# Signature of Selective Sweep Associated With the Evolution of *sex-ratio* Drive in *Drosophila simulans*

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ABSTRACT

In several Drosophila species, the XY Mendelian ratio is disturbed by X-linked segregation distorters (*sex-ratio* drive). We used a collection of recombinants between a nondistorting chromosome and a distorting X chromosome originating from the Seychelles to map a candidate *sex-ratio* region in *Drosophila simulans* using molecular biallelic markers. Our data were compatible with the presence of a *sex-ratio* locus in the 7F cytological region. Using sequence polymorphism at the *Nrg* locus, we showed that *sex-ratio* has induced a strong selective sweep in populations from Madagascar and Réunion, where distorting chromosomes are close to a 50% frequency. The complete association between the marker and the sex-ratio phenotype and the near absence of mutations and recombination in the studied fragment after the sweep event indicate that this event is recent. Examples of selective sweeps are increasingly reported in a number of genomes. This case identifies the causal selective force. It illustrates that all selective sweeps are not necessarily indicative of an increase in the average fitness of populations.

A seminal article by Morgan (1910) on sex-linked inheritance in Drosophila showed that the sex ratio of the progeny in fruit flies results from a simple XY Mendelian segregation. The observation of *sex-ratio* X chromosomes in natural populations of *Drosophila obscura* (Gershenson 1928) and *D. pseudoobscura* (Sturtevant and Dobzhansky 1936), however, revealed that genes sometimes circumvent Mendel's law. The *sex-ratio* trait has since been reported in males of a dozen Drosophila species and more recently in Diopsidae (reviewed by Jaenike 2001). It is caused by X-linked meiotic drive factors that induce the loss of Y-bearing sperm, resulting in the production of female-biased progeny. The spread of X-linked drivers induces biases in the population sex ratio and confers an advantage to parents reducing the bias in their offspring (Fisher 1930). This can be viewed as a genetic conflict between the X chromosome and the rest of the nuclear genome, which induces a selective pressure in favor of drive suppressors on the Y chromosome and the autosomes (Hamilton 1967). It has been proposed that distorters and suppressors occur as balanced polymorphisms in natural populations (discussed in Carvalho and Vaz 1999). An alternative is that *sex-ratio* systems evolve through waves of recurrent invasions, making it necessary to characterize a model species where sex-ratio events can be easily followed in natural populations.

*Drosophila simulans* is a potential model for addressing this question. *Sex-ratio* chromosomes are widespread in the African range of this cosmopolitan species, from Sao Tome Island, on the west coast, to Réunion Island, on the east coast, suggesting that the system was established long ago (Jutier *et al.* 2004). An efficient suppression system has evolved, thus maintaining an ~1:1 sex ratio, even in populations where *sex-ratio* chromosomes are at a high frequency (Atlan *et al.* 1997). There are virtually no inversions in *D. simulans*, making it easier to map sex-ratio factors and to interpret DNA variation than in *D. pseudoobscura* (Babcock and Anderson 1996; Kovacevic and Schaeffer 2000) where a characteristic "sex-ratio" chromosome arrangement occurs. When segregation distortion is completely suppressed, *sex-ratio* chromosomes are expected to be lost or fixed by random drift if neutral and to be eliminated by selection if deleterious. Their distorting ability may also degenerate. This may explain why distorting X chromosomes are rare in some African locations and frequent in others. About one-half of X chromosomes from Madagascar and Réunion are *sex-ratio*. This is the highest frequency ever recorded in a Drosophila species (Jutier *et al.* 2004) and suggests that these populations experienced a recent spread of drive factors.

No *D. simulans sex-ratio* factors have been characterized at the molecular level, making it difficult to study their dynamics in natural populations. Therefore, characterizing the genotype of individuals involves genetic tests, through counting and sexing the progeny of indi-

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vidual males in a standard background. This is achieved after several generations of crosses in the laboratory. These techniques cannot be applied on a large scale even though it would be worthwhile to distinguish the frequency of distorting elements from the distribution of their strength. In addition, they cannot provide information about past events. The molecular characterization of distorter loci would allow us to study selective events associated with the evolution of *sex-ratio* X chromosomes in populations by using the sweeping effect of selection on DNA neutral variation (Aguadé *et al.* 1989; Kaplan *et al.* 1989).

Here, we present the first molecular genetic study attempting to characterize *sex-ratio* in *D. simulans* and to trace its history. First, we used a collection of recombinant X chromosomes to fine-map the *sex-ratio* candidate region using molecular markers. Second, we looked for the molecular signature of selective sweeps within the candidate region in natural populations from Madagascar and Réunion. We used the *vermilion* gene as a neutral reference marker (Hamblin and Veuille 1999) to distinguish between local and genome-wide departure from neutral equilibrium model.

## MATERIALS AND METHODS

**Fly stocks and genetic study:** The stocks used were previously described by Montchamp-Moreau *et al.* (2001) and Montchamp-Moreau and Cazemajor (2002). The ST8 standard stock provides a reference genetic background free of distorters and drive suppressors. All crosses mentioned below were carried out using lines with an ST8 background obtained through repeated backcrossing to the ST8 stock. Males from the SR6 line carry a *sex-ratio* X chromosome ($X^{SR6}$). The *sn,lz*/C(1)RM line is a stock in which females carry the compound X chromosomes from the lz[sp]/C(1)RM,*y,w* stock (Bloomington Stock Center), whereas males carry a *standard* (nondriving) X chromosome with the *singed* and *lozenge* mutations. The $X^{sn,lz}$ chromosome was obtained by recombination of chromosomes from the lz[sp]/C(1)RM, *y w* stock with the *sn* stock (Bloomington Stock Center).

Recombinant chromosomes between the *sex-ratio* $X^{SR6}$ (producing 95% females in the ST8 background) and the standard $X^{sn,lz}$ were obtained according to the crossing scheme given in Figure 1. Phenotypically [+lz] recombinants were maintained in male lineages through repeated backcrosses with C(1)RM,*y,w* females. Each recombinant chromosome was tested for its segregation ratio in the drive-sensitive ST8 background, following the protocol of Montchamp-Moreau and Cazemajor (2002).

The $X^{SR6}$ *sex-ratio* chromosome had been extracted from a laboratory stock originating from the Seychelles, and no population sample was available. For the population study, we used X chromosomes from the same area (islands off the eastern coast of Africa). These chromosomes came from random samples of flies collected in December 1996 at La Saline (Réunion) by C. Montchamp-Moreau and in 2000 at Antananarivo (Madagascar) by M. Veuille. Iso-X lines were started in the laboratory by crossing single wild-caught males with females carrying compound C(1)RM,*y,w* X chromosomes. Line maintenance and the characterization of *sex-ratio* and *standard* phenotypes are described in Montchamp-Moreau and Cazemajor (2002) and Atlan *et al.* (2003). Males from each iso-X line were frozen and stored at −80° until use.

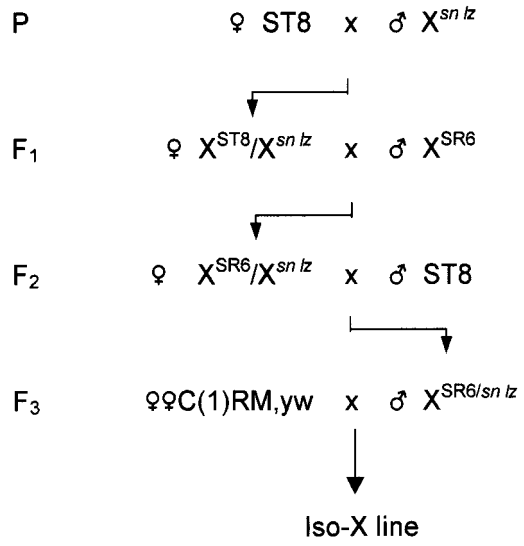


Figure 1.—Crossing scheme used to obtain recombinant X chromosomes in mapping experiments. All lines are in an ST8 background.

**DNA polymorphism survey and data analysis:** Extraction of single male genomic DNA, PCR amplification, and sequencing reactions were carried out using classical protocols (see, *e.g.*, Hamblin and Veuille 1999). The PCR amplification primers for the *Nrg, otd, otu*, and *rdgA* genes are shown in Table 1. For the *v* gene, we used formerly designed primers (Hamblin and Veuille 1999) and sequenced a region overlapping positions 654–1350 of GeneBank accession no. U27204. For the single-strand conformation polymorphism (SSCP) study, PCR products were denatured 2 min at 95° in 40% formamide and loaded on 8% polyacrylamide/7.5% glycerol nondenaturing gels in 1× TBE. Migration was run for 9 hr at 24 W at room temperature. Sequencing was carried out using an ABI-310 automated sequencer. Sequences were aligned using the Bioedit program, and the analysis of molecular variation was carried out using Allelix (Mousset *et al.* 2003), DNAsp 3.53 (Rozas and Rozas 1999), and MEGA version 2.1 (Kumar *et al.* 1994).

We checked departure from a standard neutral mutation-drift equilibrium model using several tests. The Hudson-Kreitman-Aguadé (HKA) test (Hudson *et al.* 1987) considers selection in one locus compared to a reference locus on the basis of population variation and divergence from an outgroup for the two loci. Tajima's (1989) *D* and Fu and Li's (1993) *D* statistics consider departure from neutrality in the frequency pattern of a sample of polymorphic sites by examining the consistency of several estimates of the neutral mutation parameter that are based on different predictions of the infinite site model. The *H* and *K* haplotype tests (Depaulis and Veuille 1998) consider departure from neutrality in the frequency distribution of haplotypes on the basis of information from polymorphic sites. Haplotype tests were run using a constant number of mutations (*S*) for a sample of *n* chromosomes (Depaulis *et al.* 2001, 2003, 2004; Wall and Hudson 2001). In practice, we will report results of the *H*-test, which uses haplotype heterozygosity and was run using 10,000 iterations. The *K*-test always gave results consistent with the *H*-test.

These tests may yield different results depending on whether intragenic recombination is taken into account or not. We thus ran them under both models. For tests with recombination, we used an experimentally derived recombina-

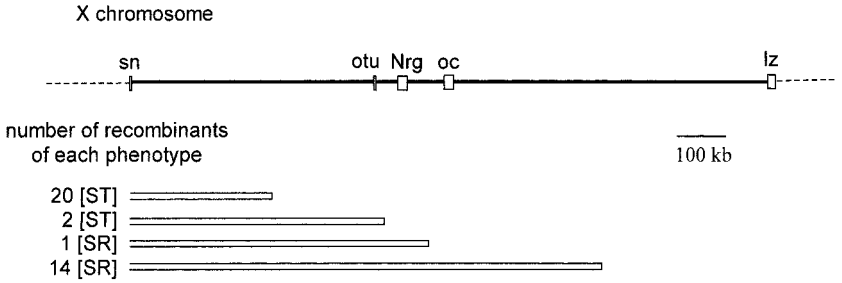


FIGURE 2.—Selecting a genetic marker for the *sex-ratio* phenotype: 37 [$sn^+$ *lz*] recombinant X chromosomes obtained from a cross between an *sn-lz* line and the $X^{SR6}$ *sex-ratio* chromosome are ranked according to a series of diallelic molecular markers; gene locations and intergenic distances are those from the standard map of *D. melanogaster*. Open boxes represent the part of the recombinant chromosomes that comes from the parental *sex-ratio* chromosome.

tion rate for *D. melanogaster* in *Nrg* ($9.96 \times 10^{-9}$ event/bp/generation) as calculated using the method of AQUADRO *et al.* (1994). This value was multiplied by 2/3 (thus assuming a 1:1 sex ratio), as our marker is X linked. We obtained a recombination rate $r = 6.63 \times 10^{-9}$/site/generation. This recombination rate was conservative for the tests being used, as the value obtained from genetic experiments in *D. simulans*, $5.8 \times 10^{-8}$, was higher (see RESULTS). For *vermilion*, we used the recombination rate estimated from *D. melanogaster* ($r = 3.60 \times 10^{-8}$ events/generation/bp). This value was higher than that for *Nrg*. However, as *v* was used as a reference neutral locus to compare with *Nrg*, using this estimate was conservative.

## RESULTS

**Characterizing the *sex-ratio* genomic region:** CAZEMAJOR *et al.* (1997) previously showed that a major *sex-ratio* effect is caused by one or more genetic factors located between the *nipped wing* (*np*) and *lozenge* (*lz*) genes. Further genetic experiments using the intervening locus *sn* (located between *np* and *lz*) showed that this or these factors are located within the *sn-lz* interval (data not shown). The recombination rate between *sn* and *lz* was 7.7 ± 2.6 cM. These experiments yielded 37 [+*lz*] X chromosomes derived from a cross between the [++] $X^{SR6}$ *sex-ratio* chromosome and the [*sn lz*] marker stock. Following the definition of sex-ratio phenotype (MONTCHAMP-MOREAU and CAZEMAJOR 2002), 14 [+*lz*] chromosomes were *sex-ratio* (yielding 77–96% female progeny), and 23 were *standard* (producing 46–66% female progeny).

Since heterozygosity in *D. simulans* intron DNA is in the $1–2 \times 10^{-2}$ range, the probability of the two parental stocks used in the mapping experiment showing different SSCP alleles in an ≈200-bp PCR fragment was very high. Association between these alleles and the *sex-ratio* phenotype in recombinant lines was recorded for mapping *sex-ratio* genes. Results are shown in Figure 2. We used PCR fragments from the *otu*, *Nrg*, *otd*, and *rdgA* loci that had been cytologically characterized before the first release of the Drosophila Genome Project. We were able to characterize a *sex-ratio* region located between markers from the *otu* and *otd* loci. Mapping ended when recombination spots in our recombinant lines were separated by the intervening *Nrg* locus. Distortion in this region would thus involve a DNA fragment from this interval.

**Selecting a marker for population studies:** A random sample of 41 X chromosomes from Réunion was surveyed for an association between the sex-ratio phenotype and SSCP variation for a 214-bp fragment (see Table 1) overlapping the second intron of the *Nrg* locus. In this sample, 21 X chromosomes were *sex-ratio* and 20 were *standard* (MONTCHAMP-MOREAU and CAZEMAJOR 2002). Results are shown in Table 2. We found six alleles, including a major allele (slow) at a frequency of 0.83. Heterozygosity among distorter X chromosomes was low ($H = 0.09$), all but one carrying the slow allele. Heterozygosity among nondistorter chromosomes was higher ($H = 0.51$). The slow allele thus showed a strong association with the *sex-ratio* trait. Statistical association, $D' = 0.72$ [calculated after LEWONTIN's (1964) coefficient of linkage disequilibrium], was significant ($P = 0.04$, Fisher's exact test after pooling all nonmajor alleles). The substantial difference in heterozygosity between the two allelic classes suggests that a selective sweep event associated with the spread of a distorter has affected polymorphism at the *Nrg* locus. This gene thus appears to be an appropriate marker of the history of *sex-ratio* in natural populations.

**Nucleotide polymorphism at *Nrg* in natural populations:** Samples of 15 X chromosomes were taken at random from each of the Madagascar and Réunion populations. Among these, 7 chromosomes from Réunion and 10 chromosomes from Madagascar were *sex-ratio*.

Sequence polymorphism was recorded for an 802-bp fragment of the *Nrg* locus overlapping two exons and three introns. Pooled data for the two populations are summarized in Table 3 and Figure 3. After discarding positions overlapping an insertion/deletion, we obtained a 774-bp aligned sequence. Of 33 nucleotide polymorphisms, 26 occurred in introns (including a three-state polymorphism), and 7 in exons (all synonymous).

The most striking feature of the data set was that 22 of the 30 chromosomes were strictly identical. They included the 10 *sex-ratio* chromosomes from Madagascar and 6 of the 7 *sex-ratio* chromosomes from Réunion. The seventh chromosome was that previously found to cause heterogeneity in the *sex-ratio* class using SSCP. It differed from the other 6 by a single nucleotide substitution. This suggests that this chromosome derives from

**TABLE 1**

**PCR primers**

| Locus | Accession no. | PCR primers | Product size (bp) |
|---|---|---|---|
| *Nrg* | AF050085 | f: 5′ ACTTGGCTAGTCCATTGTCG 3′ | |
| | | r: 5′ CATCACTGCCACCACGAAAA 3′ | 214 |
| | | r: 5′ GAATCAGTATCGCTTTCCAC 3′ | 869 |
| *otd* | X58983 | f: 5′ CAATGCCAACTATGATCGCG 3′ | |
| | | r: 5′ TTACTTCGTTGATCCTGGCG 3′ | 247 |
| *otu* | M30825 | f: 5′ CGACGCATCTTTGAGAAGGT 3′ | |
| | | r: 5′ TACAGGATAACATTGCGGCT 3′ | 263 |
| *rdgA* | D17315 | f: 5′ ATTCCGCAACATTCAGTCCA 3′ | |
| | | r: 5′ TGTTGCTACAAAGTGGTTGC 3′ | 223 |

f, forward primer; r, reverse primer.

the other *sex-ratio* chromosomes by a recent mutation or by recombination since this change (A → G) matches a polymorphism that is also present in the rest of the sample. Below we will consider it as belonging to the same haplotype family as the others, that is, to the "major haplotype." Five *standard* chromosomes, all from Réunion, also belonged to the major haplotype. All other haplotypes were very different from each other. The association between the major haplotype and *sex-ratio* was complete and highly significant in Madagascar ($D' = 1$, $P = 3.3 \times 10^{-4}$, Fisher's exact test). Considered in addition to SSCP data, the major haplotype appeared to be in association with *sex-ratio* in the two populations. A neighbor-joining tree of *Nrg* sequences is shown in Figure 4.

**Neutrality tests:** Neutrality tests are shown in Table 4. When the *sex-ratio* and standard X chromosomes were considered together, the HKA test was nonsignificant for both samples (data not shown). However, this test should be significant only when a drastic reduction in the number of polymorphic sites has occurred. This was not the case in our samples, where most standard chromosomes were very polymorphic. Under the conservative assumption of no recombination, Tajima's test was significant only for Réunion; Fu and Li's test was nonsignificant for both samples. However, when the conservative recombination rate ($r = 6.63 \times 10^{-9}$/site/generation) was used, Tajima's test became significant for both samples. The haplotype test (*H*-test; Depaulis and Veuille 1998) was always significant when no recombination was assumed. Significance was increased when recombination was assumed. This result is consistent with evidence from data inspection that one haplotype (the major haplotype) is at a high frequency, most likely due to a selective sweep. When only standard chromosomes were considered, all tests were nonsignificant with two exceptions when recombination was considered: Fu and Li's test became highly significant for Madagascar, probably because of the excess of singletons in this population; and the *H*-test for Réunion became significant, probably because of the presence of the major haplotype in six of eight chromosomes.

**Comparison with polymorphism at vermilion:** This was the first sequence variation study for *D. simulans* in Madagascar and Réunion. We thus had to confirm that

**TABLE 2**

**Association between *Nrg* SSCP alleles and the *sex-ratio* trait in Réunion**

| Allele class | *sex-ratio* ($n = 21$) | *standard* ($n = 20$) |
|---|---|---|
| Ultraslow | 0 | 2 |
| Slow | 20 | 14 |
| Medium | 0 | 1 |
| Fast | 1 | 0 |
| Ultrafast | 0 | 2 |
| Extraconformational | 0 | 1 |
| Heterozygosity | 0.09 | 0.51 |

Heterozygosity was estimated as $H = n/(n - 1)(1 - \Sigma p_i^2)$.

**TABLE 3**

**Nucleotide polymorphism and divergence in the *Nrg* region**

| | Madagascar | Réunion |
|---|---|---|
| Sample size | 15 | 15 |
| No. of sites | 779 | 778 |
| Haplotype diversity | 0.571 | 0.371 |
| No. of polymorphic sites | 28 | 15 |
| Divergence[a] | 0.083 | 0.030 |
| $\pi$ (average pairwise difference) | 0.007 | 0.003 |
| $\theta_W$ (Watterson's estimator) | 0.011 | 0.005 |
| Standard subsample | | |
| Sample size | 5 | 8 |
| $\pi$ (average pairwise difference) | 0.015 | 0.006 |
| $\theta_W$ (Watterson's estimator) | 0.017 | 0.007 |

[a] Average number of silent substitutions per site between *D. simulans* and *D. melanogaster*.

| Line | ♀(%) | Position |
|---|---|---|
| | | IIIIIISSSIIIIIIIIIIISSSSIIIIIIII |
| | | 1112222222222233356666666 |
| | | 1111127067011334666679559366679 99 |
| | | 0156737110335582478980362034931 46 |
| **R02** | **83** | **TATCTAGAAGTCTAGGTGAGTCGGCCAACACAC** |
| **R17** | **96** | ..... |
| **R104** | **94** | .....**G.** |
| **R105** | **83** | ..... |
| **R107** | **91** | ..... |
| **R110** | **85** | ..... |
| **R51** | **83** | ..... |
| R04 | 59 | ..... |
| R16 | 52 | ..... |
| R13 | 52 | .....CTG.C...TA...T.C.....G..... |
| R111 | 52 | ..... |
| R133 | 51 | ..... |
| R55 | 50 | .TCG.CT.G.....T.....G..... |
| R56 | 51 | ..... |
| R57 | 50 | ..... |
| **M01** | **86** | ..... |
| **M02** | **91** | ..... |
| **M04** | **92** | ..... |
| **M06** | **84** | ..... |
| **M07** | **84** | ..... |
| **M08** | **92** | ..... |
| **M09** | **93** | ..... |
| **M12** | **90** | ..... |
| **M14** | **71** | ..... |
| **M15** | **85** | ..... |
| M03 | 52 | ....CCT.G.....A...T..TA.A...T.AG. |
| M05 | 52 | C....CT.G.....T..T.....G. |
| M10 | 47 | C....CT.G...C...C.GT.....GG...T |
| M11 | 51 | .....G.A..TA...T....A.T...T... |
| M13 | 52 | C....CT.G..TC.A..AT.....G. |
| *D. melanogaster* | | .TC..CTGGA.....AT.C..... |

FIGURE 3.—Nucleotide polymorphism in a 774-bp fragment at *Nrg* in 30 X chromosomes from Madagascar and Réunion. Female percentage of the progeny of males was estimated in an ST8 background (from MONTCHAMP-MOREAU and CAZEMAJOR 2002); *sex-ratio* chromosomes are in boldface type.

the observed deviation from the neutral equilibrium model at *Nrg* resulted from selection at a single locus and not from a genome-wide deviation in this population. A survey of several *D. simulans* populations by HAMBLIN and VEUILLE (1999) showed that molecular variation at the *vermilion* gene (*v*) does not depart from a neutral equilibrium in Africa. The *vermilion* gene thus appeared to provide a reliable neutral reference marker. We aligned 697 bp of the *vermilion* sequence, including a 165-bp intron, in 15 chromosomes from each of the two populations. Sequence alignments are shown in Figure 5, and summary statistics are shown in Table 5. The nucleotide variation in the sample from Madagascar was higher than that in the sample from Réunion, and both values were at least twice as high as those found at *Nrg* for the same populations. Each sequence in Madagascar belonged to a different haplotype. There was no significant deficit of haplotypes using the *H*-test. Indeed, there was an excess of haplotypes, even when assuming a recombination rate of $r = 3.60 \times 10^{-8}$ (*P* value of bidirectional *H*-test $<10^{-4}$). This was due to an excess of singletons. In agreement with this, Tajima's *D* and Fu and Li's *D* were negative and became significant in simulations using recombination. In Réunion, the 15 sequences contained nine haplotypes. However, an *H*-test using recombination indicated a significant deficit of haplotypes. Tajima's *D* and Fu and Li's *D* were close to zero and were never significant.

## DISCUSSION

**Identifying a selective sweep:** This study involved three steps to characterize genes causing the *sex-ratio* phenotype in *D. simulans* and to study their dynamics in natural populations.

In a first step, using a distorting X chromosome from the Seychelles, we showed the presence of a *sex-ratio* factor in subdivision 7F, between *otu* and *otd*. In *D. melanogaster* the *otu-otd* region overlaps a 170-kb fragment, including 15 annotated genes. The organization of this genomic region in *D. simulans* should not be very


FIGURE 4.—Neighbor-joining tree of *Nrg* sequences for Madagascar and Réunion X chromosomes, using *D. melanogaster* as an outgroup. M, Madagascar; R, Réunion. ◆, *sex-ratio* X chromosomes.

different, since the X's of the two species are homosequential (LEMEUNIER and ASHBURNER 1976).

In a second step, we focused on the *otu-otd* candidate region using as a marker the *Nrg* gene, which is in the middle of this interval. A *sex-ratio* factor would be within 100 kb from *Nrg*, assuming a monofactorial genetic determinism. Using SSCP, we found a significant association between *sex-ratio* and *Nrg* in a population sample from Réunion. Moreover, there was a striking contrast in heterozygosity between the *sex-ratio* X chromosomes and the standard ones, due to the presence of a major allele that was especially predominant among distorting chromosomes. Together, the results suggested that the distorting region found for the Seychelles was also active in Réunion and that the same gene was involved.

In a third step, we recorded sequence variation at *Nrg* in Madagascar and Réunion and found that a major allele was shared by the two populations. An examination of variation at the sequence level showed that *sex-ratio* chromosomes constitute a homogeneous class of haplotypes (the major haplotype), since heterogeneity in this class involved only a singleton in one chromosome, which could be due to either a mutation or recombination. Overall, the statistical association, along with the significance of the haplotype test, confirmed that a selective sweep related to the spread of *sex-ratio* chromosomes has affected *Nrg* sequence variation. The homogeneity of the *sex-ratio* class strongly suggests that the same allele is responsible for the trait in both populations, but we cannot say at this stage whether the selective sweep occurred once in an ancestral population or in each population separately. It also indicates that the *Nrg* locus maps very close to the selective sweep factor on the chromosome or that the sweep events are recent. However, the fact that the major haplotype was also found among *standard* chromosomes shows that the fragment sequenced at *Nrg* is not itself the distorting factor, assuming a monofactorial genetic determinism.

**Alternative explanations:** The above interpretations are the most probable ones. We must, however, consider alternative explanations.

*Population bottleneck:* A population bottleneck could have caused a shift in haplotype distribution. HAMBLIN and VEUILLE (1999) studied sequence variation at the *vermilion* gene in *D. simulans* and found that non-African populations showed a decrease in both nucleotide and haplotype diversity compared to African ones. Here we found that *v* in Madagascar, as in other East African populations (Kenya, Tanzania, and Mayotte), shows no evidence of a bottleneck in *D. simulans* (range of the other African populations for the same 697-bp fragment, $\pi = 0.0119$–$0.0148$, $\theta_W = 0.0143$–$0.0207$, calculated from Hamblin and Veuille's 1999 data). However, genetic variation at the *v* locus is lower in Réunion than in other African populations and close to that of derived populations from Europe and the Antilles (range $\pi = 0.0098$–$0.0106$, $\theta_W = 0.0095$–$0.0105$, calculated from Hamblin and Veuille's 1999 data), suggesting the occurrence of a recent bottleneck or a partial selective sweep. The *v* and *Nrg* loci are located at some distance from each other on the X chromosome (at cytological positions 9F10-11 and 7F3-4, respectively) and are separated by the substantial genetic distance of 10 cM. In Madagascar, genetic variation at these two loci is in opposite directions, as *v* shows an excess of low-frequency variants, whereas *Nrg* shows a deficit of low-frequency variants. This confirms the selective sweep for *Nrg* in Madagascar. The conclusion is less firm for Réunion. We have independent evidence from other loci (E. BAUDRY, N. DEROME, M. HUET and M. VEUILLE, unpublished data) that the Madagascar population may be expanding and that the Réunion population may be the result of a recent immigration. However, the fact that

**TABLE 4**

**Neutrality tests for the *Nrg* region**

| | Madagascar | Réunion |
|---|---|---|
| Assuming no recombination | | |
| *H*-test | $P < 0.001$ | $P < 0.005$ |
| Tajima's *D* | −1.424 (NS) | −1.866 ($P = 0.018$) |
| Fu and Li's *D* | −1.816 (NS) | −1.847 (NS) |
| Assuming recombination | | |
| *H*-test | $P < 0.001$ | $P < 0.001$ |
| Tajima's *D* | $P = 0.006$ | $P < 0.001$ |
| Fu and Li's *D* | $P = 0.018$ | $P = 0.017$ |
| Standard subsample | | |
| Assuming no recombination | | |
| *H*-test | NS | $P = 0.003$ |
| Tajima's *D* | −0.725 (NS) | −1.06 (NS) |
| Fu and Li's *D* | −1.362 (NS) | −0.879 (NS) |
| Assuming recombination | | |
| *H*-test | NS | $P = 0.001$ |
| Tajima's *D* | NS | NS |
| Fu and Li's *D* | $P = 0$ | NS |

NS, not significant.

the same *Nrg* haplotype is dominant in both populations and is associated with *sex-ratio* indicates that the selective sweeps observed at this locus in Madagascar and Réunion have the same cause.

*Low local recombination rate:* Segregation distorters are often associated with a low recombination rate due to a chromosomal inversion or a pericentromeric location, which may result in reduced polymorphism and statistical association over extended regions (Hammer and Silver 1993; Babcock and Anderson 1996; Palopoli and Wu 1996). This cannot be the case for *sex-ratio* in *D. simulans*. No inversion was found on polytene chromosomes in the 7F region in both *sex-ratio/standard* heterozygous females and *D. melanogaster/D. simulans* hybrid females (F. Lemeunier, personal communication). The contrary would have been unexpected, since inversions are virtually absent from natural populations of *D. simulans* (Ashburner and Lemeunier 1975). The possibility of a minute inversion can also be ruled out, since the genetic distance observed between *sn* and *lz* in our genetic experiments was slightly higher for *D. simulans* (7.7 cM) than for *D. melanogaster* (6.7 cM), while the order of genes was apparently conserved.

**One or two sweeps in Réunion?** There was clearly a selective sweep, and this sweep was statistically associated with a *sex-ratio* selective sweep. This is encountered in the two surveyed populations. However, the SSCP study in Réunion showed that 20/21 *sex-ratio vs.* 14/20 *standard* chromosomes belonged to the major haplotype. A noteworthy fact was the deficit of minor haplotypes among *sex-ratio* chromosomes [$P(\chi^2) < 10^{-4}$, 1 d.f.]. Following the selective sweep event, we would have expected some level of recombination to have occurred between *Nrg* and the distorter gene and to have imported the *Nrg* major haplotype into nondistorting chromosomes and, conversely, to have imported minor haplotypes into *sex-ratio* chromosomes. A first explanation is that the *sex-ratio* chromosome in Réunion has been close to fixation in the past and that the proportion of the major haplotypes moved to a standard chromosome through recombination was lower than that of other haplotypes moved to *sex-ratio* chromosomes. An alternative explanation is that a nearby selective sweep, independent of *sex-ratio*, occurred in Réunion and further increased the frequency of the major haplotype. We currently have no means to test these hypotheses. Note, however, that they do not contradict the main conclusion of a selective sweep linked to *sex-ratio*.

**Age of the sweep:** The age of the selective sweep can be estimated from the fact that the major haplotype class is homogeneous or nearly so, allowing us to identify which chromosomes in the sample are descended from the selected haplotype. Assuming a star phylogeny of *sex-ratio* chromosomes, the expected number of mutations having occurred in this haplotype family after the selective sweep is $E(S) = Lnt(\theta/3N)$, where $L$ is the length of the sequence alignment, $n$ is the sample size of *sex-ratio* chromosomes, $t$ is the age of the selective sweep in generations, $q = 3N_e\mu$ is the neutral mutation parameter (for an X-linked locus), $N_e$ is the effective population size, and $\mu$ is the neutral mutation rate. This estimation is valid only for Madagascar, since an additional selective sweep is assumed to have affected the star phylogeny in Réunion. The expectation of having no mutation is $e^{-S}$. Assuming a 0.05 probability of obtaining this result yields the upper estimate $t_{0.05} < 0.181\ N_e$. It is often assumed that the effective population size of *D. simulans* is in the $10^6$ range and that there are ~10 generations

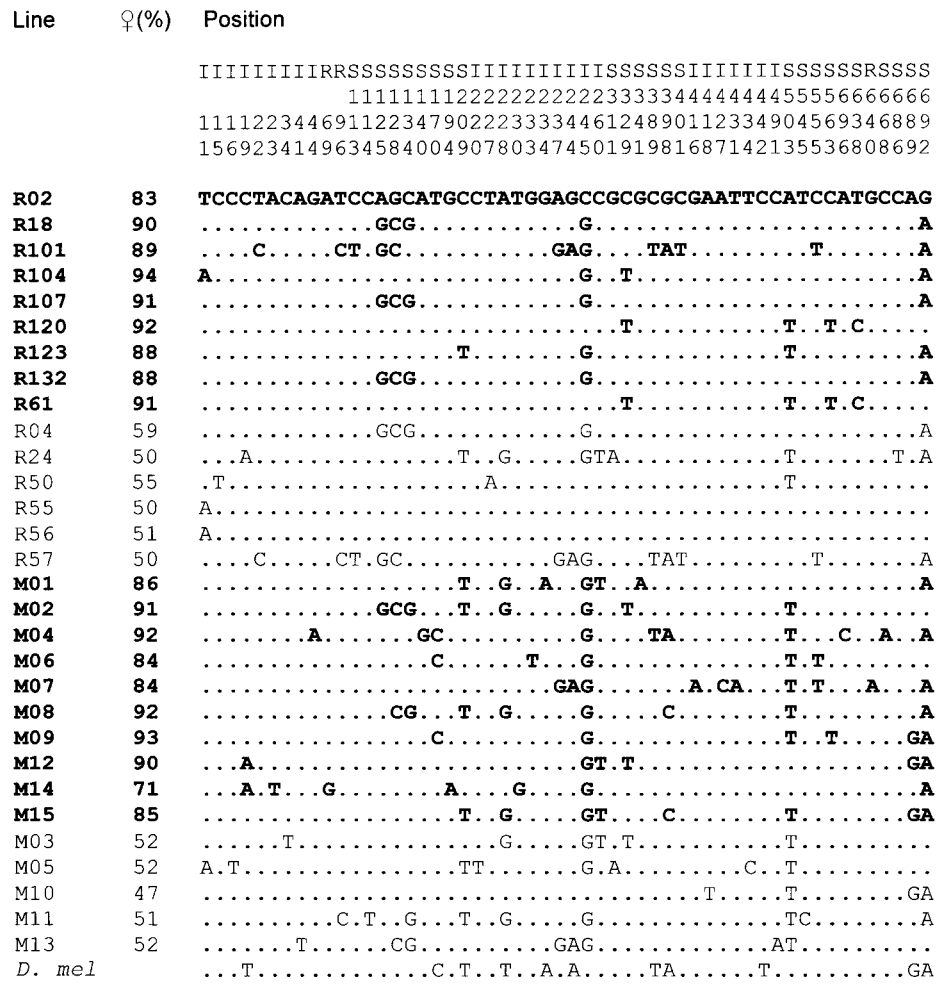


FIGURE 5.—Nucleotide polymorphism at *v* (*vermilion* gene) in a 697-bp fragment from 30 X chromosomes of Madagascar (M) and Réunion (R).

a year in the tropics. In this case, the age of the selective sweep would be <18,000 years. This upper limit is probably greatly overestimated, since the selective advantage of the distorting variant can be especially high as explained below.

**Dynamics of the *sex-ratio* event:** Given the complete association between the major haplotype and the *sex-ratio* phenotype, the *sex-ratio* selective sweeps in Madagascar and Réunion must have been strong. The distorting X chromosomes were found to produce 80–96%

**TABLE 5**

**Nucleotide polymorphism and neutrality tests for *vermilion***

| | Madagascar | Réunion |
|---|---|---|
| Sample size | 15 | 15 |
| Number of sites | 697 | 697 |
| Haplotype diversity | 0.571 | 0.371 |
| Number of polymorphic sites | 46 | 27 |
| $\pi$ (average pairwise difference) | 0.0138 | 0.0108 |
| $\theta_W$ (Watterson's estimator) | 0.0207 | 0.0119 |
| Assuming no recombination | | |
| *P* value of the *H*-test | NS | NS |
| Tajima's *D* | −1.44 (NS) | −0.38 |
| Fu and Li's *D* | −1.77 (NS) | 0.34 |
| Assuming recombination | | |
| *P* value of the *H*-test | NS | 0.016 |
| Tajima's *D* | $P < 0.05$ | $P < 0.05$ |
| Fu and Li's *D* | $P < 0.05$ | $P < 0.05$ |

NS, not significant.

female progeny in a background free of drive suppressors (Montchamp-Moreau and Cazemajor 2002; Atlan *et al.* 2003). The selective advantage of a *standard* chromosome provided by this segregation bias is so strong ($s$ = 0.30–0.46) that, assuming an initial frequency of 0.001, *sex-ratio* chromosomes would be virtually fixed in ~100 generations. It is, however, unlikely that these populations were sampled during an ongoing *sex-ratio* selective sweep, since segregation distortion was at the time inhibited by powerful suppressors. Given the low level of distortion expression (Atlan *et al.* 2003), *sex-ratio* chromosomes could invade the populations only very slowly. Moreover, repeated sampling in Réunion during the last 10 years suggests that *sex-ratio* chromosome frequency has stabilized at an intermediate level of ~50% (Jutier *et al.* 2004). We can, therefore, suppose that a strong event occurred at some time in the past on the two islands.

Drive suppressors have been found to occur together with *sex-ratio* chromosomes over a wide range of *D. simulans* populations, including East Africa, which is the likely source of emigrants to Indian Ocean islands. A first scenario is that migrants from the continent invaded *sex-ratio*-free island populations, importing both the segregation distorter and its antidote. At first, the two elements were at a low frequency in the invaded gene pool, with a low probability of occurring in the same male, allowing the distorter to benefit from its segregational advantage. An increased frequency in the suppressor secondarily occurred as a consequence of the increased frequency of *sex-ratio* chromosomes. Madagascar and Réunion may also have undergone secondary sweeps related to an arms race between suppressors and modifiers. Alternatively, a *sex-ratio* sweep may have occurred in a population ancestral to Madagascar and Reunion. However, available data do not favor this hypothesis, except in the scenario where the Réunion population is a recent invader established from Madagascar migrants.

It must be pointed out that unsuppressed *sex-ratio* chromosomes in *D. simulans* can cause detrimental effects at the individual level that exceed their segregation advantage at the gamete level, as evidenced by their rapid loss from experimental populations (Capillon and Atlan 1999). Owing to the developmental failure of Y-bearing sperm (Montchamp-Moreau and Joly 1997; Cazemajor *et al.* 2000), *sex-ratio* males can suffer more than a twofold fertility loss relative to *standard* males through sperm competition in multiple mating situations (Capillon 2000). This suggests that a rapid spread of *sex-ratio* chromosomes is possible only when males have little opportunity to mate, that is, when population density is low. While the evolution of drive suppressors and the deleterious effects on male fertility are able to stop the spread of distorters in *D. simulans*, whether or not they can hold them in a balanced polymorphism remains to be assessed. Several models show that this is a theoretical possibility (Jaenike 1996; Carvalho *et al.* 1997; Jaenike 1999; Taylor and Jaenike 2002). However, the Madagascar and Réunion data do not show evidence of balancing selection.

Our data are consistent with a recent spread of distorters across *D. simulans* populations. Together with well-documented cases of meiotic drive, including *Segregation Distorter* in *D. melanogaster*, *Sex-ratio* in *D. pseudoobscura*, and the *t*-haplotype in mice (Hammer and Silver 1993; Babcock and Anderson 1996; Palopoli and Wu 1996; Kovacevic and Schaeffer 2000), they suggest that nonequilibrium dynamics dominates the evolutionary history of meiotic drive systems. A useful property of the distorting system used in our study is that it is contained in a short fragment of the genome. Formerly studied classical cases involved large chromosome regions held in linkage disequilibrium through inversion systems or recombination inhibition by pericentromeric heterochromatin.

Since the revival of Maynard-Smith and Haigh's (1974) hitchhiking theory by Kaplan *et al.* (1989) very few, if any, of the selective sweeps that have been characterized at the molecular level over small regions have, to our knowledge, been so close to a causal explanation of the selective force involved in the process. It is often assumed that all selective sweeps are the result of Darwinian selection. Interestingly, this case involves a selective advantage for the gamete, with no evidence of a selective advantage for the zygote. In fact, studies by Capillon (2000) rather suggest that local conditions determine whether or not *sex-ratio* chromosomes are at an advantage over standard chromosomes. Note also that segregation distorters are "selfish genes" (Orgel and Crick 1980) that are potentially detrimental to the survival of populations. This suggests that selective sweep data should be critically examined before they are used to estimate the level of Darwinian selection in natural populations.

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