

# Rapid Rise and Fall of Selfish Sex-Ratio X Chromosomes in *Drosophila simulans*: Spatiotemporal Analysis of Phenotypic and Molecular Data

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## Abstract

*Sex-ratio* drive, which has been documented in several *Drosophila* species, is induced by X-linked segregation distorters. Contrary to Mendel's law of independent assortment, the *sex-ratio* chromosome ( $X^{SR}$ ) is inherited by more than half the offspring of carrier males, resulting in a female-biased sex ratio. This segregation advantage allows  $X^{SR}$  to spread in populations, even if it is not beneficial for the carriers. In the cosmopolitan species *D. simulans*, the Paris *sex-ratio* is caused by recently emerged selfish  $X^{SR}$  chromosomes. These chromosomes have triggered an intragenomic conflict, and their propagation has been halted over a large area by the evolution of complete drive suppression. Previous molecular population genetics analyses revealed a selective sweep indicating that the invasion of  $X^{SR}$  chromosomes was very recent in Madagascar (likely less than 100 years ago). Here, we show that  $X^{SR}$  chromosomes are now declining at this location as well as in Mayotte and Kenya. Drive suppression is complete in the three populations, which display little genetic differentiation and share swept haplotypes, attesting to a common and very recent ancestry of the  $X^{SR}$  chromosomes. Patterns of DNA sequence variation also indicate a fitness cost of the segmental duplication involved in drive. The data suggest that  $X^{SR}$  chromosomes started declining first on the African continent, then in Mayotte, and finally in Madagascar and strongly support a scenario of rapid cycling of X chromosomes. Once drive suppression has evolved, standard  $X^{ST}$  chromosomes locally replace costly  $X^{SR}$  chromosomes in a few decades.

**Key words:** meiotic drive, *sex-ratio*, selective sweep, natural populations, *Drosophila simulans*.

## Introduction

The term meiotic drive refers to the situation in which one member of a pair of alleles or chromosomes is preferentially recovered in the functional gametes. Such alleles are “selfish,” in that they favor their own transmission by preventing the production of gametes carrying their alternative, instead of by increasing the fitness of their carriers (Novitski and Sandler 1957; Burt and Trivers 2006). *Sex-ratio* distortion is a common type of naturally occurring meiotic drive in which driver alleles are located on the X chromosome and expressed in heterogametic XY males. The visible consequence is a strong female bias among the progeny of a male carrier. In *Drosophila*, X-linked distortion has been reported in several distantly related species (e.g., *D. obscura*, Gershenson 1928; *D. simulans*, Merçot et al. 1995; and *D. quinaria*, Jaenike 1996; see also Jaenike (2001) for a complete review), indicating its recurrent and independent evolution.

The emergence of a *sex-ratio* chromosome (hereafter  $X^{SR}$ ) is potentially deadly for a species: If it spreads to fixation, it can cause extinction due to the lack of males (Hamilton 1967). However,  $X^{SR}$  chromosomes in many species appear to be bal-

anced polymorphisms, and there has been considerable effort in understanding the conditions that allow such steady states (reviewed in Jaenike 2001; Hall 2004). This situation can arise when  $X^{SR}$  chromosomes have deleterious effects and/or Y chromosome and autosomes evolve partial drive suppression. Suppressors of drive are favored as they reduce the sex ratio bias (Fisher 1930). Interestingly, two cryptic *sex-ratio* systems have been described in *D. simulans*—Paris and Winters—in which the distorter alleles are virtually unexpressed in natural populations due to the evolution of complete drive suppression (Atlan et al. 1997; Tao, Araripe, et al. 2007; Tao, Malsy, et al. 2007). Such  $X^{SR}$  chromosomes, which have completely lost their segregation advantage, are doomed to disappear by drift, counter-selection, and/or mutational degeneration of the distorter alleles, unless they enter an arms race by recruiting drive enhancer alleles that will neutralize the suppressors. To explore this issue, we characterize in the present study the recent dynamics of Paris  $X^{SR}$  chromosomes in a geographic region where populations show complete drive suppression.

*Drosophila simulans*, originally from East Africa or Madagascar, has recently spread worldwide (Dean and Ballard

2004; Lachaise and Silvain 2004; Baudry et al. 2006; Kopp et al. 2006). In a survey of the Paris system, populations showed geographical variation in both distortion and Y-linked and autosomal suppression (Atlan et al. 1997; Jutier et al. 2004). The highest frequencies of distorters (up to 60%), along with a complete suppression, occurred on islands in the Indian Ocean. Central and East African populations showed similarly complete suppression, but lower frequencies of distorters (Atlan et al. 1997). The discovery of populations with rare distorters but complete suppression was surprising, as complete suppression is expected to evolve in response to a strong sex ratio bias (Hartl 1975; Carvalho and Vaz 1999). The presence of these populations suggests that the distorter chromosomes are in the process of disappearing due to their cost once their transmission advantage is lost.

The Paris *sex-ratio* phenotype is expressed through epistatic interactions between two X-linked elements (Montchamp-Moreau et al. 2006). The first element consists of a tandem segmental duplication including six genes (hereafter the SR duplication). The second element has been mapped to roughly 1 cM from the duplication. A molecular population genetics study on a sample collected in 2000 in Madagascar, where the highest frequency of  $X^{SR}$  chromosomes has been observed, revealed a double selective sweep indicating the recent spread of both distorter elements. The pattern of variation suggests that the sweep could have ended as recently as 100 years ago (Derome et al. 2008).

In order to further improve our understanding of the  $X^{SR}$  chromosomes evolutionary dynamics, we extended the study to two nearby populations, Mayotte and Kenya, which are connected to Madagascar by substantial gene flow (Baudry et al. 2006). All three populations exhibited complete drive suppression but had different proportions of  $X^{SR}$  chromosomes in the years 1999–2001 (Atlan et al. 1997; Jutier et al. 2004; Derome et al. 2008). Here, we tracked the frequencies of  $X^{SR}$  in the three populations and characterized the pattern of DNA sequence variation in the vicinity of the drive loci on the X chromosome. We first determined whether the signature of the selective sweep observed in Madagascar is detectable in Mayotte and Kenya. Next, in order to test whether the signature of the selective event is decreasing with time in the Madagascar population, we recharacterized molecular polymorphism in the chromosomal region containing the distorter elements using a sample taken 8 years after the one analyzed in Derome et al. (2008). The results suggest that standard chromosomes (hereafter  $X^{ST}$ ) quickly replace deleterious  $X^{SR}$  chromosomes once complete suppression has evolved. The Paris system thus offers a unique opportunity to witness an ongoing cycling of X chromosomes.

## Materials and Methods

### Sample Collection

Adult males were collected using banana traps, in Madagascar (near Antananarivo) in 2000 ( $n = 48$ ), 2004 ( $n = 27$ ), and 2008 ( $n = 67$ ); in Mayotte in 1998 ( $n = 39$ ), 1999 ( $n =$

99), and 2009 ( $n = 71$ ); and in Kenya (near Nairobi) in 1994 ( $n = 101$ ), 2001 ( $n = 81$ ), and 2009 ( $n = 67$ ).

### $X^{SR}$ Chromosome Frequency

X chromosomes from wild-caught males were kept in male lineage (X line) and suppressor-free background by crosses with females carrying compound X chromosomes (Montchamp-Moreau and Cazemajor 2002). For each X chromosome, at least five carrier males were tested for the sex ratio of their progeny. All experiments were carried out at 25 °C. Only tests producing at least 50 offspring per male parent were considered. An X chromosome was said *sex-ratio* ( $X^{SR}$ ) if it produced more than 70% females on average. When the  $X^{SR}$  chromosomes were at low frequency in a population, the 95% confidence interval (CI) was calculated after angular transformation of the data ( $\arcsin \sqrt{p}$ ). This procedure was applied to estimate the frequency of  $X^{SR}$  chromosomes among adult males collected in Madagascar (2000–2004–2008), Mayotte (1998–1999), and Kenya (1994–2001). Males of X lines were frozen for molecular analysis.

### Using the SR Duplication as a Marker of the *Sex-Ratio* Phenotype

Previous cytogenetic and molecular variation studies of the sample Madagascar 2000 (10  $X^{SR}$  and 5  $X^{ST}$ ) had shown that the  $X^{SR}$  chromosomes always carried the SR duplication, which was never found on  $X^{ST}$  chromosomes (Montchamp-Moreau et al. 2006; Derome et al. 2008). To further test this association, we designed a polymerase chain reaction (PCR) assay to determine whether or not the SR duplication is present in a given chromosome. To this end, primers allowing the simultaneous amplification of two fragments were designed (supplementary table S1, Supplementary Material online). One of these fragments straddles the junction region between the two copies of the SR duplication and can thus only be amplified from chromosomes carrying it. The second fragment is located within the *org-1* gene and is amplified on duplicated as well as standard chromosomes. Using this assay, we found a strict association of the SR duplication and the SR phenotype among the 45 X chromosomes used in the present study of molecular variation. We also investigated the whole sample of X chromosomes from Mayotte 1999 ( $n = 99$ ) and found only one chromosome carrying the duplication among 75 phenotypically standard  $X^{ST}$  chromosomes. We then used this marker to estimate the frequency of  $X^{SR}$  chromosomes in Kenya 2009 and Mayotte 2009. By using the frequency of the  $X^{SR}$  chromosomes as a proxy for SR duplication, we possibly overestimated the true value, providing a conservative estimate regarding the hypothesis of  $X^{SR}$  disappearing with time.

### Modeling Selection on $X^{SR}$ Chromosomes

Assuming 10 generations per year, we estimated the cost from the observed  $X^{SR}$  frequencies in Madagascar through a simple deterministic biallelic model. A constant selection coefficient ( $s$ ) was hypothesized to be identical in males and females such that the fitness of both males and females

genotypes are:  $w_1 = w_{ST/ST} = w_{ST/Y} = 1$ ,  $w_2 = w_{ST/SR} = 1 - h$ , and  $w_3 = w_{SR/Y} = w_{SR/SR} = 1 - s$ . The dominance coefficient  $h$  was set to either 0 (complete dominance of  $X^{ST}$  over  $X^{SR}$ ) or 0.5 (codominance = additivity). Let  $p_M$  and  $q_M$  (resp.  $p_F$  and  $q_F$ ) be the frequencies of  $X^{ST}$  and  $X^{SR}$  in males (resp. females). The frequencies in the next generation are obtained by:

$$\begin{aligned} v' &= \frac{w_1 u}{w_3} \\ u' &= \frac{2uw_1 + (u+v)w_2}{2w_3 + (u+v)w_2}, \end{aligned}$$

with  $v = p_M/q_M$  and  $u = p_F/q_F$ , respectively (Haldane and Jayakar 1964).

The value of  $s$  was estimated by fitting the decrease of  $X^{SR}$  frequency predicted from the above model to the observed frequencies in the three temporal samples from Madagascar by maximum likelihood as follows. Let  $k^{(g)}$  be the number of  $X^{SR}$  observed at generation  $g$  in a sample of total size  $n^{(g)}$ , and let  $p_M^{(g)}$  be the frequency predicted at that generation from the above model. The likelihood of  $s$  given the observed data (three generations) is then:

$$L = \prod \binom{n}{k} (p_M)^k (1 - p_M)^{(n-k)},$$

where the product is taken over the three generations  $g$  and exponent ( $g$ ) for  $n$ ,  $k$ , and  $p_M$  is omitted for clarity. Note that by doing so we do not necessarily get  $p_M = n/k$  at any of the three generations. However, this permits to design a support interval for  $s$  by considering all values of  $s$  providing a likelihood in the range ( $L_{max}$ ,  $L_{max}/100$ ) on both sides of the maximum likelihood  $L_{max}$ , that is, corresponding to a classical logarithm of the odds (LOD) drop-off of 2.

### DNA Polymorphism

Primers for 16 markers encompassing the two candidate regions were designed using the annotated *D. melanogaster* sequences and corrected when necessary with the available *D. simulans* sequences (supplementary table S1, Supplementary Material online). Twelve of these markers were previously used in Derome et al. (2008). Extraction of single male genomic DNA, PCR amplification and direct sequencing, or cloning of PCR products were carried out as previously described (Derome et al. 2004, 2008). Sequences were aligned with Multalin (Corpet 1988) then manually corrected with the Bioedit program (Hall 1999). When two different sequences were identified at markers within the duplication, they were assigned to the distal or the proximal copy by following the same procedure as in Derome et al. (2008): We retained the order which minimized the number of recombination events following the duplication event, whereas remaining compatible with the known position of alleles on the reference chromosome  $X^{SR6}$  (Montchamp-Moreau et al. 2006) and on chromosome XM04 (Ogereau D, unpublished data).

### Population Genetics Analyses

Molecular variation was analyzed using the DnaSP version 5 software package (Librado and Rozas 2009). Neutrality tests (haplotype diversity  $H$ , Tajima's  $D$ ) were conducted without recombination. The minimum number of recombination events ( $R_m$ , Hudson and Kaplan 1985) was estimated for each population.

## Results

### Spatiotemporal Distribution of $X^{SR}$ Frequencies

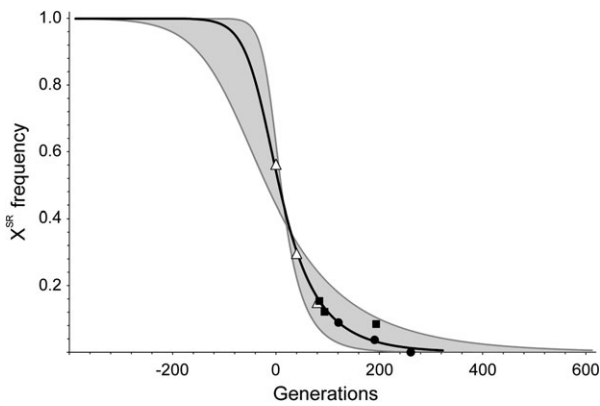
Supplementary figure S1, Supplementary Material online shows the three localities where the flies were collected along with the year of sampling and their corresponding  $X^{SR}$  frequencies (see Materials and Methods). Among the three samples collected around the same time, Madagascar 2000 showed the highest frequencies of  $X^{SR}$  chromosomes,  $56.3 \pm 14.0\%$  (95% CI), followed by Mayotte 1999 ( $12.1 \pm 6.4\%$ ) and Kenya 2001 ( $3.7$ , CI 1.2–6.6%), suggesting a southeast–northwest cline.

The temporal analysis revealed a general trend of  $X^{SR}$  frequencies decreasing through time. In Madagascar,  $X^{SR}$  frequency dropped from  $56.3 \pm 14.0\%$  in 2000 to  $29.6 \pm 17.2\%$  in 2004 and finally to  $14.9 \pm 8.5\%$  in 2008. In Mayotte,  $X^{SR}$  frequency decreased from  $15.4 \pm 11.3\%$  in 1998 to  $12.1 \pm 6.4\%$  in 1999 and finally to  $8.5 \pm 6.5\%$  in 2009. In Kenya, the sampled frequencies were  $8.9 \pm 5.6\%$  in 1994 (Atlan et al. 1997),  $3.7\%$  in 2001 (CI 1.2–6.6%), and  $X^{SR}$  chromosomes may have then suffered local extinction because they were absent from the sample collected in 2009 (CI 0–7%, Fisher's exact test  $P < 5\%$ ).

### Modeling the Decrease of $X^{SR}$ Chromosomes

The decline in frequency of  $X^{SR}$  chromosomes indicates that the carrier individual suffers a cost when the sex-ratio phenotype is totally suppressed in the populations. Assuming 10 generations per year, we estimated this cost from the three  $X^{SR}$  frequencies observed in Madagascar through a simple deterministic biallelic model with a constant selection coefficient  $s$ . Assuming recessive deleterious effects in females ( $h = 0$ ), the value that best fit the empirical data was  $s = 0.04$ , with a support interval of 0.02–0.07 corresponding to a LOD drop-off of 2 (see Materials and Methods). The predicted decline of the  $X^{SR}$  chromosomes is shown in figure 1. In Madagascar, for example,  $X^{SR}$  chromosomes are expected to disappear in 2060 at the latest, taking into consideration the uncertainty of the  $s$  value. Assuming the  $X^{SR}$  chromosomes reached fixation in the past, the decrease in Madagascar would have started around 1970. With semidominance in females ( $h = 1/2$ ) the  $s$  value was nearly identical and the curve would shift to the left by 20 years. For Kenya and Mayotte, we fit the oldest observed value of  $X^{SR}$  frequency on the maximum likelihood curve from the Madagascar data and placed the two following values on the curve as a function of the number of generations between the samples (fig. 1). The Kenya data fit the curve for a decreasing phase starting 18 years earlier than in Madagascar. The Mayotte data suggest





**Fig. 1.** Decline of the  $X^{SR}$  frequency in Madagascar ( $\Delta$ ), Mayotte ( $\blacksquare$ ), and Kenya ( $\bullet$ ). The curve was adjusted through a model applied to the Madagascar data (recessive deleterious effects of  $X^{SR}$  with constant selection coefficient  $s = 0.04$ , see text). The support interval (in gray) corresponds to a LOD drop-off of 2. The  $X^{SR}$  frequency of Madagascar 2000 was arbitrarily placed at the time 0. The  $X^{SR}$  frequencies observed in Mayotte and Kenya were plotted on the graph assuming the same dynamics as in Madagascar (see text).

a decline starting at a time intermediate between Madagascar and Kenya. However, these data did not fit the curve perfectly (although they are within the CI, see [fig. 1](#)). This may be due to sampling error or to a slower rate of decrease of the  $X^{SR}$  chromosomes on this island.

### Spatiotemporal Evolution of the Double Selective Sweep

To gain a deeper understanding of the past history of the *sex-ratio* system in this part of the world, we analyzed the patterns of molecular variation among X chromosomes sampled in the three locations. A previous study of 15 chromosomes from Madagascar 2000 ([Derome et al. 2008](#)) revealed a double selective sweep caused by the coselection of alleles at both loci responsible for the drive. A major haplotype (i.e., the haplotype found at the highest frequency at a given locus) shared by 9–12 of the 15 X chromosomes was observed at marker loci surrounding each of the drive loci. The major haplotypes were in strong association with the *sex-ratio* trait, that is, mainly, if not exclusively, carried by the  $X^{SR}$  chromosomes, resulting in strong linkage disequilibrium between markers within and between the two candidate regions, and a lack of nucleotide variation on the  $X^{SR}$  chromosomes compared with the  $X^{ST}$  chromosomes.

First, we compared the Malagasy sample and 15 chromosomes sampled in Mayotte roughly at the same time (1999) to determine whether the molecular data were consistent with a more ancient spread and decline of  $X^{SR}$  chromosomes in Mayotte. [Figure 2](#) summarizes the distribution of haplotypes in the two samples across 15 markers (total of 7,114 bp) scattered along  $\sim 300$  kb and including the SR duplication and the second drive locus. Note that markers F', I', and M had not been previously analyzed in Madagascar 2000. The Mayotte sample shared the major haplotypes previously found in Madagascar, supporting the hypothesis that  $X^{SR}$  chromosomes in these populations

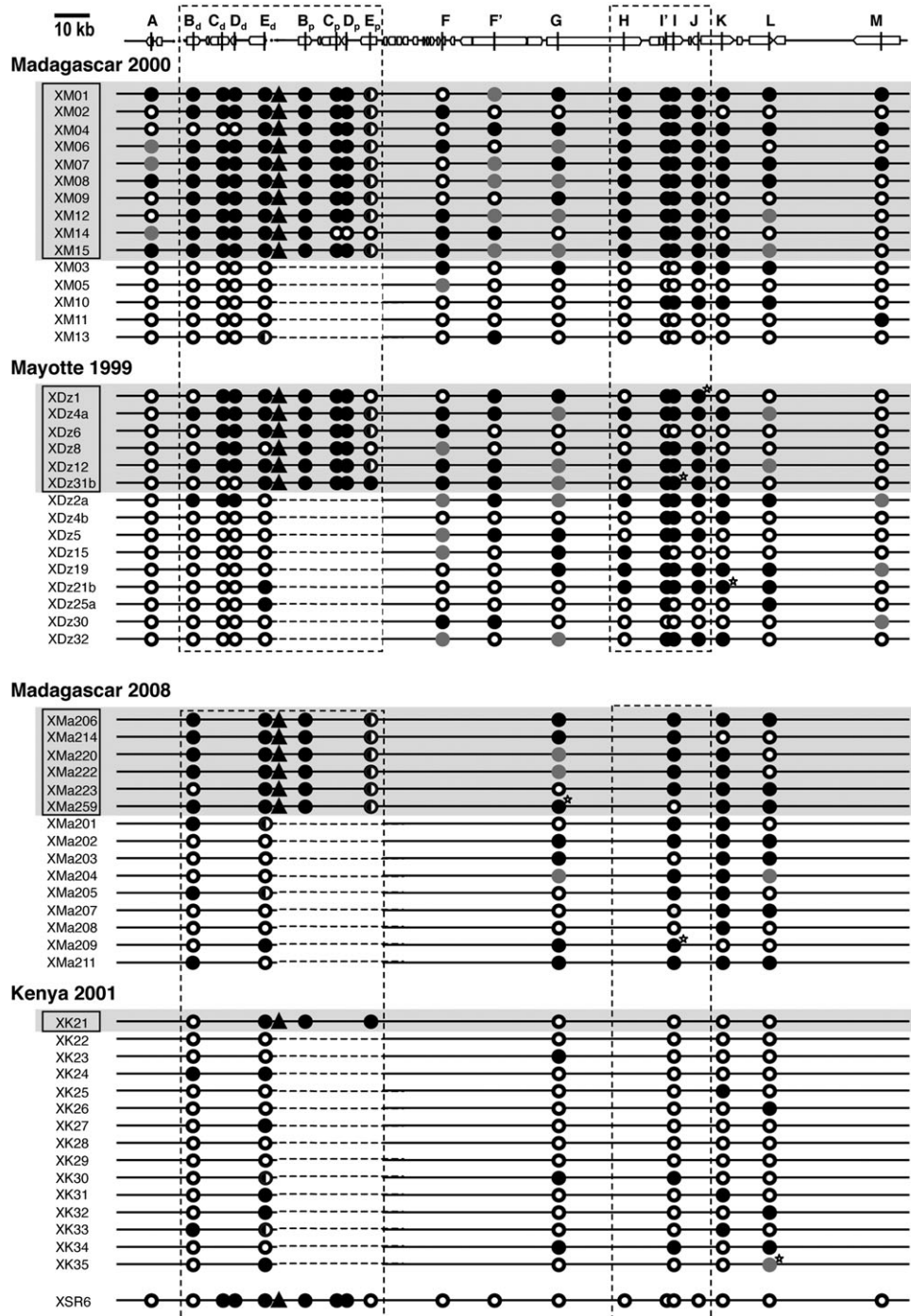
originate from the same ancestral  $X^{SR}$  rather than from independent evolutionary events.

In the Mayotte 1999 sample, the association of the major haplotypes with the  $X^{SR}$  chromosomes was significant only with the markers within the SR duplication and marker F, and significant linkage disequilibria were nearly exclusively observed between these markers ([fig. 3A](#) and [supplementary fig. S2A, Supplementary Material](#) online). Consistently, marker loci showed maximum linkage disequilibrium ( $D' = 1$ , [Lewontin 1964](#)) less often in Mayotte 1999 than in Madagascar 2000. The Mayotte sample—six  $X^{SR}$  and nine  $X^{ST}$ —differs from a random sample, which would have had only two or three  $X^{SR}$  chromosomes among 15 given their population frequency ( $12.1 \pm 6.4\%$ ). However, we checked by simulations (data not shown) that this was conservative with respect to the comparison between the Mayotte and Madagascar samples: The only possible effect of the biased sample (considering six  $X^{SR}$  instead of two or three) is to increase the significance of the association tests if linkage disequilibrium was already present in the population (due either to a selective sweep or to another cause). Hence, the association estimated in Mayotte from a truly random sample could only be weaker than that in the Madagascar sample. Thus, the contrast in the strength of association and linkage disequilibria between Mayotte and Madagascar reveals real differences between the two populations.

For the four markers within the SR duplication, corresponding to the first of the two distorting elements, the signature of a sweep associated with the spread of  $X^{SR}$  chromosomes was observed in both populations:  $\pi_{SR}/\pi_{ST} = 0$  at markers Ed and Bp in Madagascar 2000, at markers Bp, Cp, and Dp in Mayotte 1999 ([fig. 4](#)). Although Mayotte  $X^{SR}$  chromosomes had more variation, several markers showed a significant deficit of haplotype diversity ( $Hd$ ) and significantly negative Tajima's  $D$  ([supplementary tables S2 and S3, Supplementary Material](#) online). The lack of haplotype diversity in Mayotte was also significant when we considered all four markers simultaneously ( $Hd = 0.933$ ,  $P < 0.05$ ). On the other hand, among the  $X^{ST}$  chromosomes, there was no significant deviation from neutrality.

Around the second driving element, the situation was very different: While  $X^{SR}$  chromosomes in Madagascar 2000 showed significant lack of variation at four markers (H, I', I, and J) and strong association with the major haplotypes, in Mayotte 1999, only marker I had lower variation among the  $X^{SR}$  chromosomes ( $\pi_{SR}/\pi_{ST} < 1$ , [fig. 4](#)), and major haplotypes were scattered among  $X^{SR}$  and  $X^{ST}$  chromosomes. Consequently, low values of  $Hd$  were observed among both types of chromosomes, at markers I, J, and above all at marker I' (highly significant). Consistently, Tajima's  $D$  was negative and significant at markers I', J, and K when the two types of chromosomes were pooled, and only at marker I' among the  $X^{ST}$  chromosomes when they were treated separately.

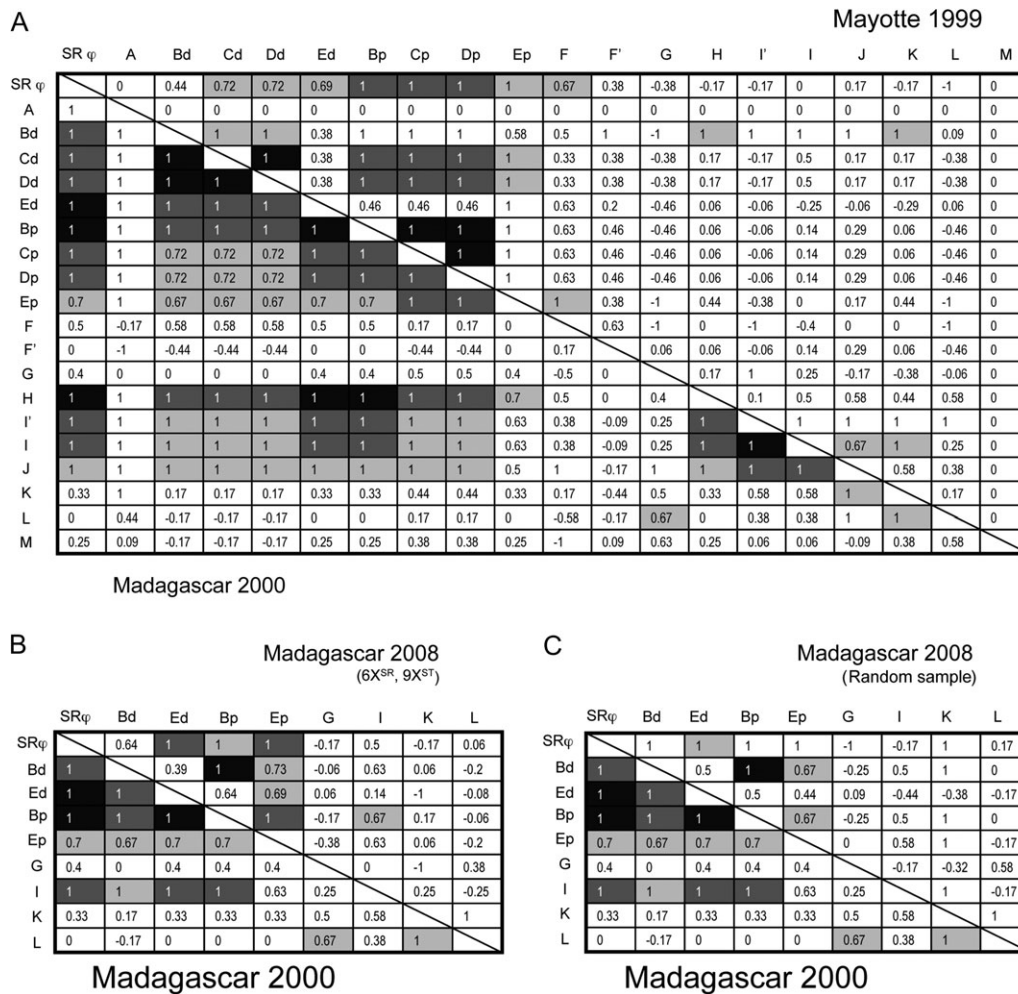
Together, the data suggest that more recombination events had occurred between  $X^{SR}$  and  $X^{ST}$  chromosomes in the Mayotte 1999 sample than in the Madagascar 2000 sample, which in turn suggests that, in Mayotte, either



**Fig. 2.** Haplotype distribution of the surveyed markers in the four samples of X chromosomes.  $X^{SR6}$ : reference sex-ratio chromosome from the Seychelles; ● and ○: major haplotypes = haplotypes at the highest frequency among Madagascar and Mayotte samples (arbitrarily chosen at marker A where two haplotypes were equally represented), the striped symbol refers to the major haplotype of the proximal copy of the E marker which is different from that of the distal copy. ●: haplotypes at intermediate frequency (present in at least three sequences among Madagascar and Mayotte samples), ○: other haplotypes; ☆: haplotype with singleton mutation. The solid boxes on the left and the gray background denote the  $X^{SR}$  chromosomes. The dashed boxes around the markers denote the candidate regions for the distorter elements. The unbroken lines between the boxes are roughly proportional to the distance between markers in *Drosophila melanogaster*. The dotted lines on  $X^{ST}$  chromosomes fill in the gap corresponding to the duplication present on  $X^{SR}$  chromosomes. The sequenced fragments are positioned according to the *D. melanogaster* genome map and are located in the following genes, from the telomere (left) to the centromere (right): A (*CG10555*), B (*Trf2*), C (*CG1440*), D (*CG12123*), E (*org-1*), F (*Cp36*), F' (*CG33181*), G (*Nrg*), H (*Nrg*, in Derome et al. 2004), I' and I (*CG11265* = *Trf4-1*), J (*spirit*, previously *CG2056*), K (*CG12065*), L (*Crag*), and M (*oc*).

more time has elapsed since the spread of  $X^{SR}$  or the  $X^{SR}$  chromosomes spread more slowly. However, it is worth noting that there are singletons on the major haplotype back-

ground at markers I, J, and K in Mayotte 1999 (fig. 2), which supports the hypothesis that more time has elapsed since the spread of  $X^{SR}$  in the Mayotte population.



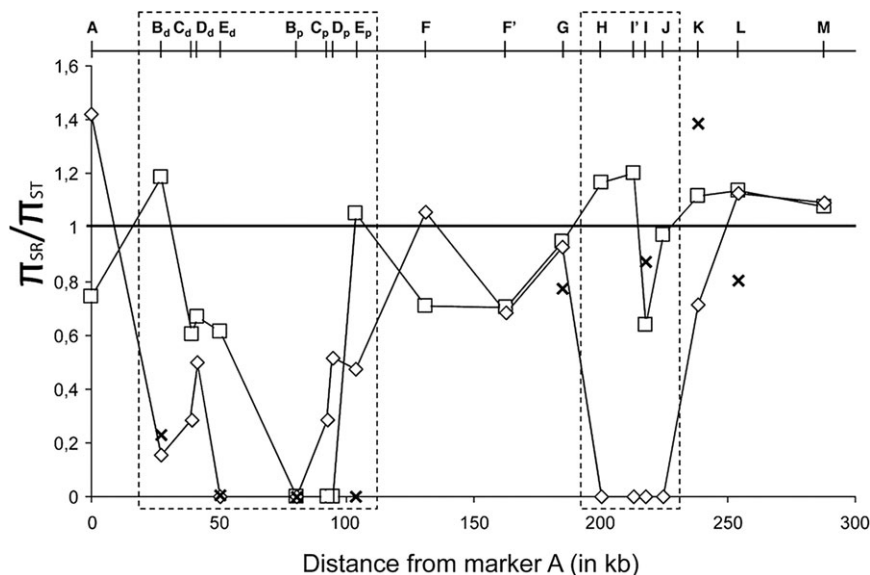
**FIG. 3.** Values of linkage disequilibrium between marker loci and association with the *sex-ratio* phenotype as measured by  $D'$  (Lewontin 1964), and level of significance as assessed by Fisher's exact test (white: nonsignificant, light gray:  $P < 0.05$ , dark gray:  $P < 0.01$ , and black:  $P < 0.001$ ).  $P$  values are given in [supplementary figures S2 and S3B, Supplementary Material online](#).

We then compared the Madagascar 2000 sample with a sample collected 8 years later at the same location (Madagascar 2008). We also compared Madagascar 2008 and Mayotte 1999, which had similar  $X^{SR}$  frequencies (observed values 15% vs. 12%). We used a subset of markers located in positions that were informative with respect to the selective sweep. First, we considered a Madagascar 2008 sample where  $X^{SR}$  and  $X^{ST}$  were present in the same numbers as in Mayotte 1999 (6 and 9, respectively). We did not detect significant association of  $X^{SR}$  with any major haplotype except for those at the two markers within the SR duplication. Linkage disequilibria between marker loci were weaker than in Madagascar 2000 and similar to those encountered in Mayotte 1999, that is, significant disequilibria occurred mainly between markers within the duplication ([fig. 3B](#)), and values of  $\pi_{SR}/\pi_{ST} = 0$  were observed only at these markers ([fig. 4](#)). In contrast, the  $\pi_{SR}/\pi_{ST}$  ratios at the four markers around the second element (including I) were close to or above the neutral expectation of 1, resembling the situation in Mayotte 1999, and indicating a loss of association between the major haplotypes and the  $X^{SR}$  chromosomes over time. In the Madagascar

2008 sample,  $Hd$  values of the markers Ed, Ep, K, and L significantly departed from neutrality, whereas, similarly to Mayotte 1999, only K showed a significant negative value of Tajima's  $D$ . In the  $X^{SR}$  subsample, haplotype diversity was significantly lowered within the duplication ( $Hd = 0.333$ ,  $P < 0.001$  when concatenating all the markers) and at markers I, K, and L. In the  $X^{ST}$  subsample, significant value was found only at marker K. Tajima's  $D$  were never significant when considering  $X^{SR}$  and  $X^{ST}$  separately.

To determine to what extent the overrepresentation of  $X^{SR}$  in the Madagascar 2008 sample affected the results of the neutrality tests, we randomly removed four of the six  $X^{SR}$  chromosomes and replaced them by four additional  $X^{ST}$  chromosomes ([supplementary fig. S3A, Supplementary Material online](#)). Unsurprisingly, the number of significant associations was lowered by about one half compared with the nonrandom sample ([fig. 3C and supplementary fig. S3B, Supplementary Material online](#)). When neutrality tests were performed on the whole sample,  $Hd$  remained significant only at marker K, Tajima's  $D$  was still significantly negative at marker K, but the degree of significance was lowered ([supplementary tables S2 and S3, Supplementary](#)





**FIG. 4.** Ratio of nucleotide diversity in the *sex-ratio* subsample ( $\pi_{SR}$ ) to nucleotide diversity in the standard subsample ( $\pi_{ST}$ ) over the surveyed marker loci. Markers are ordered as in figure 2 and positioned according to *Drosophila melanogaster* genome data.  $\diamond$ : Madagascar 2000,  $\square$ : Mayotte 1999, and  $\times$ : Madagascar 2008.

Material online). Thus, after the decline of  $X^{SR}$  chromosomes due to counter-selection, the signature of the past selective sweep became hardly detectable in a random sample of this size.

Finally, we studied a sample from Kenya collected in 2001. It included one  $X^{SR}$  chromosome carrying the SR duplication and 14  $X^{ST}$  chromosomes. The major haplotypes shared by the Madagascar and Mayotte samples were present in Kenya at markers within the duplication on the  $X^{SR}$  chromosome. They were also found at all markers among the  $X^{ST}$  chromosomes, but at low frequency except for marker E (five  $X^{ST}$  chromosomes with the major haplotype of the distal copy of E and two with the major haplotype of the proximal copy). Once again, this supports the hypothesis that  $X^{SR}$  chromosomes originated from the same ancestral  $X^{SR}$  as the Malagasy and Mayotte  $X^{SR}$ . None of the markers had a significant  $Hd$  nor Tajima's  $D$  value (supplementary tables S2 and S3, Supplementary Material online), in concordance with the prediction that the signature of selective sweep fades with time when  $X^{SR}$  chromosomes are counter selected.

## Discussion

The most important finding of our phenotypic and molecular analysis is that Paris  $X^{SR}$  chromosomes are quickly decreasing in populations where complete drive suppression has evolved. In addition, the geographic pattern suggests that the Paris system evolved from the African continent toward Madagascar via Mayotte. Finally, we describe a discrepancy in the evolution of the two elements necessary for drive to occur.

### Rapid Loss of $X^{SR}$ in Natural Populations

Analyzing the chromosomes sampled in 2000, Derome et al. (2008) obtained estimates of the time since the se-

lective sweep in Madagascar, which can roughly be considered as the time since the  $X^{SR}$  started to decrease in frequency, using two different methods. Considering patterns of linkage disequilibrium, Derome et al. (2008) estimated this time at roughly 88 years; using instead the mutation rate, the maximum time was 1,182 years ( $P < 0.05$ ). Under the deterministic model used here, the  $X^{SR}$  chromosomes were supposed to have reached fixation around 1950–1970 at the latest and then are predicted to disappear within less than 100 years. Supposing that the  $X^{SR}$  chromosomes did not reach fixation, but rather a frequency of  $\sim 80\%$  as suggested by the frequencies of the major haplotypes around the second drive locus (see below), then their decrease should have started around 1990. These values are compatible with the estimates obtained by Derome et al. (2008). Although the frequency data from Mayotte suggest a slower decrease in this population than in Madagascar, they are compatible with the lowest limit of the selective cost estimated from Madagascar data. The scenario of general decrease of  $X^{SR}$  chromosomes in this geographic area is also supported by data from Réunion Island, where there is also complete suppression (Atlan et al. 1997; Jutier et al. 2004), and their frequency dropped by about one half during the last decade (Atlan et al. 1997; Jutier et al. 2004 and supplementary table S5, Supplementary Material online). The decrease of  $X^{SR}$  chromosomes in populations where suppression is complete indicates that they have deleterious effects other than those that are a direct consequence of the drive. These can be due to the distorter alleles themselves, through pleiotropic effects, or to deleterious variants linked to the compound drive locus. In that respect, the evolution of the Paris system in *D. simulans* is in striking contrast with that of the Winters *sex-ratio* recently described in this species (Tao, Araripe, et al. 2007; Tao, Malsy, et al. 2007).

Despite the evolution of complete drive suppression several thousand years ago, the Winters distorter alleles still persist in populations (Kingan et al. 2010), thus suggesting no or negligible deleterious effect.

### Discrepancy in Evolution of the Two Elements

The number of chromosomes carrying the major haplotypes relative to the number of  $X^{SR}$  chromosomes ( $N_{maj}/N^{X^{SR}}$ ) in the samples Madagascar 2008 and Mayotte 1999 showed a striking contrast between the SR duplication and the region containing the second drive element. In both samples, drawn from populations with moderate frequencies of  $X^{SR}$  chromosomes, the major haplotypes at markers around the second element were still at high frequencies regardless of the phenotype of the X chromosomes (illustrated by  $\pi_{SR}/\pi_{ST}$  values oscillating around 1). Depending on the marker, between 56% and 88% of the  $X^{ST}$  chromosomes carry the major haplotype. This results in high values of  $N_{maj}/N^{X^{SR}}$  (1.75 on average), which is not the case for the markers within the SR duplication (1.09 on average). This result suggests that the counterselection acts mainly, if not exclusively, on the SR duplication or at loci harboring deleterious element(s) nearby. Because the distorter allele at the second locus does not seem to be associated with deleterious effects, the observed frequency of major haplotypes linked to this locus could be close to the maximum frequency attained by  $X^{SR}$  chromosomes before they started decreasing. We can infer that in the past the  $X^{SR}$  chromosomes had reached similar high frequencies in Mayotte and Madagascar.

### Rapid Replacement of Deleterious $X^{SR}$ by $X^{ST}$ Chromosomes

Once suppression is complete, the  $X^{ST}$  chromosomes locally increase in frequency, which may lead to their fixation. This has likely already occurred in Kenya and could happen in Mayotte and then in Madagascar. There is little genetic differentiation between the three populations of Madagascar, Mayotte, and Kenya (Baudry et al. 2006) thus indicating a high gene flow, which must allow a rapid dispersal of the  $X^{SR}$  and  $X^{ST}$  chromosomes. The three populations shared major haplotypes at the studied markers, which strongly supports the hypothesis of a common ancestor distorter chromosome invading the three populations. The change in  $X^{SR}$  frequencies suggests a scenario where the *sex-ratio* phenotype invaded first Kenya, then Mayotte, and finally Madagascar. This scenario is also supported by the patterns of linkage disequilibrium and by the estimates of the minimum number of recombination events ( $R_m$ ): Madagascar 2000 had the smallest  $R_m$  values among four samples for all markers except K, and all markers, except K, show a linear correlation with the *sex-ratio* distortion phenotypic differences between the four samples (supplementary table S4, Supplementary Material online). Two other observations support the scenario that  $X^{SR}$  chromosomes spread in Mayotte prior to the invasion of Madagascar. First, in spite of repeated cloning and typing efforts, one of the  $X^{SR}$  chromosomes

in the Mayotte sample (XDz31b) showed no sign of sequence variation at marker E, indicating that the proximal (Ep) and distal (Ed) copies of the duplication carry the same sequence. This chromosome XDz31b sequence was the major haplotype usually found at the proximal copy, thus it should represent the ancestral state. Moreover, the Kenyan  $X^{SR}$  chromosome shows the same pattern. Although this pattern may alternatively result from secondary gene conversion between the two copies, the observation of 18 nucleotides and 13 indels polymorphisms scattered along 479 bp differentiating Ed and Ep major haplotypes renders the gene conversion hypothesis very unlikely (supplementary fig. S1, Supplementary Material online in Derome et al. 2008). Second, among four singletons detected on major haplotypes in the four studied samples, three were in the Mayotte 1999 sample and one in the more recent Madagascar 2008 sample (fig. 2). Taken together, these results strongly suggest that the *sex-ratio* phenotype occurred first in Kenya, before spreading in Mayotte, and finally in Madagascar.

### Alternative Scenario

We found no indication in the molecular data that  $X^{SR}$  chromosomes drifted in the past to high frequency in Kenya. The major haplotypes found in Mayotte and Madagascar were at low frequencies in the Kenyan sample, except at marker E. Thus, either the effect of counterselection against the SR duplication (or a nearby locus) extends up to, or beyond, marker L or the distorter chromosomes have only occurred in Kenya at low frequencies.

An alternative scenario would be that, due to differences in drive/suppression and fitness parameters,  $X^{SR}$  chromosomes did have different dynamics of invasion in the three populations. If the  $X^{SR}$  chromosomes invasion went slowly or if they were stopped before reaching high frequency, recombination events with  $X^{ST}$  chromosomes would have then occurred more often. Consequently, the molecular signature of the selection would have been softened, thus mimicking an older selective sweep signature. Basically, as the three populations are connected by substantial gene flow (Baudry et al. 2006),  $X^{SR}$  chromosomes from Madagascar and Mayotte could diffuse passively to Kenya, accompanied by their own suppressors. However, if  $X^{SR}$  chromosomes never rose to high frequency in Kenya, the strong autosomal suppression recorded in this population (Atlan et al. 2003) is difficult to explain. Indeed, autosomal suppressors are thought to evolve to counteract a biased sex ratio, which requires a high frequency of  $X^{SR}$  chromosomes. Consequently, the scenario involving drive suppressors passively diffusing to a population harboring few or no driving chromosomes would be possible if suppressors have a low cost, if any. However, complete suppression is also widespread in sub-Saharan Africa, for example, in Congo (sampled in 1989) and Tanzania (sampled in 1992), where X chromosomes exhibited little or no ability to drive (Atlan et al. 1997). This is hardly compatible



with the scenario of passive diffusion of suppressors because sub-Saharan populations of *D. simulans* are structured into several distinct groups (Baudry et al. 2006; Schöfl and Schlötterer 2006). Because the suppressors appeared able to spread across these groups, we can reasonably assume that  $X^{SR}$  chromosomes preceded them and swept to sufficiently high frequency, possibly in a patchy manner. This would imply that either the *sex-ratio* system has a rather long history in continental Africa or it can evolve astonishingly quickly.

The molecular data suggest that the  $X^{SR}$  chromosomes present in the three locations studied here originated in the recent past. All the major haplotypes, within the duplication and around the second distorter element, are derived alleles (fig. 3 in Derome et al. 2008). In addition, unless extensive gene conversion occurred between its two copies, the SR duplication itself should be very young. It shows the same major haplotypes at both copies of markers B, C, and D, and no variation occurred on background of the major haplotypes at these markers. A low number of recombination events occurred within the duplication as witnessed by the  $\pi_{SR}/\pi_{ST}$  ratio, despite experimental evidence that recombination is possible in heterozygous  $X^{SR}/X^{ST}$  females (Montchamp-Moreau et al. 2006). Assuming a long history of the Paris *sex-ratio* system in Africa, the  $X^{SR}$  chromosomes described here should correspond to a new type, secondarily arisen. Further molecular analysis, extended to other geographic regions, should allow their history to be traced back through time.

## Conclusion

Similarly to the evolution of *P* transposable element that spread throughout the worldwide range of *D. melanogaster* in only 30 years (Anxolabéhère et al. 1988), the rise and fall of  $X^{SR}$  chromosomes in the Paris system occurred extremely quickly, at least in the surveyed area. This is the first report of rapid change in frequency of  $X^{SR}$  chromosomes on such a short time scale. Also, while past selective events have recently been characterized in other meiotic drive systems, thanks to their genomic signatures (Presgraves et al. 2009; Kingan et al. 2010), the Paris system offers the opportunity to witness an ongoing cycle. What will happen next in Africa merits attention: the death of the system if the  $X^{SR}$  completely disappeared and the suppressors decreased in turn, or a novel wave of advance of distorter alleles thanks to the evolution of X-linked enhancer genes enabling them to evade suppression? In addition, outside of Africa, some populations display moderate frequencies of  $X^{SR}$  chromosomes along with no or slight suppression (Atlan et al. 1997; Jutier et al. 2004), which should allow  $X^{SR}$  to invade new areas.

## Supplementary Material

Supplementary tables S1–S5 and figures S1–S3 are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

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