Abnormal spermiogenesis is associated with the X-linked *sex-ratio* trait in *Drosophila simulans*

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The *sex-ratio* trait, known in several *Drosophila* species, results from X-linked meiotic drive that affects Y-bearing sperm and causes males to produce female-biased progeny. We describe spermiogenesis in three types of *D. simulans* males: wild-type, *sex-ratio*, and males that bear driver X chromosomes but do not express the *sex-ratio* trait because of autosomal and Y-linked suppression. *Sex-ratio* males show numerous spermatid nuclei in abnormal positions throughout their cysts, which occurs rarely, if ever, in the two other types. The degree of the spermiogenic failure in *sex-ratio* males is correlated with the bias towards females in their progeny. This proves that the trait is a case of meiotic drive and strongly suggests that the abnormal spermatids are Y-bearing ones. The number of cysts per testis, hence the production of X-bearing sperm, is not increased in *sex-ratio* males. Implications for the spread of the *sex-ratio* trait in natural populations of *D. simulans* are discussed.

Keywords: *Drosophila simulans*, *sex-ratio*, spermiogenesis.

Introduction

The *sex-ratio* trait is a case of meiotic drive caused by X-linked factors and occurs naturally in several *Drosophila* species. Males bearing a driver X chromosome, so called *sex-ratio* males transmit predominantly X-bearing sperm which results in only or mostly female progeny. *Sex-ratio* males were first described within the subgenus *Sophophora*, in species belonging to the *Drosophila obscura* group: *D. affinis* (Morgan *et al.*, 1925), *D. obscura* (Gershenson, 1928), *D. pseudoobscura*, *D. persimilis*, *D. athabasca*, *D. azteca* (Sturtevant & Dobzhansky, 1936), and *D. subobscura* (Jungen, 1968). They are also known in unrelated species belonging to the subgenus *Drosophila*: *D. paramelanica* (Stalker, 1961), *D. mediopunctata* (De Carvalho *et al.*, 1989) *D. testacea* (James & Jaenike, 1990).

When cytologically investigated, the *sex-ratio* trait was found to be related to spermatogenic defects (for a review see Lyttle, 1991). In *D. subobscura* a clear sign of sperm dysfunction is the abnormal position of about half the spermatid nuclei, presumably Y-bearing ones. This occurs in short and long cysts of *sex-ratio* males in this sperm-heteromorphic

species (Hauschteck-Jungen & Maurer, 1976). Abnormal behaviour of the Y chromosome during meiosis was described in *D. pseudoobscura sex-ratio* males (Sturtevant & Dobzhansky, 1936; Novitsky *et al.*, 1965; Cobbs *et al.*, 1991); however, Y-bearing spermatids are probably eliminated during the individualization process (Policansky & Ellison, 1970).

In *Drosophila simulans*, a species of the *melanogaster* subgroup, which is monomorphic for sperm length (Joly *et al.*, 1989), *sex-ratio* males were first reported by Faulhaber (1967), who observed abnormal shape but normal position of spermatid nuclei in elongating cysts of these males. Strikingly in this case, the trait appeared to be caused by a recessive autosomal gene. Given that, theoretically, a recessive autosomal *sex-ratio* driver cannot be selected for, Hurst & Pomiankowski (1991) suggested that a 'hidden' X-linked driver was in fact responsible for the trait whose expression was suppressed by an autosomal factor. This hypothesis was backed up by the recent report in *D. simulans* of an X-linked *sex-ratio* trait that is rarely if ever expressed within strains or natural populations because of the presence of autosomal and/or Y-linked suppressors (Merçot *et al.*, 1995; Atlan *et al.*, in press; Montchamp-Moreau *et al.*, in press). The *sex-ratio* trait described by Faulhaber is expressed at 16°C and not at 25°C whereas that described by Merçot *et*

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al. (1995) is well expressed at the latter temperature. It cannot be excluded that two different *sex-ratio* systems occur in *D. simulans*.

If sperm are not in limited supply, driver X chromosomes are transmitted by males at a higher rate than normal X and consequently are expected to invade the populations rapidly (Gershenson, 1928). However, they are usually found at low frequency in natural populations of *Drosophila* (Sturtevant & Dobzhansky, 1936; Dobzhansky & Epling, 1944; Jungen, 1968; Hauschteck-Jungen, 1990; James & Jaenike, 1990). A reduced fertility of *sex-ratio* males is one of the factors that might prevent the spread of driver X chromosomes. This probably occurs in *D. pseudoobscura* (Beckenbach, 1978; Wu, 1983a,b). By contrast, in *D. subobscura*, *sex-ratio* males produce as many offspring as wild-type males even under multiple mating conditions (Hauschteck-Jungen *et al.*, 1987). The loss of Y-bearing sperm appears to be compensated by an increased production of cysts (Bircher & Hauschteck-Jungen, 1994).

Here we present data on spermiogenesis related to the X-linked *sex-ratio* trait in *D. simulans* (Merçot *et al.*, 1995). Driver X chromosomes in this species were found to occur at high frequency, at least in some island populations, which suggests that the fertility of *sex-ratio* males in this species is only slightly or not at all reduced (Atlan *et al.*, in press). Our purpose was: (i) first to look for spermiogenesis abnormalities in *sex-ratio* males and to assess whether these abnormalities were similar to those described by Faulhaber (1967); and (ii) to establish whether the number of cysts was increased in *sex-ratio* males, which could promote the spread of driver X chromosomes in natural populations.

Materials and methods

D. simulans stocks

ST: a standard strain, free of driver X chromosomes and of drive suppressors (Merçot *et al.*, 1995); its Y chromosomes are susceptible to the driver X chromosomes of the SR strain (see below).

SR: a strain derived from flies collected on Mahé Island, it carries driver *sex-ratio* X chromosomes together with autosomal and Y-linked drive suppressors. Thus, SR males do not express the *sex-ratio* trait and produce equal numbers of male and female progeny (Merçot *et al.*, 1995; Montchamp-Moreau *et al.*, submitted).

SR5: a strain carrying X-linked drive factors from the SR strain, in an ST genetic background. SR5 males express the *sex-ratio* trait because they do not bear drive suppressors (Montchamp-Moreau *et al.*, in press).

All the experiments were performed at 25°C on a cornmeal medium.

Sex-ratio males

Sex-ratio distortion is expressed in F₁ males from crosses between ST males and SR or SR5 females (denoted as F₁ (SR × ST) and F₁ (SR5 × ST), respectively). These males usually produce between 75 per cent and 100 per cent of females in their progeny, and are called *sex-ratio* males.

Cyst observations

The testes were dissected in Ringer solution then transferred to a drop of 4',6-Diamidino-2-phenylindole (DAPI) for staining of nuclei. Cysts were allowed to spread out and a coverslip was added. Lengths of cysts were measured at the beginning of individualization, using a video camera connected to a Macintosh computer. We used the public domain NIH IMAGE program (written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous ftp from zypki.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868). The DAPI-stained spermatid nuclei in fully (or nearly fully) elongated cysts were counted under a fluorescence microscope after gently squashing the slide. The shape of the nuclei observed varied from lanceolate to needle-shaped corresponding to P and Q stages of spermatogenesis (Fuller, 1993, p. 76).

Student's *t*-test was used to compare the mean cyst lengths and the mean numbers of cysts per testis for the different types of males studied. The comparisons of the numbers of spermatid heads per cyst between the different types of males were performed using nonparametric methods.

Cyst abnormalities and distortion strength

Newly emerged F₁ (SR × ST) *sex-ratio* males were individually mated with virgin ST females and kept together for 48 h in vials with cornmeal medium. Males were then removed, killed and dissected to determine, as previously described: (i) the number of cysts per testis; and (ii) the number and the localization of spermatid heads in fully elongated cysts. Females were allowed to lay eggs for four additional days. The emerging adults were counted until the vials were exhausted. The sex ratio was established for individual progenies of 50 offspring at least. This parameter shows an extra-binomial variance among *sex-ratio* males (data not shown), thus the correlations between the distortion strength and (i) the cyst number, and (ii) the number of

normal spermatids per cyst, were tested using the Spearman rank correlation coefficient.

Results

Cyst length

Lengths of fully elongated cysts were measured in ST and SR males, in *sex-ratio* F_1 ($SR \times ST$), and in reciprocal hybrids from the cross between ST females and SR males, denoted as F_1 ($ST \times SR$). In each case, we observed monomorphism for cyst length. The mean length was obtained from 100 cysts measured on both testes of three males (Table 1). ST males had significantly longer cysts than SR males (Table 2). Cyst lengths were not statistically different between F_1 males from the two reciprocal crosses, which indicated that the expression of the *sex-ratio* trait in the F_1 ($SR \times ST$) males had no effect on cyst length. Moreover, it implied that the difference in cyst length between ST and SR males was not related to the presence of driver chromo-

Table 1 Means and standard errors for cyst length ($N = 100$) and numbers of cysts per testis ($N = 50$, one testis per male) for *Drosophila simulans* ST, SR and F_1 hybrid males (for the origin of the F_1 hybrid males the maternal strain is indicated first)

	Cyst length (mm)	Cyst number/testis	
		<24 h	>48 h
ST	1.164 ± 0.006	51.98 ± 1.55	66.80 ± 2.61
SR	1.145 ± 0.007	41.84 ± 1.10	44.36 ± 1.35
F_1 ($SR \times ST$)*	1.206 ± 0.008	47.50 ± 1.25	59.12 ± 1.68
F_1 ($ST \times SR$)	1.206 ± 0.006	50.22 ± 1.60	62.70 ± 2.25

*Males expressing the *sex-ratio* trait.

Table 2 P -values of t -tests for cyst length and cyst number comparisons between *Drosophila simulans* ST, SR and the reciprocal F_1 hybrid males (for the origin of the F_1 hybrid males the maternal strain is indicated first)

	Cyst length <24 h	Cyst number	
		<24 h	>48 h
ST vs. SR	0.042	7.2×10^{-7}	6.4×10^{-11}
ST vs. F_1 ($ST \times SR$)	1.4×10^{-6}	0.431	0.236
ST vs. F_1 ($SR \times ST$)	4.7×10^{-5}	0.026	0.015
SR vs. F_1 ($SR \times ST$)	1.9×10^{-8}	9.9×10^{-4}	7.3×10^{-10}
SR vs. F_1 ($ST \times SR$)	7.5×10^{-11}	4.3×10^{-5}	6.9×10^{-1}
F_1 ($ST \times SR$) vs. F_1 ($SR \times ST$)	0.949	0.183	0.236

somes in the latter. On the other hand, both types of F_1 males had longer cysts than those of the parental strains which suggests an overall heterosis effect.

Number of cysts per testis

Cysts more than 0.5 mm length were counted in males less than 24 h old and in males between 48 and 72 h old (Table 1). The number of cysts per testis in ST, F_1 ($SR \times ST$) and F_1 ($ST \times SR$) males increased significantly between 24 and 48 h ($t_{98} = 4.887$, $P < 0.001$; $t_{98} = 5.557$, $P < 0.001$; $t_{98} = 4.523$, $P < 0.001$, respectively). It also increased, but not significantly, in SR males ($t_{98} = 1.445$, $P = 0.15$). ST males had significantly more cysts per testis than SR ones, regardless of age (Table 2). At both ages F_1 males from the two reciprocal crosses showed intermediate numbers of cysts and did not differ significantly from each other. Thus, the difference in cyst number between ST and SR males was not related to the presence of driver chromosomes in the latter. There was no correlation between the number of cysts per testis and the distorting ability of 40 *sex-ratio* males (Spearman rank correlation coefficient $r_{s38} = 0.197$, $P = 0.22$).

Abnormal spermatids in *sex-ratio* males

In *D. simulans*, a cyst normally contains 32 Y- and 32 X-bearing spermatids. In ST males, 70 per cent of the 50 elongated cysts observed had the proper number of heads (64) in the normal (anterior) position (Fig. 1a). In the other cysts (30 per cent), at most one or two heads were lacking, and in one cyst two heads were located in the tail region (Table 3). Similarly, 86 per cent and 82 per cent of the cysts from the SR and F_1 ($ST \times SR$) males appeared normal. The others showed slight defects similar to those of ST males, except for one cyst from one SR male that showed numerous heads (21) in the tail region. Finally, with respect to the numbers of misplaced heads, the three types of non *sex-ratio* males were not statistically different (Smirnov test, $P > 0.05$). By contrast, in all three types of *sex-ratio* males, F_1 ($SR \times ST$) <24 h, F_1 ($SR \times ST$) >48 h and F_1 ($SR \times ST$), numerous heads were repeatedly found in the tail region (Fig. 1b). Misplaced heads occurred in 82 to 100 per cent of the cysts and their number varied widely between cysts (Table 3). They are significantly more numerous in the three types of *sex-ratio* males than in the non *sex-ratio* ones (Smirnov test, $P < 0.001$). The numbers of heads in the tail region were not statistically different between the two types of *sex-ratio* males less than 24 h-old (Smirnov test, $P > 0.05$), but *sex-ratio* males more than 48 h-old appeared significantly less affected.

ted when compared with both F_1 ($SR \times ST$) < 24 h (Smirnov test, $P < 0.05$) and F_1 ($SR5 \times ST$) < 24 h (Smirnov test, $P < 0.001$).

With respect to the total number of heads per cyst, no significant difference was found between the three types of non *sex-ratio* males (Smirnov tests, $P > 0.05$). The three types of *sex-ratio* males showed significantly lower numbers of heads per cyst when compared with non *sex-ratio* ones (Smirnov tests,

$P < 0.01$). Again, *sex-ratio* males more than 48 h-old were less affected when compared with both F_1 ($SR \times ST$) < 24 h and F_1 ($SR5 \times ST$) < 24 h (Smirnov tests, $P < 0.01$).

Within a cyst of a *sex-ratio* male, heads in the normal position did not show any peculiar heterogeneity for condensation or elongation of nuclei, when compared to those of ST males (Fig. 1a,b), but nuclei in the tail region frequently showed a lesser

Fig. 1 Fully elongated cysts of *Drosophila simulans* stained with 4',6-Diamidino-2-phenylindole (DAPI). (a) Cyst of ST male with 64 heads in normal position. (b) Cyst of *sex-ratio* male, showing heads in anterior and tail regions. (c) Posterior end of a cyst of *sex-ratio* male with degenerating spermatid nuclei. The bars represent 30 μm.

Table 3 Mean numbers of heads in the anterior and tail regions per fully elongated cyst in *Drosophila simulans* ST, SR and F_1 hybrids (for the origin of the F_1 maternal strain is indicated first). For SR5 strain see Materials and methods section. SE = standard error; minimum and maximum numbers of heads are given within parentheses. For each type of male, 50 cysts were examined on 10 males

	Anterior region		Tail region		Total	
	Mean $\pm$ SE	(Min–max)	Mean $\pm$ SE	(Min–max)	Mean $\pm$ SE	(Min–max)
ST < 24 h	63.56 $\pm$ 0.104	(62–64)	0.04 $\pm$ 0.040	(0–2)	63.60 $\pm$ 0.099	(62–64)
SR < 24 h	63.48 $\pm$ 0.422	(43–65)	0.46 $\pm$ 0.421	(0–21)	63.94 $\pm$ 0.044	(63–65)
F_1 (ST $\times$ SR) < 24 h	63.68 $\pm$ 0.116	(60–64)	0.10 $\pm$ 0.052	(0–2)	63.78 $\pm$ 0.082	(61–64)
F_1 (SR $\times$ ST) < 24 h*	45.50 $\pm$ 1.452	(19–64)	13.74 $\pm$ 1.292	(0–41)	59.24 $\pm$ 0.790	(40–65)
F_1 (SR5 $\times$ ST) < 24 h*	44.62 $\pm$ 0.901	(32–57)	15.20 $\pm$ 0.836	(5–28)	59.82 $\pm$ 0.502	(50–64)
F_1 (SR $\times$ ST) > 48 h*	53.80 $\pm$ 0.984	(38–64)	8.48 $\pm$ 0.987	(0–24)	62.28 $\pm$ 0.282	(56–64)

*Males expressing the *sex-ratio* trait.

degree of condensation and/or elongation, and sometimes signs of degeneration (Fig. 1c).

Abnormal spermatids and distortion strength

We examined 21 *sex-ratio* males for their distorting ability and the position of the spermatid heads in 10 fully elongated cysts per male. The number of heads in the normal (anterior) position varied widely between cysts from the same male (data not shown). The largest variation was for a male that showed one cyst with all 64 heads, and another cyst with only 35 heads, in the anterior position. However, the between-male variations were significantly wider than the within-male ones (Kruskal–Wallis one-way analysis of variance by ranks: $\chi^2_{20} = 61.89$, $P = 3.6 \times 10^{-6}$).

We tested the correlation between the extent of spermiogenic failure and the distorting ability of the 21 *sex-ratio* males. They had between 71 and 98 per cent of females in their progeny and the mean number of heads in the anterior position was between 34.4 and 60.8, depending on the male. A significant negative correlation was found between these two parameters (Fig. 2, Spearman rank correlation coefficient $r_{s19} = -0.695$, $P < 0.001$). This strongly suggests that the abnormal spermatids are Y-bearing ones and will degenerate. Under this hypothesis, the expected frequency of females in the progeny of a *sex-ratio* male can be deduced from the mean number (n) of heads in the normal position in its cysts (Lyttle *et al.*, 1986): 32 are assumed to be X-bearing and $(32 - n)$ Y-bearing. Thus the expected frequency of females in the progeny is $32/n$, which corresponds to the regression slope in Fig. 2. All of the experimental points lie upon this slope, which means that some of the normal looking spermatids were nevertheless defective.

Discussion

Spermiogenic failure

The X-linked *sex-ratio* trait in *D. simulans* appeared to be associated with the presence of numerous spermatid heads in the tail region of the cysts, and with a reduced number of total spermatids per cyst. This spermiogenic failure, similar to that reported in *D. subobscura* *sex-ratio* males (Hauschteck-Jungen & Maurer, 1976), definitively indicates that the trait results from meiotic drive. We observed abnormal spermatids in F_1 (SR $\times$ ST) and F_1 (SR5 $\times$ ST) *sex-ratio* males. The former bear driver X chromosomes from the SR strain, Y chromosomes from the ST strain and autosomes from both strains, whereas the F_1 (SR5 $\times$ ST) males carry an ST genetic background except for the X-linked drive factors that come from the SR strain. It follows that abnormal spermiogenesis does result from the presence of the X-linked drive loci themselves and not from other elements of the driver X chromosomes. This is in accordance with the correlation observed between the degree of spermiogenic failure and the bias towards females.

We rarely observed spermiogenic failure in SR males. This is consistent with the fact that SR males bear driver X-chromosomes but rarely if ever express the *sex-ratio* trait, usually producing progeny with equal sex ratio (Merçot *et al.*, 1995). This indicates that autosomal and Y-linked factors that prevent the expression of the trait in SR males act as suppressors. This contrasts with the *male sex-ratio* modifying genes described in *D. pseudoobscura* which induce spermiogenic failure and suppress the trait by an overproduction of sterile XO males (Cobbs, 1987; Cobbs *et al.*, 1991).

The observed excess of females in the progeny of *sex-ratio* males is always higher than expected from

Fig. 2 Mean number of anterior heads per cyst in 21 *sex-ratio* males of *Drosophila simulans* (calculated on 10 cysts per male) plotted against female percentage in their progeny. Vertical bars represent the standard error.

the observed spermatid defects. Thus, some of the normal-looking spermatids must be defective. Abnormal or lacking spermatids are significantly more numerous in newly emerged than in sexually mature *sex-ratio* males. However, we did not detect any age effect on the percentage of females among the progeny of *sex-ratio* males (unpublished data). Thus, increased spermiogenic failure observed in newly emerged males may rather be related to a delayed sexual maturity. In addition, in newly emerged *sex-ratio* males, fewer than 32 heads in the normal position were found in three among the 50 cysts observed (Table 3). This suggests that the X-bearing sperm must also be affected, to a lesser extent, by the *sex-ratio* trait and that abnormal spermatids might be only a by-product of the meiotic drive. Alternatively another independent phenomenon might be responsible for the extensive degeneration of the spermatids observed in some cysts. Similar failure of more than half of the spermatids in a cyst occasionally occurs in *D. subobscura* *sex-ratio* males (Hauschteck-Jungen & Maurer, 1976).

The *sex-ratio* trait we described here in *D. simulans* is cytologically different from that described in the same species by Faulhaber (1967). The most striking cytological feature described by this author was a within-cyst dimorphism of spermatid nuclei, whereas he did not report any abnormal position of spermatid heads. In our case, the spermatid heads in

the normal position within a cyst of a *sex-ratio* male do not show any peculiar morphological heterogeneity when compared to those of ST males. By contrast, heads in the tail region frequently show a lesser degree of condensation and/or elongation, and sometimes signs of degeneration. This is an additional similarity to the spermiogenic failure described in *D. subobscura* *sex-ratio* males (Hauschteck-Jungen & Maurer, 1976). The *sex-ratio* trait described as autosomal by Faulhaber is possibly X-linked (Hurst & Pomiankowski, 1991), but it differs from ours both by its thermosensitivity and by its cytology. Therefore we can conclude that at least two different *sex-ratio* systems occur in *D. simulans*.

Maintenance of the sex-ratio trait in natural populations of D. simulans

The fertility of *sex-ratio* males is one of the factors that determine the fate of the trait, which will spread through populations only if *sex-ratio* males produce more female progeny than non *sex-ratio* ones. This must be the case in *D. simulans*, where driver X chromosomes are widespread, sometimes at a high frequency, and have induced the selection of suppressors which almost completely prevent drive expression (Merçot *et al.*, 1995; Atlan *et al.*, in press). We did not detect any significant effect of meiotic drive X-linked factors on cyst number or cyst length, which implies that *sex-ratio* and non *sex-ratio* males do produce roughly equal quantities of X sperm. A higher production of females by *sex-ratio* males can then be achieved if sperm production is not a limiting factor for *sex-ratio* male fertility in this species. Data on the sibling species *D. melanogaster* support this hypothesis (reviewed in Gromko *et al.*, 1984; discussed in Beckenbach, 1978).

Our results show that the *sex-ratio* trait in *Drosophila* might spread according to different selective processes depending on the opportunities offered by the species. From data on the *obscura* group species, Bircher *et al.* (1995) proposed that the prerequisite for the development of the *sex-ratio* trait in a population may have been the existence of sperm heteromorphism. Indeed, in *D. subobscura*, the testes of *sex-ratio* males contain twice as many cysts as those of controls (Bircher & Hauschteck-Jungen, 1994), via an increased production of short cysts at the expense of long cysts (Bircher *et al.*, 1995). In this species the trait is associated with chromosomal inversions spanning the X chromosome and thus females homozygous for driver X chromosomes are expected to have a low fitness (Curtsinger & Feldman, 1980). In addition, the sperm production

is possibly a limiting factor for male fertility (Beckenbach, 1978; Hauschteck-Jungen *et al.*, 1987). It follows that an increase in sperm production may be necessary for the *sex-ratio* trait to evolve in *D. subobscura*, which is not the case in *D. simulans* where it has spread despite the lack of sperm length heteromorphism.

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