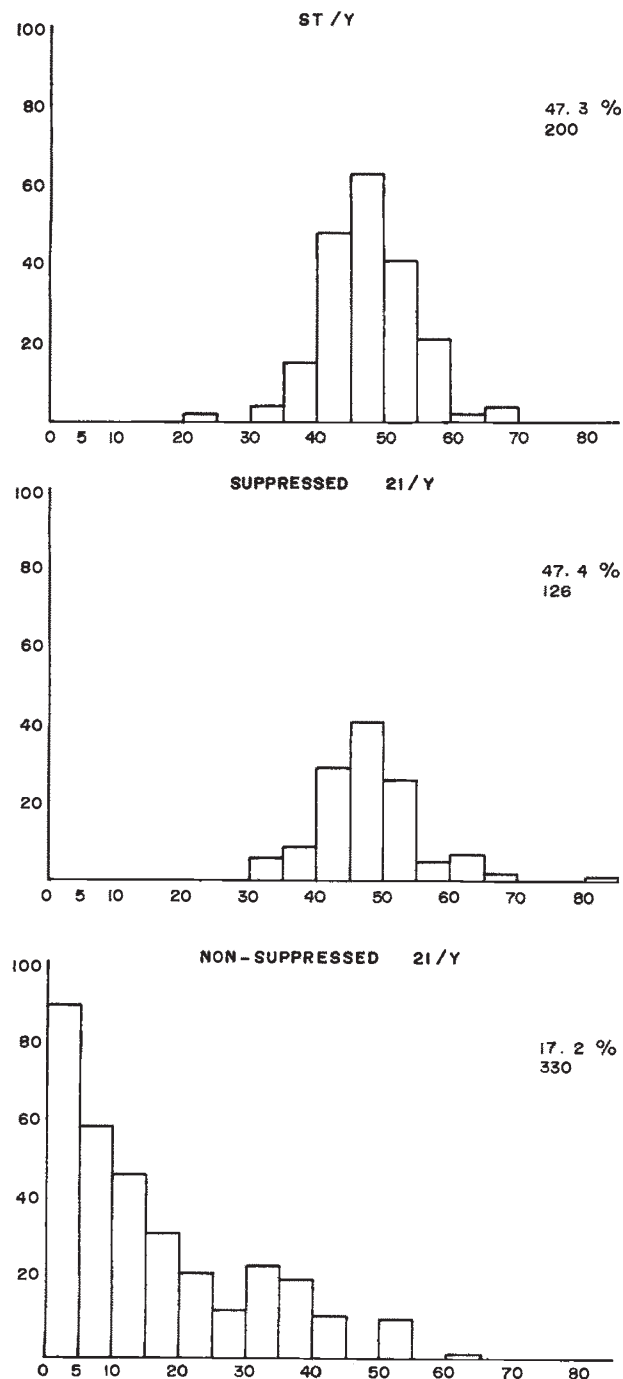


Fig. 4 Progenies of ST/Y, suppressed 21/Y and non-suppressed 21/Y males. The male genotype is indicated in each histogram. Axes are the same as in Fig. 2.

Discussion

Our results demonstrate that *D. mediopunctata* has a strong Y-linked suppressor of *sex-ratio* expression. This suppressor system is complex, with at least two types of Y chromosomes (suppressor and nonsuppressor) and two types of X:21 chromosomes (suppressible

and unsuppressible). Suppression results from a specific interaction between a suppressible X:21 and a suppressor Y. In our experimental conditions *sex-ratio* expression was lost in males with this genotype; the three other genotypes ($21^{\text{suppressible}}/Y^{\text{nonsuppressor}}$, $21^{\text{unsuppressible}}/Y^{\text{suppressor}}$, $21^{\text{unsuppressible}}/Y^{\text{nonsuppressor}}$) produced female-biased progenies.

The analysis of these results must take into consideration the limitations (and potentialities) of the method we have used. The main limitation of our method comes from its inability to control all the autosomal background, which could be particularly problematical given that autosomal suppressors of *sex-ratio* have been described in *D. mediopunctata* (Carvalho & Klaczko, 1993). This meant that we could not unambiguously detect subtle variations in the sex chromosomes: the effect of a weak Y suppressor, for example, cannot be distinguished from autosomal suppressors 'diluted' by the successive backcrosses to the 'clean' laboratory background. So the existence of additional types of Y and X:21 chromosomes such as weak Y suppressors could not be ruled out.

Y-linked suppressors of *sex-ratio* expression have been studied in detail only in *D. paramelanica* which is strikingly similar to *D. mediopunctata* in this aspect: the two Y types (Northern and Southern) and the two SR types (Northern and Southern) of *D. paramelanica* also interact specifically; only males with the SR Northern/Y Southern genotype are suppressed. However, in *D. paramelanica* an intrapopulational polymorphism for SR chromosomes was not found since the two types have distinct geographical distributions (Stalker, 1961). A specific interaction between Y and SR chromosomes is also present in *D. affinis* (Voelker, 1972).

If meiotic drive is the sole evolutionary force acting in the *sex-ratio* system of *D. mediopunctata*, X:21 is at an advantage in relation to X:ST, in the same way as $21^{\text{unsuppressible}}$ is in relation to $21^{\text{suppressible}}$ and $Y^{\text{suppressor}}$ is in relation to $Y^{\text{nonsuppressor}}$. Because of Fisher's Principle, the autosomal suppressor alleles are also at an advantage. One would expect, thus, the fixation of at least some of the favoured alleles. However, there is an extensive polymorphism in *D. mediopunctata* for all these 'loci' (Carvalho *et al.*, 1989; Carvalho and Klaczko, 1993; this paper). An equilibrium between meiotic drive and other fitness components could stabilize some of these polymorphisms, as has been demonstrated in *D. pseudoobscura* for X:ST and X:SR (Wallace, 1948; Curtsinger, 1991), but this is much more difficult with the Y polymorphism (Clark, 1987). We do not know if these polymorphisms are stable, transient or even almost neutral relics of a past history of intense oscillations in genic frequencies.

In order to obtain some answers to these questions we are not typing Y and X:21 chromosomes from natural populations (with a reference suppressible X:21 and a reference suppressor Y, respectively), controlling all the autosomal background and the age of the males (the experiments we report here were done in 1987–1988, before we knew the effect of male ageing). At present, we know that the frequency of Y suppression among 21/Y males is approximately 10 per cent (35 per cent of the wild-caught 21/Y males produced suspected progenies; two males in seven suspects tested were suppressed by Y).

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