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ORIGINAL ARTICLE

Interspecific Y chromosome variation is sufficient to rescue hybrid male sterility and is influenced by the grandparental origin of the chromosomes

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Y chromosomes display population variation within and between species. Co-evolution within populations is expected to produce adaptive interactions between Y chromosomes and the rest of the genome. One consequence is that Y chromosomes from disparate populations could disrupt harmonious interactions between co-evolved genetic elements and result in reduced male fertility, sterility or inviability. Here we address the contribution of 'heterospecific Y chromosomes' to fertility in hybrid males carrying a homozygous region of *Drosophila mauritiana* introgressed in the *Drosophila simulans* background. In order to detect Y chromosome–autosome interactions, which may go unnoticed in a single-species background of autosomes, we constructed hybrid genotypes involving three sister species: *Drosophila simulans*, *D. mauritiana*, and *D. sechellia*. These engineered strains varied due to: (i) species origin of the Y chromosome (*D. simulans* or *D. sechellia*); (ii) location of the introgressed *D. mauritiana* segment on the *D. simulans* third chromosome, and (iii) grandparental genomic background (three genotypes of *D. simulans*). We find complex interactions between the species origin of the Y chromosome, the identity of the *D. mauritiana* segment and the grandparental genetic background donating the chromosomes. Unexpectedly, the interaction of the Y chromosome and one segment of *D. mauritiana* drastically reduced fertility in the presence of Y*sim*, whereas the fertility is partially rescued by the Y chromosome of *D. sechellia* when it descends from a specific grandparental genotype. The restoration of fertility occurs in spite of an autosomal and X-linked genome that is mostly of *D. simulans* origin. These results illustrate the multifactorial basis of genetic interactions involving the Y chromosome. Our study supports the hypothesis that the Y chromosome can contribute significantly to the evolution of reproductive isolation and highlights the conditional manifestation of infertility in specific genotypic combinations.
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INTRODUCTION

The Y chromosome of *Drosophila simulans* and other closely related species harbor a high density of transposable elements and megabase long segments of repetitive sequences (Carvalho and Clark, 2005; Smith *et al.*, 2007), with few protein-coding genes and nearly absent protein-coding polymorphism. The Y chromosome is required for male fertility in most *Drosophila* species (Ashburner *et al.*, 2005) even though it does not contain a male-determining gene. Instead, most if not all of the ~14 protein-coding genes present on the Y chromosomes of *Drosophila melanogaster*, *D. simulans* and other closely related species have male-specific functions and are exclusively expressed in the testis (Gepner and Hays, 1993; Carvalho *et al.*, 2000; Carvalho *et al.*, 2001; Vibranovski *et al.*, 2008). Nevertheless, a suite of studies have shown that genetic variation present on the Y of *Drosophila* underlie phenotypic variation in male fitness (Chippindale and Rice, 2001; Yee *et al.*, 2015), sex ratio distortion (Carvalho *et al.*, 1997; Montchamp-Moreau *et al.*, 2001; Branco *et al.*, 2013a, b), tolerance to temperature extremes (Rohmer *et al.*, 2004; David *et al.*, 2005), behavior (Stoltenberg and Hirsch, 1997; Huttunen and Aspi, 2003), gene expression (Lemos *et al.*, 2008; Sackton *et al.*, 2011; Branco *et al.*, 2013a, b) and chromatin states in somatic tissues (Lemos *et al.*, 2010).

Y-linked regulatory variation (YRV) is a source of gene expression diversity in Drosophila (Ashburner *et al.*, 2005; Lemos *et al.*, 2008). Moreover, analyses of Y chromosome variation in wild-type backgrounds and in genotypes with loss-of-function mutations revealed that the manifestation of YRV is exquisitely sensitive to the genomic background (Jiang *et al.*, 2010; Branco *et al.*, 2013a, b). Moreover, these studies support the expectation that Y-linked variation preferentially affects genes with male-biased expression and modifies male fertility. Interestingly, YRV affects genes with greater expression divergence between *D. melanogaster* and *D. simulans* and higher level of expression polymorphism within species (Lemos *et al.*, 2008; Sackton *et al.*, 2011). It is reasonable to expect that YRV will modulate the evolution of fast-evolving genes and contribute to speciation and hybrid incompatibility in *Drosophila*. Indeed, the Y chromosome of *Drosophila* has been shown to be involved in hybrid genome incompatibilities. For example, protein binding to the Y chromosome in hybrids of *D. simulans* and *D. mauritiana* is linked to local

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chromatin condensation and hybrid sterility (Bayes and Malik, 2009). Albeit in a different hybrid species system, similar mechanisms might underlie earlier observations that *Drosophila mojavensis* males with a Y chromosome from *Drosophila arizonae* are sterile (Vigneault and Zouros, 1986; Pantazidis *et al.*, 1993). Collectively, these results are in agreement with the notion that epistatic interactions between the Y chromosome and the genetic background are prevalent for components of male fitness (Johnson *et al.*, 1992, 1993; Chippindale and Rice, 2001; Sackton *et al.*, 2011; Yee *et al.*, 2015). Molecularly, a variety of mechanisms—involving both RNA mediators and non-coding DNA sequences—have been suggested for Y chromosome modulation of autosomal and X-linked gene expression that is, in turn, expected to modulate male fertility (reviewed in Francisco and Lemos, 2014).

Here we report a quantitative assay of the contribution of a heterospecific Y chromosome to male fertility in hybrid genotypes involving three sister species: *D. simulans*, *D. mauritiana*, and *D. sechellia*. Crosses between any two of these species produce an F1 of fertile females and sterile males (Lachaise *et al.*, 1986; Ashburner *et al.*, 2005). The construction of mixed-genotype lines in the laboratory provides an ideal system to test whether hybrid male fertility deficiency results from specific interactions among defined genomic regions. Intraspecific variation in the Y chromosome can be responsible for modulating hybrid phenotypes (Johnson *et al.*, 1992, 1993; Sackton *et al.*, 2011; Cutter, 2012). However, subtle effects on fertility may go unnoticed and are particularly difficult to ascertain when single-species systems are investigated. The three-species hybrid system has the potential of inciting less subtle effects, which may reveal novel aspects of the Y chromosome contribution to fertility.

Introgression lines of *D. mauritiana* into the *D. simulans* background were constructed by multiple generations of backcrossing hybrid females with *D. simulans* males. Aided by molecular markers, these introgressed segments of *D. mauritiana* are well delimited (see Tao *et al.*, 2001, 2003). We crossed seven pairs of introgression lines, each carrying a different segment of *D. mauritiana*'s chromosome 3, in order to produce homozygotes of certain portions of these segments. Along the crossing scheme, we also incorporated an exogenous Y chromosome (from *D. simulans* or *D. sechellia*) to the introgression genotypes and produced focal males from three sets of grandfathers. Males with different grandfathers, different combinations of homozygous introgressions and the Y chromosome of either *D. simulans* or *D. sechellia* were individually tested for fertility.

We found complex interactions between the Y chromosome and *D. mauritiana* segments that are manifested in male fertility. Notably, one segment of *D. mauritiana* is more detrimental in the presence of the Y*sim* (Y of *D. simulans*) than in the presence of the Y*sech* (Y of *D. sechellia*), in spite of most of the autosomal and X-linked genome being from *D. simulans*. The segment reduces fertility in the Y-simulans background but the infertility is partially rescued by the Y chromosome of *D. sechellia*. Moreover, the genotype of the grandparental line donating the Y chromosome displayed a significant contribution to male fertility. The complex interactions revealed in our study support the hypothesis that the heterochromatic Y chromosome participates in co-adapted associations within populations and may contribute significantly to the evolution of reproductive isolation as well as to the conditional manifestation of infertility in specific genotypic combinations.

MATERIAL AND METHODS

Drosophila stocks

D. simulans stocks *w; e* (kindly provided by J Coyne) and *simB* (*w; nt; III*) were described in Tao *et al.*, (2003). In the *simB* stock, the third chromosome is isogenic to the third chromosome of the highly inbred stock *13w 1* X *1 JJ* (constructed by sib-pair mating for 20 generations from *13w*—Liu *et al.*, 1996). This chromosome does not carry a phenotypic marker, while the first and second chromosomes carry markers *white* (*w*) and *net* (*nt*), respectively (Tao *et al.*, 2003). *Drosophila mauritiana* stocks (*w*; *P* [*w*+]) contain independent *P*-element inserts on the third chromosome, which carry a functional copy of the gene *white* (True *et al.*, 1996). Numerical identifiers of each of these stocks are according to Tao *et al.* (2003). These stocks were used elsewhere for the construction of introgression lines that allowed the fine mapping of sex-ratio distorter and suppressor genes (*Dox* and *Nmy*—Tao *et al.*, 2007a, b), as well as one candidate gene for hybrid male sterility (*agt*—Araripe *et al.*, 2010). This is a reliable and well-studied system in which the manifestation of incompatibility phenotypes can be tracked to study the influence of Y chromosome variation. All flies were reared on cornmeal–molasses–agar medium sprinkled with yeast grains at room temperature (21 ± 1 °C).

Introgression lines

Segments of the third chromosome of *D. mauritiana* were introgressed in the genomic background of *D. simulans* stock *simB* by repeated backcrosses (see Tao *et al.*, 2003 for details). Aided by molecular markers, these introgressed segments of *D. mauritiana* had their lengths well delimited in previous work (see Tao *et al.*, 2001, 2003). All introgression lines used here have the genotype of *simB, w; nt; P/ III* (*Nmy*), and each has a different segment of *D. mauritiana*'s third chromosome with the semidominant *P*-element transgene *P* [w^+] inserted (True *et al.*, 1996) in positions that were previously mapped by Araripe *et al.* (2006). If the introgression overlaps with the suppressor of the Winters sex-ratio distortion, *Nmy* (Tao *et al.*, 2007a, b), the line will be carrying the lack of function mutant, *nmy*.

Stocks used as sources of Y chromosomes

The Y chromosome of *D. sechellia* (Y*sech*) (stock 3588, Dermitzakis *et al.*, 2000) was introgressed into stocks A14, SR12-2-7 and G23 of *D. simulans* (Tao *et al.*, 2007a, b). *D. simulans* stocks A14, SR12-2-7 and G23 were chosen because they carry different combinations of the Winters sex-ratio distortion gene (*Dox*) and the suppressor of sex-ratio distortion (*Nmy*), as shown in Table 1 and Figure 1.

Construction of hybrid and triple-hybrid males

Seven pairs of the heterozygote introgression lines (3.4 and 8.4; 21.12 and 26.14; 38.9 and 38.6; 32.2 and 27.2; 32.2 and 33.3; 27.2 and 26.14; 33.5 and 42.2) were studied. To exemplify the procedure, we describe the steps to construct three of those lines that were examined in greater detail. Males of stocks A14, SR12-2-7 and G23, carrying the exogenous Y chromosome (Y*sech* or Y*sim*), were crossed to females of each one of the three heterozygous

Table 1 Grandparental genotypes and distortion phenotypes used as sources for the construction of hybrid and triple hybrid males

Line	*Genotype*	*Sex-ratio distortion*	*Detailed genotype*
A14/Y*sim* and A14/Y*sech*	*dox/nmy*	No	*w dox*/ Y; *nt*; *nmy* 1427
SR12-2-7/Y*sim* and SR12-2-7/Y*sech*	*Dox/nmy*	Yes	*Dox*/ Y; *nt*; *nmy* 1427
G23/Y*sim* and G23/Y*sech*	*Dox/Nmy*	No	*w*/ *C(1)yw; nt; Nmy*
simB/Y*sim*	*Dox/Nmy*	No	*Dox*/ Y; *nt*; *Nmy*

Grandparental genotype is A14, SR12-2-27 and G23. Y chromosome is from *D. simulans* (Y*sim*) or *D. sechellia* (Y*sech*).

introgression lines. For this first step, we arbitrarily chose lines 8.4, 21.12 and 38.6 (Figure 2). The F1 males from these crosses carry the X chromosome of *simB* (from the female with *D. mauritiana* introgression), the Y chromosome provided by one of the three Y*sim* grandparental genotypes or one of the three Y*sech* grandparental genotypes described above, and the third chromosome heterozygote with one copy of 8.4, 21.12 or 38.6 *P*-elements and one copy of the third chromosome of *simB* (Figure 3). F1 males with colored eyes ($P[w^+]$) were then crossed to females of each one of the three other heterozygous introgression lines: 3.4, 26.14, and 38.9. The focal males were F2 males with either Y*sech* or Y*sim* and homozygote introgressions paired as 3.4/8.4, 21.12/26.14 and 38.6/38.9, adding to a total of 18 genomic combinations to be tested (Figure 3). Focal males were selected by the dark red eyes given by $2P$ $[w^+]$.

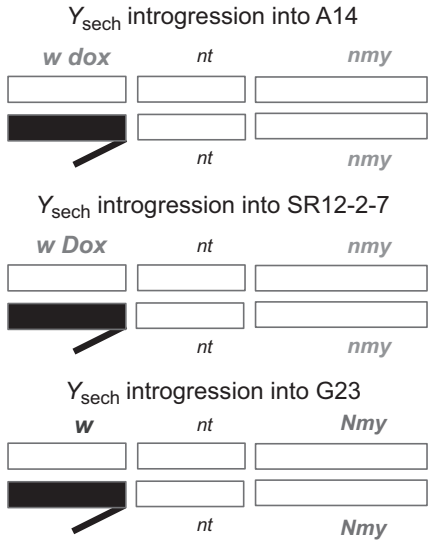

Figure 1 Grandparental genotypes of males with the Y chromosome of *D. sechellia* introgressed (A14/Y*sech*, SR12-2-7/Y*sech* and G23/Y*sech*). Grandparental strains with Y*sim* have the same genotype in the X chromosome/autosome background but are not shown here. Grandparental females of the G23 stock carry the attached X chromosome [C(1)RM]. Y chromosomes switch between males and females each generation along the G23 lineage. White bars represent chromosomes of *D. simulans*, black bars represent the Y chromosome of *D. sechellia*.

Fertility tests

We focused on quantitative decrease or increase in male fertility, rather than on complete male sterility, as the phenotype resulting from hybridization. Fertility tests were carried out by mating one male with three virgin *w; e* females for 7 days before being discarded. The progeny was counted up to the twentieth day after cross setup, when all adults had emerged. Fertility was defined as the number of progeny produced (Tao *et al.*, 2003). Fertility tests were conducted with ~15 single males per genotype. To qualitatively validate our observations, crosses made to create the described genotypes were independently conducted a second time for each genotype and essayed for fertility in an experiment with fewer males tested. All fertility tests were performed at room temperature (21 ± 1 °C).

RESULTS

The construction of lines with various combinations of Y chromosome and incompatibility genes could reveal new genomic interactions involving the Y chromosome. These genotypes could also shed light on the contribution of Y chromosome–autosomal epistasis to the process of reproductive isolation and speciation. Here we hypothesized that Y chromosomes of *D. simulans* or *D. sechellia* origin might exhibit differential responses to incompatibility elements present in the genomic background. To address the issue, seven pairs of the heterozygote introgression lines (3.4 and 8.4; 21.12 and 26.14; 38.9 and 38.6; 32.2 and 27.2; 32.2 and 33.3; 27.2 and 26.14; 33.5 and 42.2) were crossed in order to make specific regions of the third chromosome homozygote for *D. mauritiana*. Although the introgression of the whole third chromosome of *D. mauritiana* into *D. simulans* causes

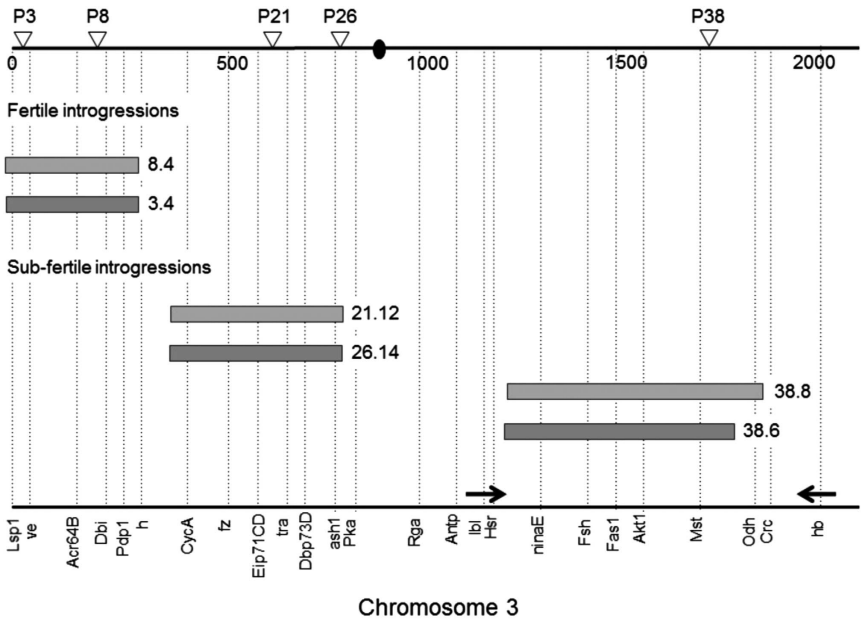

Figure 2 Positions of the *D. mauritiana* chromosomal segments introgressed into the *D. simulans* background, on the third chromosome (modified from Tao *et al.*, 2003). Each introgression is tagged by a P $[w^+]$ insert (open triangles on the top), followed by an individual number. The introgression ends are defined with the aid of ASO markers (names at the bottom and dotted lines). The ASO markers and P $[w^+]$ inserts are positioned according to polytene bands. Cytological positions are based on the standard map of *D. melanogaster* (Lefevre, 1976), but *D. simulans* differs from *D. melanogaster* by an inverted segment (93F6-7 to 84F9, the two arrows facing each other) (Horton, 1939). Filled oval: the centromere.

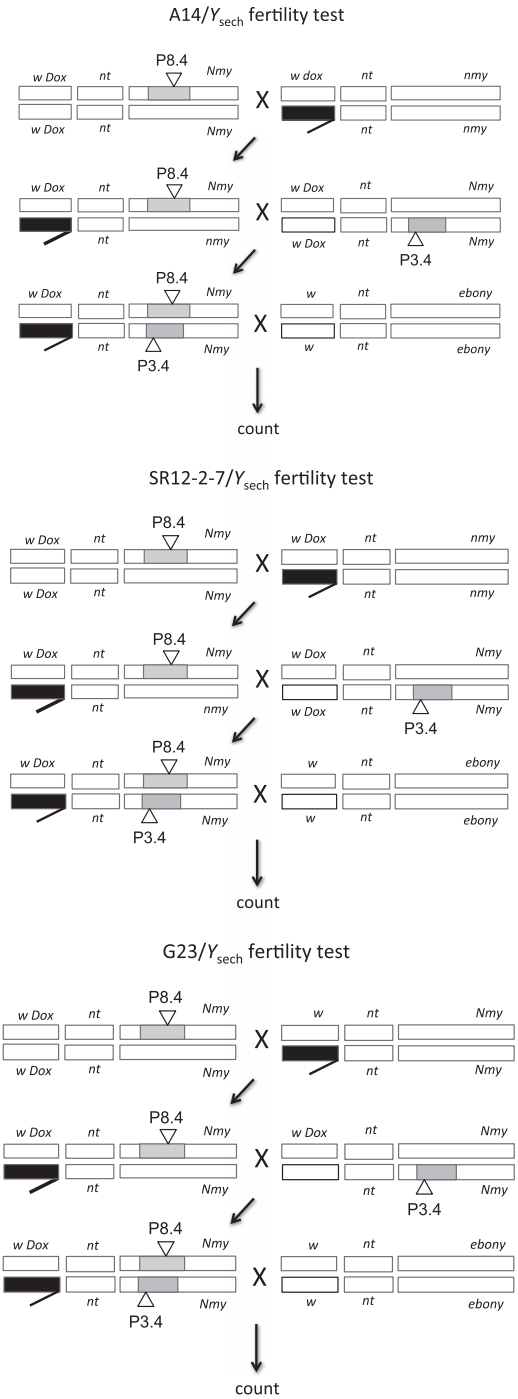

Figure 3 Schematics of the construction of focal males for fertility tests for one pair of *D. mauritiana* introgressions (8.4/3.4) with the Y*sech*. Identical crosses and male fertility tests with the *Ysim* chromosome were performed simultaneously (not shown here). Shown here is A14/Y*sech* in the background genome of *simB* with homozygous *D. mauritiana* introgressions 8.4/3.4. Males from lines SR12-2-7/Y*sech* and G23/Y*sech* also provided Y*sech* chromosomes for assay with 8.4/3.4, 21.2/26.14 and 38.6/38.8. Males from lines A14/Y*sim*, SR12-2-7/Y*sim* and G23/Y*sim* provided Y*sim* chromosomes for the same introgression combinations cited above. Crosses and fertility assays were performed simultaneously for all treatments. Note that the focal male is identical across all cases. Male fertility was tested by crossing one male with three *D. simulans w; e* females for 7 days and subsequently counting the progeny. White bars represent chromosomes of *D. simulans*, black bars represent chromosomes of *D. sechellia* and gray bars represent segments of *D. mauritiana* third chromosome.

males to be fully sterile, the seven *D. mauritiana* homozygote segments described above were shown to generate subfertile-to-fertile males (Tao *et al.*, 2003). This is key for our study, as it allows for quantitative effects of Y chromosome substitution on the hybrid fertility phenotype to be noticed in both directions, that is, either as an increase or a decrease in fertility.

We initially performed a screen for fertility variation emerging from the interaction of each of the seven pairs of *D. mauritiana* introgressions and Y*sim* and Y*sech* chromosomes originating from the A14 grandparental background (Figures 1 and 3). This initial screen with all seven pairs revealed that four of them did not exhibit fertility differences between Y*sim* and Y*sech* (mean and s.e.m. of progeny number in 32.2/27.2: Y*sim* = 235.1 ± 13.7 offspring vs Y*sech* = 210.6 ± 25.6 offspring; 32.2/33.3: Y*sim* = 222.0 ± 38.9 vs Y*sech* = 189.6 ± 20.3; 27.2/26.14: Y*sim* = 176.3 ± 35.3 vs Y*sech* = 208.2 ± 20.7; 33.5/42.2: Y*sim* = 193.0 ± 17.5 vs Y*sech* = 218.4 ± 22.3; $P > 0.05$ for all cases, Student's *t*-test). On the other hand, three pairs of introgressions displayed significant differences between Y*sim* and Y*sech* (8.4/3.4: Y*sim* = 246.1 ± 15.6 vs Y*sech* = 72.0 ± 14.1; 21.12/26.14: Y*sim* = 6.5 ± 2.1 vs Y*sech* = 69.0 ± 11.3; 38.9/38.6: Y*sim* = 23.0 ± 7.9 vs Y*sech* = 0.0 ± 0.0; $P < 0.05$ for all cases, Student's *t*-test). Together these observations highlight the relevance of the genetic background in the emergence of fertility differences mediated by the Y chromosome.

In view of these observations, we chose the three pairs of introgression segments displaying Y chromosome variation in fertility for further analysis. Specifically, these introgressions were further studied in greater detail in 18 focal males that were classified according to: (1) the species origin of the Y chromosome (*D. simulans* or *D. sechellia*) and (2) the grandparental genomic background of origin (*D. simulans* of A14, SR12-2-7 or G23; Figure 1; and the introgressed *D. mauritiana* segment on *D. simulans* third chromosome (3.4/8.4, 21.12/26.14 or 38.9/38.6; Figure 2). As expected from the initial screen, differences in progeny number are significant between males carrying Y chromosome from the line A14/Y*sim* and Y chromosome from the line A14/Y*sech* for all three hybrid backgrounds re-tested: 8.4/3.4 ($t = 4.351$, $P < 0.001$), 21.12/26.14 ($t = -3.815$, $P < 0.001$), and 38.9-/38.6 ($t = 2.458$, $P < 0.05$) (Figure 4, Table 2). Similarly, as expected from the initial screen, the A14/Y*sech* exhibited greater fertility than A14/Y*sim* in the introgression 21.12/26.14. Surprisingly, however, we observed that while the species origin of the Y chromosome had a clear influence on male fertility when originating from some grandparental backgrounds it displayed a milder effect when originating from other backgrounds. For instance, progeny number of SR12-2-7/Y*sim* males is not significantly different from the progeny number of SR12-2-7/Y*sech* males (Figure 4). The variation illustrates the multifactorial nature of genomic interactions involving the Y chromosome and autosomes and is further detailed below.

The observation that Y*sech* chromosome exhibits greater fertility than Y*sim* in the presence of introgression pair 21.12/26.14 is particularly unexpected. Triple-hybrid males, which carry Y*sech* and homozygous *D. mauritiana* introgressions in a *D. simulans* background, are expected to be less fertile than bi-hybrid males, which carry Y*sim* instead of Y*sech*. This is because the former may show three-way interspecific incompatibilities among all three species. Indeed, this was the case for most of the genotype combinations in which the two Y chromosomes were contrasted (Figure 4, Table 2). For instance, the A14/Y*sim*; 8.4/3.4 bi-hybrid males produced on average 234.4 offspring while the A14/Y*sech*; 8.4/3.4 tri-hybrid males produced 116.7 offspring ($t = 4.353$, $P < 0.001$). Surprisingly, however, we observed that the tri-hybrid A14/Y*sech*; 21.12/26.14 produced on average much more offspring per male (129.9) than the bi-hybrid

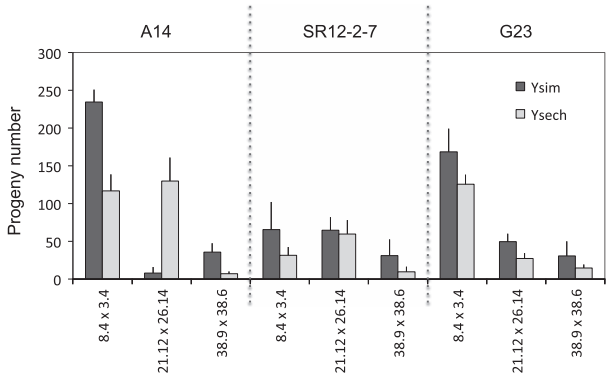

Figure 4 Progeny number for males carrying either Y*sim* or Y*sech* and three pairs of introgressions from *D. mauritiana*. (for example, 8.4×3.4 refers to the final *D. mauritiana* genotype in the focal male). A14, SR12-2-7 and G23 refer to the grandparental genotype donating the chromosomes to the focal male in which the assays were performed. (Left panel) A14/Y*sim* vs A14/Y*sech*, (Center panel) SR12-2-7/Y*sim* vs SR12-2-7/Y*sech* and (Right panel) G23/Y*sim* vs G23/Y*sech*. Error bars: 1.96× s.e.m.

Table 2 Average progeny number sired by hybrid males with every arrangement of three factors: Grandparental Genotype (GG), Introgression Combination (IC), and Y chromosome Origin (YO)

Grandparental Genotype (GG)	*Introgression Combination (IC)*	*Y chromosome Origin (YO)*			
		*Y*sim	*Y*sech	*df*	t
A14	8.4×3.4	234.4	116.7	17	4.353***
	21.12×26.14	8.0	129.9	18	−3.815**
	38.9×38.6	35.6	6.9	17	2.458*
	8.4×3.4	65.3	31.5	7	0.166^{NS}
SR12-2-7	21.12×26.14	64.9	59.8	18	0.204^{NS}
	38.9×38.6	31.2	9.7	18	0.965^{NS}
	8.4×3.4	168.4	125.6	17	1.235^{NS}
G23	21.12×26.14	49.5	27.4	18	1.73^{NS}
	38.9×38.6	30.5	14.5	18	0.804^{NS}

ANOVA	*df*	*F*
YO	1	5.896*
GG	2	10.461***
IC	2	67.208***
YO×GG	2	0.179^{NS}
YO×IC	2	15.801***
GG×IC	4	5.745***
YO×GG×IC	4	4.669**
Within	148	
Total	165	

Abbreviations: ANOVA, analysis of variance; NS, not significant. Averages were compared between Y*sim* and Y*sech* males using a Student's *t*-test. Three-way ANOVA reveals significant interactions among factors (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

A14/Y*sim*; 21.12/26.14 (8.0, $t=-3.815$, $P<0.01$). This result suggests that the Y*sech* is more compatible than the Y*sim* to the 21.12/26.14 portion of *D. mauritiana* genome and to the background genome of line A14. Evidently, Y chromosomes from *D. simulans* and *D. sechellia* must differ in some causative Y-linked sequence that mediates the ability of the *D. sechellia* Y chromosome to rescue the infertility phenotype.

The *D. simulans* grandparental background also has a profound effect on fertility of the hybrid and tri-hybrid males. For example, the males constructed with Y chromosomes deriving from the line SR12-2-7 showed low fertility overall, irrespective of the species origin of the Y chromosome and the introgression background (on average 53.8 flies produced by the Y*sim* and 33.7 produced by the Y*sech*). These data suggest that the genotype *Dox*/*nmy* (*Dox* distorter/*nmy* lack-of-function suppressor) has a predominant effect on fertility regardless of the Y chromosome. Interestingly, Branco *et al.* (2013a) have shown that the *D. simulans* Y chromosome is polymorphic for its capacity to modulate sex ratio distortion in genotype SR12-2-7. These authors used the same stock SR12-2-7 as a background for the introgression of 78 Y chromosomes from several *D. simulans* populations and found that there is significant diversity in Y chromosome resistance to sex-ratio distortion: some Y chromosomes carry YRV that overrides the effects of the distorter *Dox*, while other Y chromosomes are even more sensitive to *Dox* than the original Y chromosome present in SR12-2-7.

Finally, males with the homozygous introgressions 38.9/38.6 show the lowest fertility (on average 34.4 offspring flies produced by males carrying Y*sim* and 10.4 produced by males carrying Y*sech*), irrespective of the Y chromosome species and the genomic background. The 38.9/38.6 introgressions have the hybrid male sterility factors and sex-ratio distortion suppressor *too much ying* (*tmy*) (Tao *et al.*, 2001, 2003). When homozygous, tmy^{mau} males (the *tmy* region from *D. mauritiana* in an otherwise *D. simulans* background) have very low fertility and offspring with female-biased sex ratio (~75% female). Interestingly, Y*sech* did not show a 'rescuing' effect for male sterility caused by tmy^{mau}. Similarly, Tao *et al.* (2007a, b) observed that Y*sech* and Y*sim* show the same sensitivity to the sex-ratio distortion by *Dox*; the same sex ratio is seen in *Dox; nmy* males regardless of the origin of the Y chromosome.

DISCUSSION

Genetic incompatibilities manifesting within and between populations could provide essential variation leading to speciation. YRV affects rapidly evolving testis-specific genes and is expected to be involved in adaptive processes, with Y chromosome-mediated incompatibility possibly emerging from the disruption of co-adapted networks of genes within populations. These networks are built by interactions between autosomal and sex-linked genes; the contribution of X–Y interactions to the emergence of disrupted hybrid phenotypes has been especially considered (Haldane, 1932; Coyne, 1985; Johnson *et al.*, 1992, 1993; Zeng and Singh, 1993). The identification of specific loci on the X chromosome and autosomes that are incompatible with specific Y chromosome variants could illuminate processes mediating variable fertility. In turn, the extent of Y chromosome-mediated fertility breakdown in hybrid genotypes may reveal mechanisms responsible for a broader spectrum of phenotypic variation.

Genome-wide gene expression in genotypes differing only in Y chromosome origin revealed cryptic Y chromosome diversity (Lemos *et al.*, 2008; Lemos *et al.*, 2010; Jiang *et al.*, 2010; Branco *et al.*, 2013a). These include changes in the expression level of genes involved with functions as varied as metabolism, cell division, immune response and chromatin structure. In *D. simulans*, Branco *et al.* (2013a) observed a range of sex ratios in Y chromosome substitution lines of the Winter's sex-ratio system. The data indicate that polymorphic variation residing in the Y chromosome causes resistance to an X-linked distorter. These observations recapitulate previous observations by Montchamp-Moreau *et al.* (2001) with the Paris system. In addition to sex-ratio distortion, other phenotypes associated with reproductive isolation and hybrid incompatibilities may be modulated by interactions with

genic and regulatory sequences of the Y chromosome. These phenotypes, including fertility and YRV, may emerge from non-genic elements on the Y chromosome heterochromatin and may be under selection (Lemos *et al.*, 2010; Francisco and Lemos, 2014). The piRNA pathway is a candidate to mediate Y chromosome effects in hybrids (Castillo *et al.*, 2011; Kelleher *et al.*, 2012) and could reconcile the low polymorphism in Y-linked protein-coding sequences (Zurovcova and Eanes, 1999; Larracuente and Clark, 2013) with YRV. Differences in the extensive blocks of heterochromatin are given by the kinds and quantities of satellite DNA and transposable elements. These elements are predicted to be key players in the phenomenon of YRV, and the mechanisms for their manifestation are likely to be varied (Francisco and Lemos, 2014).

The three sister species in the *D. simulans* clade are able to intercross, but postzygotic incompatibilities such as hybrid male sterility are evident. One gene involved in the reproductive isolation of *D. simulans* and *D. mauritiana* is the fast-evolving X chromosome gene *Odysseus site homeobox*, first described by Ting *et al.*, (1998). Further investigation found that the protein OdsH binds to Y chromosome heterochromatic sites in hybrids, affecting local chromatin condensation and leading to hybrid sterility (Bayes and Malik, 2009). In the *D. simulans* sister-species clade, the binding sites of OdsH differ for hybrids of different pairs of species. For instance, the Y chromosomes of *D. sechellia* and *D. simulans* were enriched for the protein OdsH of *D. mauritiana*, whereas the protein of *D. simulans* did not bind to any of the three Y chromosomes. Given that the divergence of the *D. simulans* species clade is recent (~250 000 years, Kliman *et al.*, 2000), these results are consistent with the hypothesis that the rise of genomic incompatibilities may be partly driven by the rapid evolution of heterochromatic DNA (Brideau *et al.*, 2006).

Here we studied nine genomic combinations and showed that one segment of the *D. mauritiana* genome (21.12/26.14) interacts with the Y chromosome of *D. simulans* and *D. sechellia* in a unique way, resulting in unexpected reversal of the direction of variation. Moreover, the reversal was conditional on the grandparental genotype. As shown in Figure 2, introgression fragments 21.12 and 26.14 are located on chromosome 3L, between genes *hairy* (h) and *Pka-R1*. These introgression fragments overlap through a common segment between 7.8 and 12.2 Mb in length (Tao *et al.*, 2003). At least three hypotheses may guide identification of the genetic elements in the segment that might differentially interact with the Y chromosome of *D. simulans* and *D. sechellia*. First, regulatory factors in the introgressed *D. mauritiana* segment may differentially modulate the expression of Y-linked coding elements (genes, transposable elements, piRNAs and so on). Second, satellite sequences may serve as a differential sink for the binding of protein-coding genes/small RNAs in these chromosomes and may become unavailable in other sites of the genome, including the introgressed segment. Third, interspecific divergence in blocks of repeated sequences embedded in heterochromatin may affect fertility by abnormally affecting chromosome segregation. This appears to be the cause of hybrid female lethality from crosses between *D. simulans* females and *D. melanogaster* males, where *Zhr* gene is actually a 359-bp stretch of satellite sequences on the heterochromatic region of the X chromosome of *D. melanogaster* that is not present in *D. simulans* (Ferree and Barbash, 2009).

Our results show that not only the species origin of the Y chromosome generates subfertility in hybrid males but also the magnitude and direction of the effect depends on the genomic background of the grandparent donor line. The influence of the grandparental genome on the modulating capacity of the Y chromosome is implied when we compare the progeny number of males A14/Y*sech*; 21.12/26.14 with the progeny number of males SR12-2-7/Y*sech*; 21.12/26.14. Both the Y*sech* and the autosomic background are the same among these lines, whereas the Y chromosome donor line (A14 vs SR12-2-7) is different. Noteworthy, the second chromosomes from all lines came from the same *D. simulans* donor and all carry the phenotypic marker *net*. However, the origin is not strictly immediate and we cannot rule out that new mutations in it might have contributed to the difference in progeny number across grandparental backgrounds. On the other hand, sex chromosomes are sensitive to parental origin in Drosophila (Golic *et al.*, 1998; Maggert and Golic, 2002), including substantial consequences to testis-specific gene expression and epigenetic states elsewhere in the genome (Greil and Ahmad, 2012; Zhou *et al.*, 2012; Lemos *et al.*, 2014). Collectively, our observations raise the prospect that genetic or epigenetic variation acquired during the making of the focal males is partially responsible for modulating hybrid male sterility.

Finally, our results are in concordance with the expectation that the extent of reproductive isolation between a pair of species and hybrid male fertility attributes may be the by-product of a multifactor genomic environment. Relevant components include rapidly evolving repetitive sequences as well as rapidly evolving pathways implicated in the maintenance of heterochromatic chromosomes (Ferree and Barbash, 2009; Bayes and Malik 2009; Castillo *et al.*, 2011). The observation that the heterochromatic Y chromosome from *D. sechellia* can rescue the fertility loss of a *D. mauritiana* segment in a *D. simulans* genome background is unexpected and displays the evident complexity of epistatic interactions with the Y chromosome. We conclude that investigating the diversity of phenotypic outcomes in hybrid genotypes is an important step to uncover the full spectrum of Y chromosome modulation of endogenous regulatory processes.

DATA ARCHIVING

Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.n2v20.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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