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Contrasting patterns of X-chromosome divergence underlie multiple sex-ratio polymorphisms in stalk-eyed flies

K. A. PACZOLT*[1], J. A. REINHARDT†[1] & G. S. WILKINSON*

*Department of Biology, University of Maryland College Park, College Park, MD, USA
†Department of Biology, SUNY Geneseo, Geneseo, NY, USA

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genomic conflict;
recombination;
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Abstract

Sex-linked segregation distorters cause offspring sex ratios to differ from equality. Theory predicts that such selfish alleles may either go to fixation and cause extinction, reach a stable polymorphism or initiate an evolutionary arms race with genetic modifiers. The extent to which a sex ratio distorter follows any of these trajectories in nature is poorly known. Here, we used X-linked sequence and simple tandem repeat data for three sympatric species of stalk-eyed flies (*Teleopsis whitei* and two cryptic species of *T. dalmanni*) to infer the evolution of distorting X chromosomes. By screening large numbers of field and recently laboratory-bred flies, we found no evidence of males with strongly female-biased sex ratio phenotypes (SR) in one species but high frequencies of SR males in the other two species. In the two species with SR males, we find contrasting patterns of X-chromosome evolution. *T. dalmanni-1* shows chromosome-wide differences between *sex-ratio* (X^{SR}) and standard (X^{ST}) X chromosomes consistent with a relatively old *sex-ratio* haplotype based on evidence including genetic divergence, an inversion polymorphism and reduced recombination among X^{SR} chromosomes relative to X^{ST} chromosomes. In contrast, we found no evidence of genetic divergence on the X between males with female-biased and nonbiased sex ratios in *T. whitei*. Taken with previous studies that found evidence of genetic suppression of sex ratio distortion in this clade, our results illustrate that sex ratio modification in these flies is undergoing recurrent evolution with diverse genomic consequences.

Introduction

Whereas most genes obey Mendel's law of equal segregation, segregation distorter alleles violate this rule by being transmitted to more than half of the gametes. Such distorter alleles can be viewed as being 'selfish' in that they will increase in frequency at the expense of other alleles and thereby create conflict within the genome (Burt & Trivers, 2006). Segregation distortion alleles may become fixed or persist as stable polymorphisms; in either case, they can have significant genomic consequences (Nam *et al.*, 2015). Some basic genetic processes, such as recombination, may have evolved as an ancient genomic defence against segregation distortion on both autosomes and sex chromosomes (Haig & Grafen, 1991; Barton & Charlesworth, 1998). Segregation distortion has also been linked to a variety of evolutionary processes, such as the origin of new sex chromosomes (Patten, 2014; Ubeda *et al.*, 2014) and speciation (Frank, 1991; Hurst & Pomiankowski, 1991; McDermott & Noor, 2010; Phadnis, 2011).

A major goal in the study of segregation distortion was to understand the evolutionary dynamics of active distorters in natural populations (Lindholm *et al.*, 2016). Sex-linked segregation distorters are of particular interest because they directly influence offspring sex ratio. In most X-chromosome segregation distortion systems, the distorter allele acts during spermatogenesis to destroy or disable Y-bearing sperm. These so called 'sperm killer' alleles create *sex-ratio* (SR) males that produce primarily female offspring (Jaenike, 2001;

Correspondence: Kimberly Paczolt, 1210 Biology Psychology Building #144, College Park, MD 20742, USA.
Tel.: 301 405 2693; fax: 301 314 9358;
e-mail: kpaczolt@umd.edu
[1]These authors contributed equally to this work.

Presgraves, 2008; Lindholm *et al.*, 2016). In the absence of opposing forces, the transmission advantage of an X-linked sex ratio distortion allele will insure that it increases in frequency, resulting in an increasingly female-biased population sex ratio and a larger population size until extinction occurs due to lack of males (Hamilton, 1967). Thus, for an X-chromosome distortion allele to persist, either selection must act against the allele or suppressing alleles, which counter the sex ratio distortion effect, or resistant Y chromosomes, which are unaffected by the SR allele, must arise and spread (Carvalho & Vaz, 1999). If selection intensity against SR increases with allele frequency, then a stable polymorphism can arise. For example, if SR males pass fewer sperm than males with 1 : 1 offspring sex ratios (called standard or ST males), then ST males should be able to mate (or remate) with more females as the sex ratio becomes increasingly female-biased (Jaenike, 1996; Taylor & Jaenike, 2002). Alternatively, selection may act against distorter-carrying females, perhaps as a consequence of genetic factors that are linked to the locus by virtue of being within an inversion (Wallace, 1948; Edwards, 1961; Curtsinger & Feldman, 1980). For example, females carrying two copies of the distortion allele may have reduced fecundity in comparison with females lacking distortion alleles (Beckenbach, 1996).

The intragenomic conflict that arises in systems with sex ratio distorters may initiate an arms race between either autosomal suppressors or resistant Y chromosomes and X-linked modifiers that escape suppression or enhance the strength of distortion (Meiklejohn & Tao, 2010). Observations of several X-linked segregation distorters are consistent with this arms race scenario. In some active distorter systems, both driving and suppressor alleles are present at intermediate frequencies (Carvalho *et al.*, 1997; Montchamp-Moreau *et al.*, 2001; Kingan *et al.*, 2010; Unckless & Clark, 2015). In other cases, distortion is completely masked by fixation of a suppressor (Tao *et al.*, 2007a,b; Bastide *et al.*, 2011). Complete suppression creates a 'cryptic' or 'extinct' distortion system that is then only revealed in progeny from crosses to individuals from a different population or strain that lacks genetic defence against the distortion allele, as has been observed in multiple cases (Merçot *et al.*, 1995; Reed *et al.*, 2005; Phadnis & Orr, 2009; Cocquet *et al.*, 2012; Wilkinson *et al.*, 2014).

Inversion polymorphisms may aid in the persistence of active distorters by creating linkage disequilibrium between a segregation distortion locus and nearby modifiers. Inversion polymorphisms can reduce or prevent genetic exchange with homologous chromosomes, or with standard X chromosomes in the case of X-linked distortion (Jaenike, 2001). Although many distorter-associated inversions have been described (Dobzhansky & Sturtevant, 1938; Novitski, 1947; Kovacevic & Schaeffer, 2000; Dyer *et al.*, 2007; Machado *et al.*, 2007; Unckless & Clark, 2015), they are not universal among active sex-chromosome distortion systems (Montchamp-Moreau *et al.*, 2006). Distortion-associated inversions are thought to capture modifiers that enhance distortion and enable associations with sexually antagonistic alleles (Hartl, 1975; Patten, 2014; Rydzewski *et al.*, 2016). However, the presence of distortion-associated inversion polymorphisms can have drastic effects on the X chromosome, including loss of genetic variation (Dyer *et al.*, 2007; Christianson *et al.*, 2011; Pieper & Dyer, 2016), and an accumulation of deleterious alleles (Andolfatto *et al.*, 2001). The fate of such chromosomes may be eventual extinction if the negative effects outweigh the advantage conferred by segregation distortion, whereas maintenance of genetic variation through recombination may enable longer persistence.

Strong sex ratio distortion is present in at least two *Teleopsis* species (*T. dalmanni* and *T. whitei*) of stalk-eyed flies from South-East Asia (Presgraves *et al.*, 1997). In these species, SR males typically sire more than 90% female offspring, whereas ST males sire equal numbers of male and female offspring. The defect in sperm development caused by sex ratio distortion in these two species differs (Wilkinson & Sanchez, 2001; Johns *et al.*, 2005), suggesting that although SR males in these species exhibit similar sex-ratio phenotypes, sex ratio distortion may be caused by different developmental mechanisms. Additionally, the sex ratio distortion X chromosome (X^{SR}) of *T. dalmanni* exhibits widespread divergence from the standard X chromosome (X^{ST}) (Christianson *et al.*, 2011; Cotton *et al.*, 2014; Reinhardt *et al.*, 2014), and recombination is rare or absent between the two types of X chromosomes (Johns *et al.*, 2005).

Previous phylogeographic studies on *T. dalmanni* collected from South-East Asia (Swallow *et al.*, 2005) indicate the presence of at least two clades that exhibit little phenotypic divergence but complete reproductive isolation (Christianson *et al.*, 2005; Rose *et al.*, 2014), and thus likely represent distinct species. These cryptic species have not been formally described, so we refer to them here as '*T. dalmanni-1*' and '*T. dalmanni-2*'. When mated, *T. dalmanni-1* and *T. dalmanni-2* produce only sterile hybrid offspring in the laboratory (Christianson *et al.*, 2005; Rose *et al.*, 2014). In contrast, a suppressed sex ratio distorter is exposed in backcrosses between some populations of *T. dalmanni-1* (Wilkinson *et al.*, 2014). Whereas males producing female-biased sex ratios have been found in most samples of *T. dalmanni* and *T. whitei* collected from South-East Asia, the degree of sex ratio distortion varies among sites (Wilkinson *et al.*, 2003). Consequently, at present, it is unknown whether variation in female-biased sex ratios is due to multiple X chromosomes within and between these species or results from a common distorting X chromosome that is modified by different suppressors in each species or population.

Here, we reconstruct the evolutionary history of X chromosomes in these three closely related species of stalk-eyed flies to determine which of these scenarios is most probable. First, we characterize X-linked sequence variation from SR and ST males from all three species collected at five sites in central peninsular Malaysia using a phylogenetic approach on a subset of scored males and then a genotype-clustering approach on copy number variation in simple tandem repeats from a larger sample of scored males. From these data, we ask how many times X-chromosome distortion has arisen and whether it is suppressed by characterizing patterns of genetic differentiation among species and types of X chromosomes (X^{SR} and X^{ST}) across collection sites. We also estimate the age and population size of X^{SR} and X^{ST} in *T. dalmanni-1* using coalescent simulations. Finally, we estimate recombination and identify structural changes between X^{SR} and X^{ST} in *T. dalmanni* by assaying haplotype-specific markers within crosses between two X^{SR} or two X^{ST} chromosomes. We find strikingly dissimilar patterns of genetic differentiation among X chromosomes within each species, with evidence that sex ratio distortion is caused by a small region in one case, but associated with a large, relatively old inversion in the other.

Materials and methods

We collected 635 *Teleopsis dalmanni* and *T. whitei* stalk-eyed flies using hand nets along streams at five sites in peninsular Malaysia in August 2012. These sites include two places where *T. dalmanni* from each of the major clades had previously been collected (Gombak and Langat, Swallow *et al.*, 2005) as well as three additional locations, Janda Baik (3°18′27.97″N, 101°53′20.51″E), Tua (3°18′48.33″N, 101°41′55.85″E) and Kanching (3°17′6.66″N, 101°38′51.31″E). Male and female flies of all species collected from the same site were initially group-housed and then shipped alive under USDA APHIS permits to the University of Maryland to establish laboratory colonies. Cursory visual inspection of the collections revealed *T. dalmanni* (either or both *T. dalmanni-1* or *T. dalmanni-2*) and *T. whitei* at all sites.

We scored offspring sex ratios of males captured in the field or recently bred from field-caught flies (Table S1). Flies were reared using standard techniques (Lorch *et al.*, 1993), and offspring sex ratios were calculated as the proportion of male progeny. Males were paired with two or three virgin females, and screening cages were fed pureed corn twice per week following standard protocols (Lorch *et al.*, 1993). Food cups were collected for at least three weeks; offspring were collected from these food cups and sexed at eclosion. Forty-eight *T. dalmanni* males were first paired with virgin females from a stock *T. dalmanni-1* population and then paired with virgin females from their own population as F1 virgin females were produced. When offspring were produced from both females, origin of the female did not influence offspring sex ratio (as noted in Presgraves *et al.*, 1997). Thus, all offspring collected from each male were used to estimate progeny sex ratio. After screening, males were stored in 70% ethanol prior to DNA extraction using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany).

To determine the extent to which X-chromosome genes have a different history from autosomal and mitochondrial genes, we sequenced X-linked, autosomal and mitochondrial genes from males with and without biased sex ratios in each species. Using sex-ratio-screened males from each site, we sequenced at least two SR males (> 90% female offspring) and at least two ST males (approximately 50% female offspring) from each collection site. In addition, we sequenced a few males with partially female-biased progeny (70–80% female offspring). All individuals were sequenced at a mitochondrial locus (16S rRNA) and three X-linked loci (*wingless, dynamin-associated protein 160* or *dap160,* and *echinoid* or *ed*). For a subset of individuals, an autosomal locus (*glutathione S transferase D1* or *GstD1*) was also sequenced. Three of these genes were targeted for sequencing because they were found on the same scaffolds in a draft genome assembly as mapped markers (see Baker & Wilkinson, 2010; Wilkinson *et al.*, 2014) on the X (*ed* near ms071, and *Dap160* near ms167) and on an autosome (*GstD1* on a scaffold with ms262Y, ms262Z, and ms217A). Ms071 and ms167 were previously mapped to opposite ends of the standard X chromosome (Baker & Wilkinson, 2010); thus, these genes were chosen for sequencing to investigate whether patterns of divergence differ across the X. *Wingless* was used in prior phylogenetic studies (Swallow *et al.*, 2005). PCR was performed using standard reagents and conditions. Products were cleaned using Exo-SAP-IT (Affymetrix, Santa Clara, CA, USA) and sequenced on an ABI3730 (ABI, Foster City, CA, USA) either at the University of Maryland or at Eton Biosciences (Newark, NJ, USA). 16S and *wingless* primers were used as described (Baker *et al.,* 2001). Nucleotide sequence from a draft *T. dalmanni* genome assembly and a *T. whitei* transcriptome (Bioproject PRJNA241109) was used to identify conserved exonic sequences, and then primers for *GstD1, dap160* and *ed* (see Table S2) were designed using Primer3 (Untergasser *et al.,* 2012). Examination of *ed* sequences revealed that the two cryptic *T. dalmanni* species could be discriminated on the basis of intronic indels that differed in size (Fig. S1). Therefore, we also designed a second set of *ed* primers (ed-seq2) to detect intron size and identify the two cryptic *T. dalmanni* species. In *T. dalmanni-2*, all individuals had alleles larger than 564 bp, and in *T. dalmanni-1*, all individuals carried alleles smaller than 560 bp. After sequencing, low-quality bases were manually masked and sequences were aligned using MAFFT (–auto). Alignments were inspected, trimmed and concatenated using MESQUITE

v3.04 (Maddison & Maddison, 2017) to produce a 2129-bp data matrix.

The evolutionary history of the X chromosome across the three species was then reconstructed by first estimating the maximum-likelihood tree using RAxML v.8.1.2 (-f a -#100 –m GTRGAMMA) using the concatenated X-linked sequence and then conducting a multispecies coalescent simulation using BP&P v. 3.2 (Yang & Rannala, 2010; Yang, 2015) under the assumption of equal mutation rates along all lineages. The guide tree was (*T. whitei* (*T. dalmanni*-2 (*T. dalmanni*-1 standard, *T. dalmanni*-1 sex ratio))), that is assuming the origin of X^{SR} in *T. dalmanni*-1 is not older than the divergence with *T. dalmanni*-2. This assumption was tested using the A01 model, which searches for the best species tree, assuming all individuals have been grouped correctly. Then, the A10 model was used to estimate the likelihood that the nodes in the species tree represent real species delimitation as opposed to artificial splitting of a truly panmictic lineage (over-splitting). Finally, the A00 model was used with the best-supported tree to estimate τ and θ for all nodes and branches in the tree. A burn-in time of 50 000 steps was used, followed by a 50 000 step run.

To test the hypothesis that the X-chromosome haplotype associated with sex ratio distortion (X^{SR}) arose once vs. multiple times in *T. dalmanni-1*, we compared three phylogenetic hypotheses using X-linked and mitochondrial sequence data from 21 flies collected across all sites. These males produced either equal (ST) or highly female-biased (SR) broods and were confirmed to be *T. dalmanni*-1 using the ed 'seq 2' marker. The alternative phylogenetic hypotheses differ with regard to the degree to which genetic exchange occurs across geographical sites vs. alternative X (i.e. SR vs. ST) chromosomes. Hypothesis 1 states that geography is the primary factor limiting gene flow, so individuals collected from the same location will be most closely related, regardless of their sex-ratio phenotype. Under hypothesis 2, sex ratio distortion has multiple, recent origins in *T. dalmanni-1*, so individuals with similar sex ratios should be closely related to each other, but this relationship should be nested within geographic sites. Hypothesis 3 postulates that divergence of SR haplotypes from ST haplotypes predates geographic divergence, and thus, individuals should cluster by sex-ratio status rather than by collection location. We generated five randomly permuted bifurcating trees consistent with each of these hypotheses (Table S3). We then used RAxML to produce site-wise log-likelihoods (-m GTRGAMMA -f g) for the mitochondrial and X-linked (three gene concatenated) alignments and each hypothesis tree. Consel (Shimodaira & Hasegawa, 2001) was then used to perform phylogenetic hypothesis tests (AU-test, SH-test) and to rank the trees for each data set.

We confirmed the patterns of X-chromosome divergence observed in the sequence data by genotyping X-linked simple tandem repeats (STR) in a much larger sample of males screened for progeny sex ratio from all three species (Table S1). *T. dalmanni* specimens were then genotyped at up to nine STR loci that span the X^{ST} chromosome. In order to capture the greatest amount of genetic variation, screened males collected in the field or from generations closest to the field and those with rare SR phenotypes were prioritized for genotyping (Table S1). These nine markers were co-amplified in three sets of three and analysed on an ABI 3730xl (co-amplified marker sets: ms125, ms244, ms395; CG31738, ms106, ms071; ms054, ms070, CRCtrr; Wright *et al.*, 2004; Birge *et al.*, 2010). Five polymorphic markers were also genotyped in 37 *T. whitei* males that produced sufficient offspring to estimate offspring sex ratio (ms106, ms054, CRC, BUN and CG31738). Alleles were called using GeneMapper software (ThermoFisher Scientific, Waltham, MA, USA).

We characterized X-linked divergence in STRs by estimating the number of genetic clusters in *T. dalmanni* and *T. whitei* using STRUCTURE (Pritchard *et al.*, 2000). Screened individuals with genotypes at five or more loci were included in the STRUCTURE model of *T. dalmanni*. STRUCTURE was run using an admixture model and correlated allele frequencies with no *a priori* information provided on collection site or sex-ratio phenotype. The model was run five times for each value of K, ranging from 1 to 6, each time using 100 000 burn-in steps followed by a run of 100 000 steps. To confirm that the divergence between X^{SR} and X^{ST} within *T. dalmanni-1* is, in fact, chromosome-wide and not driven by a single highly diagnostic locus, the model was run a second time without ms395. This X-linked microsatellite has been shown to be diagnostic of sex ratio distortion (Johns *et al.*, 2005; Wilkinson *et al.*, 2006; Cotton *et al.*, 2014). In our data, ms395 alleles form a bimodal distribution; alleles larger than 227 base pairs were found predominantly in males exhibiting offspring sex ratios of 10% male or less (mean = 0.068), whereas alleles smaller than 221 base pairs were found predominantly in males not exhibiting such highly skewed offspring sex ratios (mean = 0.451). STRUCTURE was also run over K values of 1 to 4 with *T. whitei* males screened for progeny sex ratio that had genotype data at three or more of the five loci assayed. CLUMPAK (http://clumpak.tau.ac.il/index.html) was used to identify the best K value (following the method of Evanno *et al.*, 2005) for each analysis and to summarize replicate runs. At the best value of K, we reported the expected heterozygosity as a measure of diversity within each cluster and net nucleotide distance as a measure of genetic distance between clusters, as calculated in STRUCTURE. Net nucleotide distance is a measure of the difference in allele frequencies between clusters corrected by the average expected heterozygosity within populations (Pritchard *et al.*, 2000). POPHELPER was used to create figures summarizing across

replicate runs (Francis, 2017). Individuals were assigned to the genetic cluster for which they had the highest proportion of ancestry.

The divergence (Reinhardt *et al.*, 2014) and lack of recombination (Johns *et al.*, 2005) between *T. dalmanni-1* X^{SR} and X^{ST} chromosomes raises the question of how X^{SR} chromosomes persist without accumulating deleterious mutations. To determine whether X^{SR} chromosomes undergo recombination when a pair is present in a female, we genotyped the sons of females carrying two X^{SR} chromosomes. In three families (G171, G185, G192), a two-generation cross was conducted. An X^{SR} male was crossed to a female carrying one X^{ST} and one X^{SR} chromosome. Female progeny were collected and genotyped to confirm they inherited an X^{SR} chromosome from each parent (both ms395 alleles > 220). All F1 sisters carrying two X^{SR} chromosomes will have inherited the same two X^{SR} haplotypes (one from each of their parents), as recombination between X^{SR} and X^{ST} chromosomes in heterozygous females is extremely rare (Johns *et al.*, 2005). To confirm this result, we also tested for evidence of recombination in descendants of X^{SR}/X^{ST} heterozygous mothers using three or more X-linked microsatellites. In two additional families (G182, G190), females bearing two X^{SR} (ms395 > 220) haplotypes were obtained. These X^{SR}/X^{SR} females were mated to X^{ST} males from established laboratory colonies. Parents and their sons were genotyped at seven STR loci that span the X chromosome (ms071, ms395, ms070, Bun, CG31738, MS244 and CRCtrr). To address whether recombination among X^{SR} chromosomes occurs, we genotyped 10 or more sons of X^{SR}/X^{SR} females from these five families using at least three maternally heterozygous markers from among the seven listed above. For the one-generation crosses (G182 and G190), the most common haplotypes were assumed to be parental rather than recombinant, whereas for G171, G185 and G192 parental X^{SR} haplotypes were determined directly by genotyping the grandparents of the cross. To estimate the order of markers on X^{SR} and the frequency of recombination, we genotyped 96 sons from one family (G185) at seven microsatellite markers. We compared results to genotyping data from 59 sons from a cross within a control population of standard flies from the Gombak population (Johns *et al.*, 2005). Map distances (Haldane's) and recombination frequencies were estimated using Joinmap 4 (Van Ooijen, 2006).

Results

Phylogenetic analysis of X-linked (Fig. 1) and mitochondrial (Fig. S2) loci revealed two lineages with 100% bootstrap support in *T. dalmanni*, each of which contained individuals from every collection site, but only a single undifferentiated group in *T. whitei* (Fig. 1). However, the tree produced using three X-linked loci (*ed*, *dap160*, and *wingless*) revealed an additional, though somewhat less (80% bootstrap) well-supported, lineage within *T. dalmanni* comprised entirely of SR males. By incorporating *wingless* and 16S sequences from a prior study (Swallow *et al.*, 2005; Fig. S3), we find that all SR and some ST males are in the same *T. dalmanni* clade as flies previously collected from the Gombak site, near Kuala Lumpur, as well as several sites in Sumatra. Meanwhile, the other *T. dalmanni* clade, comprised solely of ST males, includes flies previously collected from the Langat and Cameron Highland sites in Peninsular Malaysia, as well as from Bogor, Indonesia on the island of Java. As previous work (Christianson *et al.*, 2005) demonstrated that flies from these two well-supported *T. dalmanni* clades are reproductively isolated but yet undescribed species, we refer to them as *T. dalmanni-1* and *T. dalmanni-2*, respectively.

Consistent with these topologies, average pairwise distance for X-linked sequence between SR and ST male *T. dalmanni-1* is much greater than that present within either group (within SR: 0.0040, within ST: 0.0068, between SR and ST: 0.0122). In contrast, pairwise distances within and between SR and ST males are very similar for the mitochondrial gene (within SR: 0.0015, within ST: 0.0015, between SR and ST: 0.0015) and the autosomal gene (within SR: 0.0085, within ST: 0.0023, between SR and ST: 0.0062). Average X-linked pairwise divergence between all individuals (i.e. combining SR and ST samples) from *T. dalmanni-1* and *T. dalmanni-2* was 0.080, and between *T. dalmanni-1* and *T. whitei*, 0.121 (within each species, average pairwise distance was < 0.01).

Additional support for a single origin of X^{SR} in this species, consistent with the phylogeny shown in Fig. 1, is provided by direct comparison of the three potential hypothetical relationships among the *T. dalmanni-1* samples using Consel. The 95% confidence set for the X-linked sequence data (AU-test 0.992) included a single tree (Table S3, AU-test, $P_{tree15} = 0.990$) generated under the hypothesis that individuals are related first by *sex ratio*, and then by collection site. In contrast, the 95% confidence set for the mitochondrial data included three trees (Table S3, $P_{tree10} = 0.968$, $P_{tree5} = 0.244$, $P_{tree11} = 0.114$), one generated under each of the three hypotheses. These results demonstrate that the 'sex-ratio' clade in *T. dalmanni-1* is supported by X-linked, but not mitochondrial, sequence.

Using a coalescent approach (Yang, 2015), we find good support (posterior support = 1.0) for a model in which the four major X-linked clades are genetically distinct. We further estimate that X^{SR} chromosomes from *T. dalmanni-1* as a group have an effective population size 6.1 times smaller than X^{ST} chromosomes from the same population – *T. dalmanni-1* (Fig. 2, X^{SR} median $\theta = 0.00251$, X^{ST} median $\theta = 0.01524$). The estimated coalescent time for *T. dalmanni-1* X^{SR} and X^{ST} (median $\tau = 0.0015$) was three-fourth of the coalescent time for

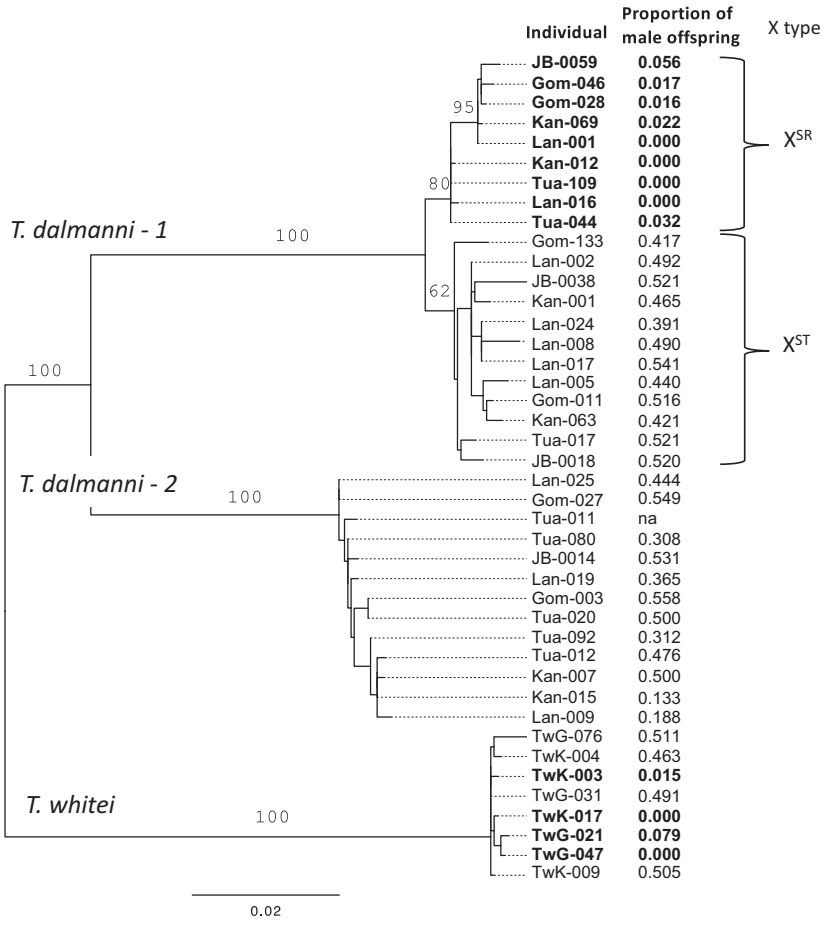

Fig. 1 Maximum-likelihood phylogeny generated by RAxML using 2129 bp of sequence from three genes on the X chromosome for males with species names, progeny sex ratios (mean progeny = 86.6 ± 10.1), inferred X-chromosome types and bootstrap values indicated. SR males are indicated in bold. Note that *T. whitei* males form a single clade independent of progeny sex ratio or collection site. Bootstraps < 60 are not shown.

T. dalmanni-1 and *T. dalmanni-2* (median $\tau = 0.002$) implying an ancient origin of X^{SR} in this species.

A STRUCTURE analysis of nine X-linked loci genotyped in screened male *T. dalmanni* revealed three genetic clusters independent of collection site but dependent on sex-ratio phenotype (Fig. 3). One cluster included mostly individuals with strongly female-biased offspring sex ratios ($N = 79$, mean offspring sex ratio = 0.058, Fig. 4) including all SR males assigned to the *T. dalmanni-1* X^{SR} clade in the phylogenetic analysis (Fig. 1). However, five individuals within this cluster exhibited offspring sex ratios close to 1 : 1 (Fig. 4a). The other two clusters are concordant with the *T. dalmanni-1* X^{ST} and *T. dalmanni-2* clades, respectively (Fig. 1). Individuals in the *T. dalmanni-1* X^{ST} cluster ($N = 122$, Fig. 4b) mostly exhibited standard progeny sex ratios, except for two males that had broods with less than 10% male offspring. The *T. dalmanni-2* cluster contained males with nearly all progeny sex ratios greater than 30% male offspring ($N = 66$, Fig. 4c). Offspring sex ratios of males in the *T. dalmanni-1* X^{ST} and *T. dalmanni-2* clusters did not differ significantly from each other (mean offspring sex ratios, 0.446 vs. 0.452, Student's *t*-test, $P = 0.75$) but each differed from *T. dalmanni-1* X^{SR} (Student's *t*-tests; *T. dalmanni-1* X^{SR} vs. X^{ST}, $t_{193} = 0.3636$, $P < 0.0001$; *T. dalmanni-1* X^{SR} vs. *T. dalmanni-2*, $t_{125} = 0.351$, $P < 0.0001$). We estimated the frequency of X^{SR} chromosomes in the wild using the results of our STRUCTURE analysis. Of the 64 field-collected *T. dalmanni* that were successfully scored for progeny sex ratio and genotyped in our experiment, seven clustered with *T. dalmanni-1* X^{SR}, 31 with *T. dalmanni-1* X^{ST} and 26 with *T. dalmanni-2*. Thus, X^{SR} chromosomes represent 18% of the *T. dalmanni-1* X chromosomes sampled from the field.

The STRUCTURE analysis revealed patterns of X-linked differentiation that match the sequence analysis. The *T. dalmanni-1* X^{SR} and *T. dalmanni-1* X^{ST} clusters are more closely related to each other (distance = 0.1795) than either cluster is to *T. dalmanni-2* (*T. dalmanni-1* X^{SR} to *T. dalmanni-2* distance = 0.3472, *T. dalmanni-1* X^{ST} to *T. dalmanni-2* distance = 0.2419). The *T. dalmanni-1* X^{ST} cluster had greater diversity at STR loci (expected heterozygosity, 0.8035) than either the *T. dalmanni-1* X^{SR} or the *T. dalmanni-2* cluster (expected heterozygosity, *T. dalmanni-1* X^{SR}: 0.5764, *T. dalmanni-2* X: 0.5664); however, reduced diversity at these loci in *T. dalmanni*-2 may be influenced by an

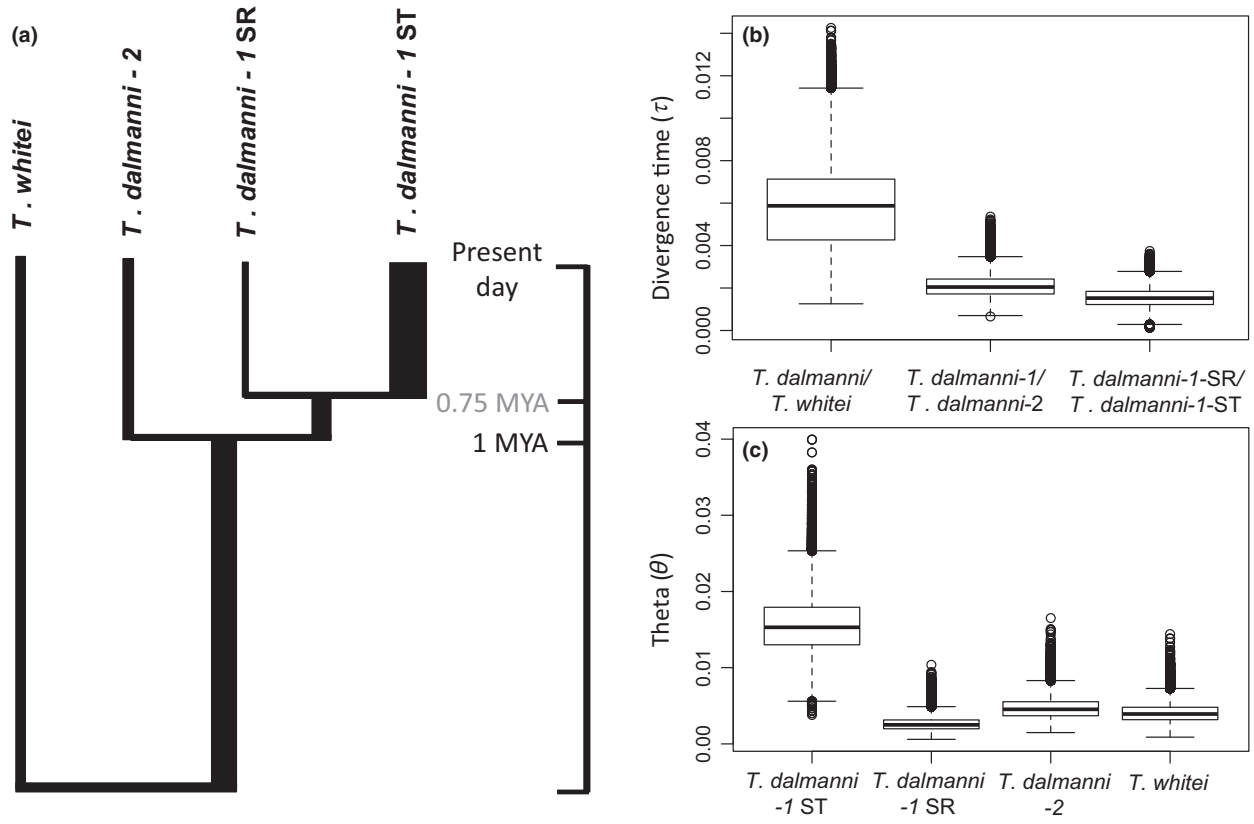

Fig. 2 (a) The best-supported tree from a coalescent analysis of the X-linked sequence data (Fig. 1) with branch thickness proportional to diversity (θ) and conversion of divergence times to years based on Swallow *et al.* (2005). (b) Box plots showing medians, quartiles and outliers from 50 000 estimates of divergence time (τ) and (c) diversity (θ).

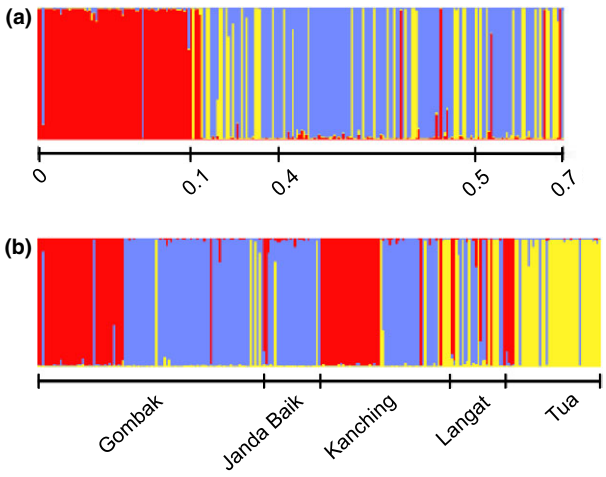

Fig. 3 STRUCTURE cluster assignments using X-linked genotypes for *T. dalmanni* males ordered by (a) offspring sex ratio (excluding individuals with unassigned offspring sex ratios) or (b) by site and then by offspring sex ratio, with unassigned individuals at the right. Individual bars are shaded based on the probability of assignment to the cluster predominated by *T. dalmanni-1* X^{SR} (red) *T. dalmanni-1* X^{ST} (blue) or *T. dalmanni-2* (yellow) individuals.

ascertainment bias, as previously described (Wright *et al.*, 2004). The STRUCTURE results were also highly concordant with single gene markers diagnostic for *T. dalmanni-2* (ms106, ms070, and ed-seq2). Of the 181 individuals where both STRUCTURE group assignment and *T. dalmanni-2* diagnostic marker genotypes were available, one fly had differing assignments. *Ed-seq2* sequencing of this individual confirms it is *T. dalmanni-2* (Fig. 1), rather than *T. dalmanni-1* as suggested by its ms070 or ms106 genotypes.

To confirm that the observed genetic structure between *T. dalmanni* X^{SR} and X^{ST} is driven by chromosome-wide divergence rather than by a single diagnostic marker for X^{SR}, we ran a second STRUCTURE analysis excluding ms395, which is diagnostic of sex ratio (Cotton *et al.*, 2014). When ms395 is excluded, STRUCTURE still best supports three genetic clusters and shows similar estimates of divergence between *T. dalmanni-1* X^{SR} and X^{ST} (distance = 0.1800). Individual group assignment likewise did not change substantially. These results support the inference that the entire X^{SR} chromosome is evolving independently from X^{ST}.

Consistent with the phylogenetic analysis, STRUCTURE detected no evidence of divergence between SR

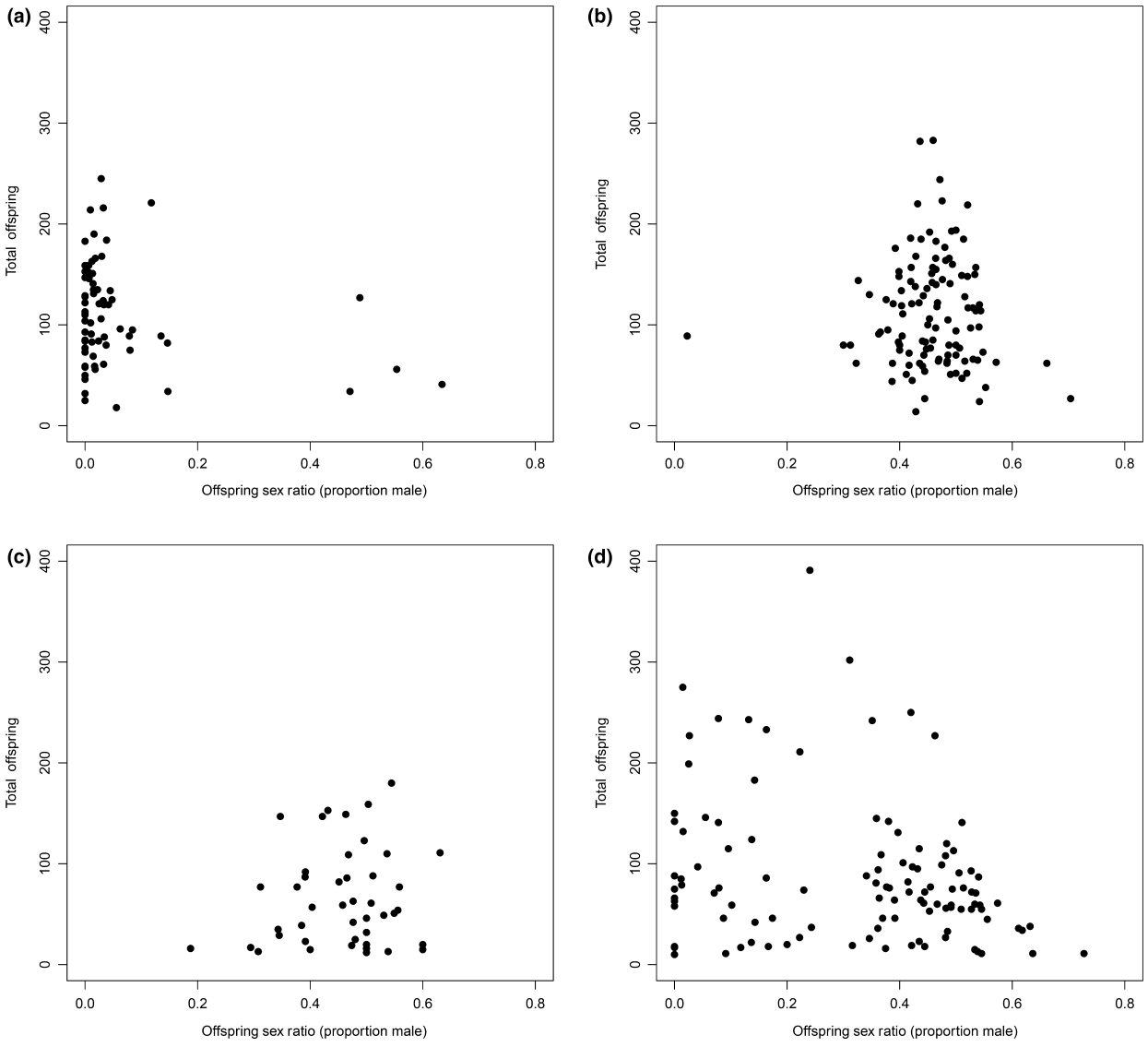

Fig. 4 Progeny sex ratio (proportion male) plotted against number of offspring per male for the genetic clusters identified by STRUCTURE in Fig. 3 (a) *T. dalmanni-1* X^{SR}, (b) *T. dalmanni-1* X^{ST}, (c) *T. dalmanni-2* and (d) for *T. whitei*.

and ST in *T. whitei* despite a similar range of progeny sex-ratio phenotypes as in *T. dalmanni-1* (Fig. 4). STRUCTURE best-supported one genetic cluster (Fig. S4); at all values of K, individuals were assigned equally to all possible clusters. Because SR status shows no evidence of genetic divergence on the X chromosome, we could not estimate the frequency of SR in the wild as we did for *T. dalmanni-1*. Instead, we estimated the frequency of the SR phenotype among screened field-collected and F1 males for one collection site (Gombak). Of the 76 screened *T. whitei* males that produced sufficient progeny to estimate offspring sex ratio, 14.5% ($N = 11$) produced offspring sex ratios of 10% male or less.

Given the presence of sequence and STR variation among *T. dalmanni-1* X^{SR} chromosomes, we investigated the role recombination between X^{SR} chromosomes might play in maintaining diversity. We found no evidence for recombination between X^{SR} and X^{ST} chromosomes, as 12 daughters of X^{SR}/X^{ST} heterozygous mothers in three-two-generation crosses inherited intact haplotypes from both parents (Table S4). However, recombination did occur between X^{SR} chromosomes, as at least one son produced by an X^{SR}/X^{SR} female in each of five families carried a recombinant X chromosome. The frequency of recombination between X^{SR} chromosomes, though, appears to be lower than between X^{ST} chromosomes given that the map length

for the X^{SR} chromosome was two-third the length of the X^{ST} chromosome (Fig. 5), which is a significant difference (Welch's *t*-test, $t_{355} = 2.77$ $P = 0.007$). Furthermore, the order of the markers differed between the two maps, indicating the presence of an inversion spanning at least one-third of the total map length.

Discussion

Sex-ratio distortion alleles create strong genomic conflict that can be resolved by extinction, selection maintaining a polymorphism or the evolution of modifiers, which can result in an evolutionary arms race (Lindholm *et al.*, 2016). Using phylogenetic and population genetic analyses on large samples of three closely related and sympatric species of stalk-eyed flies, *T. whitei, T. dalmanni-1* and *T. dalmanni-2,* we attempted to reconstruct the evolutionary history of X-chromosome segregation distortion in these species. X-chromosome distortion in two of these species causes similar female-biased progeny sex ratios, with a substantial proportion of males (up to 30%) from the wild producing fewer than 10% sons (Presgraves *et al.*, 1997; Wilkinson *et al.*, 2003), whereas a third species shows evidence only of weakly female-biased offspring sex ratios. Despite phenotypic similarities, we found that the X-distortion systems in *T. dalmanni-1* and *T. whitei* appear to have originated independently and have different genomic effects. *T. dalmanni-1* exhibits strong divergence between sex ratio (X^{SR}) and standard (X^{ST}) X chromosomes, whereas *T. whitei* exhibits no detectable distorter-related divergence on the X chromosome. This comparison illustrates how X-chromosome segregation distortion can persist with or without being part of a large inversion (Jaenike, 2001).

Sex-chromosome segregation distortion in *T. dalmanni-1* is associated with an X-chromosome lineage (presumably associated with one or more inversions) that is old enough to contain considerable genetic variation and to have diverged substantially from X chromosomes carried by individuals from the same populations exhibiting standard sex ratios. These chromosomes were found within all populations of *T. dalmanni-1* that we sampled. This genetic structure was well-supported in analyses using both X-linked sequence and STR data. Previous work on *Drosophila recens* (Dyer *et al.*, 2007) reported chromosome-wide LD between X^{ST} and X^{SR} chromosomes but almost no genetic variation among sampled X^{SR} chromosomes. In contrast to this case, whereas *T. dalmanni-1* X^{SR} chromosomes carry less genetic variation than X^{ST} chromosomes, they exhibit more diversity than estimated previously from a smaller sample (Christianson *et al.*, 2011). X-chromosome segregation distortion in *T. dalmanni-1* is thus more similar to other Drosophila species, in which homozygous X^{SR} females are viable, such as *Drosophila neotestacea* (Dyer *et al.*, 2013; Pieper & Dyer, 2016).

We estimate that X^{SR} chromosomes comprise roughly 18% of all sampled X chromosomes in *T. dalmanni-1*.

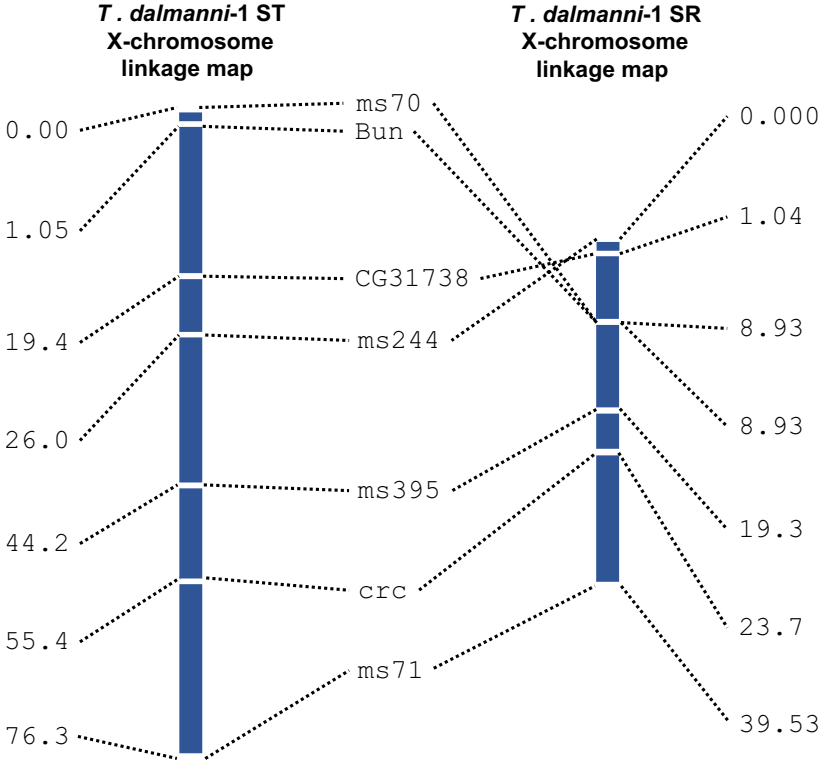

Fig. 5 Linkage maps generated from two F2 intercross experiments in which the F1 female either carried two X^{ST} chromosomes or two X^{SR} chromosomes with seven informative microsatellite markers on each chromosome. Marker name, order and cumulative distances (Haldane's) are shown. The X^{ST}-X^{ST} map was created using data from Johns *et al.* (2005).

With the assumption of no genetic exchange between X^{SR} and X^{ST} since the time X^{SR} 'emerged', the effective population size of an X^{SR} chromosome is expected to be 1/5–1/4 that of an X^{ST} chromosome in the same population (e.g. Pieper & Dyer, 2016). When we compare estimates of diversity (H_e) on X^{SR} and X^{ST} chromosomes using nuclear markers, we find that the variation currently present in X^{SR} is more comparable to X^{ST} than would be expected under this simple hypothesis, indicating that the anticipated impact on genetic diversity is less severe than expected. However, the inclusion of a second *T. dalmanni* species allows us to better contextualize the evolution of the X^{SR} chromosome in *T. dalmanni*. Using a multispecies coalescent approach with the X-chromosome sequence data from *T. dalmanni* and *T. whitei*, we found the estimate of θ for X^{SR} chromosomes was roughly six times smaller than for X^{ST} chromosomes (Fig. 2b). Sex ratio distortion chromosomes are expected to experience extreme local selective pressures, particularly when they first originate and rapidly increase in frequency (Hamilton, 1967). Subsequently, inversions associated with strong sex ratio distorters may experience a bottleneck as they sweep and displace phenotypically weaker haplotypes. Thus, although genetic variation is now present among X^{SR} chromosomes in *T. dalmanni-1*, these past events have likely shaped the overall pattern of genetic diversity relative to other lineages. Furthermore, diversity may be shaped by other forces, including differences in mutation or recombination rates, either within or between chromosome types at present or in the past. More detailed analysis will be needed to determine which forces drove the observed differences we see among these haplotypes.

Relative divergence time estimates (Fig. 2c) using three X-linked loci support a fairly ancient origin of X^{SR} in *T. dalmanni*-1; τ between X^{SR} and X^{ST} within this species is about three-fourth of that between *T. dalmanni-1* and *T. dalmanni*-2. These two species (*T. dalmanni-1* and *T. dalmanni-2*) were previously estimated to have diverged roughly 1MYA (Swallow *et al.*, 2005). If these estimates are accurate, then the *T. dalmanni-1* X^{SR} is over 500 000 years old (see Fig. 2a). However, given the large uncertainties associated with both analyses, this estimate should be viewed with caution. Previous molecular analyses of X^{SR} in *D. pseudoobscura* have produced very different estimates for its age depending on which loci and populations are analysed (Babcock & Anderson, 1996; Kovacevic & Schaeffer, 2000).

Prior studies have reported evidence for suppression of sex ratio distortion in *T. dalmanni* (Presgraves *et al.*, 1997; Wilkinson *et al.*, 2014). In this screen, we found a small number of individuals with 'X^{SR}-like' haplotypes that did not exhibit female-biased offspring sex ratios (Fig. 4). These would be consistent with suppression of *sex ratio*. However, at this time, we cannot distinguish whether these rare cases of genotype/phenotype mismatch are due to autosomal suppression, Y resistance, mutation in the STRs or sex ratio distortion locus, or rare recombination events between X^{SR} and X^{ST} chromosomes. Our results indicate that *sex ratio* is strongly phenotypically penetrant in this species, with multiple lines of evidence for consistent genetic structure between X^{SR} and X^{ST} chromosomes (Figs 1 and 3). Future genetic studies of such individuals will be useful in determining whether they may be carrying resistant Y chromosomes or autosomal suppressors.

Our mapping crosses in *T. dalmanni* confirm that (1) at least one inversion polymorphism exists between X^{SR} and X^{ST} X chromosomes, (2) genetically divergent X^{SR} chromosomes recombine with one another in X^{SR}-homozygous females, and (3) X^{SR} recombination occurs less often than X^{ST} recombination (roughly half as many recombinant progeny were recovered when compared to a previous cross). The lower rate of recombination among X^{SR} chromosomes could be caused by several factors, for example by inversion polymorphisms among X^{SR} chromosomes preventing genetic exchange, by differences in the overall rate of recombination or by synthetic lethal relationships between alleles carried on X^{SR} but not X^{ST} chromosomes. Together with the evidence that both X^{SR} and X^{ST} carry substantial genetic variation, we conclude that in *T. dalmanni-1,* these two X chromosomes are evolving nearly independently within an otherwise shared genomic background. As recently reported, a similar situation exists in *Drosophila neotestacia* (Dyer, 2012; Pieper & Dyer, 2016). In that species, the X^{SR} and X^{ST} chromosome lineages are distinct and recombination now appears to be rare. However, ancestral gene conversion or recombination was detected, indicating that evolution of the X^{SR} and X^{ST} chromosomes is not entirely independent. Because we sequenced only two mapped genes on the X chromosome, we would not be able to detect regions of ancestral recombination as reported by Pieper & Dyer (2016) and we cannot rule out such events.

Despite the lack of observable divergence between X chromosomes carried by SR and ST males in *T. whitei,* the frequency of SR males appears to be remarkably stable over time. In this study, 14.5% of males collected from the Gombak site had offspring sex ratios less than 10% compared to 14% of 64 males collected from the same site 23 years earlier (Wilkinson *et al.*, 2003). The lack of divergence between X^{ST} and X^{SR} in *T. whitei* has several potential causes that will require further work to disentangle. First, this pattern would be expected if our markers are not close to the *sex-ratio* element or in an associated inversion. If sex ratio distortion is not in an inversion and caused by a small region, as in *D. simulans* 'Paris' X-drive (Bastide *et al.*, 2011; Fouvry *et al.*, 2011), and then many more markers will be needed to localize it. Secondly, sex ratio distortion may have arisen so recently that variation in nearby markers is insufficient to detect differentiation. Consistent with

this possibility, *T. whitei* samples showed little divergence between either collection sites or progeny sex-ratio phenotype for X-linked sequence (Fig. 1) or STR data (Fig. S4). Finally, divergence between a putative sex ratio distorter and standard X chromosomes might be difficult to detect whether polymorphic autosomal suppressors or resistant Y chromosomes mask some distorter chromosomes. Given that sperm development in *T. whitei* SR males differs cytologically (Wilkinson & Sanchez, 2001) from either active or cryptic X-chromosome segregation distortion (Wilkinson *et al.*, 2014) in *T. dalmanni-1*, we infer that X-linked sex ratio distortion has appeared at least three times in this genus. It is conceivable that a single genetic factor has triggered the evolution of sex ratio distortion on multiple independent occasions. In support of this hypothesis, the microsatellite marker ms395 is strongly associated with sex-ratio phenotypes both for cryptic (Wilkinson *et al.*, 2014) and active sex ratio distortion (Cotton *et al.*, 2014 and present analysis) in *T. dalmanni*.

We also discovered that *T. dalmanni* likely comprises a sympatric cryptic species complex and that the *sex-ratio* X previously characterized in *T. dalmanni* is found exclusively in *T. dalmanni-1*. Both *T. dalmanni-1* and *T. dalmanni-2* were found at all sites, indicating that these species are sympatric in central peninsular Malaysia. It remains to be seen to what extent their ranges may differ elsewhere on the Sunda Shelf. These two species, whereas reproductively isolated (Christianson *et al.*, 2005; Rose *et al.*, 2014), are phenotypically similar and so far undescribed. The sympatric distribution of these clades explains the large proportion of apparently sterile males observed in our sex ratio screen of *T. dalmanni* compared to the screen in *T. whitei* (24% vs. 8%). The most likely explanation for this is a cryptic species mismatch between males and females randomly paired in the sex-ratio screen. The most notable difference between these species is revealed by their offspring sex ratios. We found no *T. dalmanni-2* males with extremely female-biased sex ratios (< 10% males), suggesting that such extreme sex ratio distortion is either absent or at very low frequencies at this species. We did qualitatively observe a larger number of individuals with weakly female-biased sex ratios in *T. dalmanni-2* compared to *T. dalmanni-1* (Fig. 4). A very similar sex-ratio pattern, that is only males with weakly female-biased sex ratios, was also found for *T. dalmanni-2* flies collected in Bogor, Java (Wilkinson *et al.*, 2003). The apparent absence of males with extreme progeny sex ratios in this species is notable in a clade where sex ratio distortion has arisen repeatedly and is worthy of further investigation.

Despite the similarity in progeny sex-ratio phenotypes and population frequencies, we find that X-chromosome segregation distortion in *T. dalmanni-1* and *T. whitei* have had very different effects on patterns of chromosome divergence. As yet, it is unclear why these two distorter systems differ so much, but their close evolutionary relationship to each other, and to a suppressed distortion allele in the *T. dalmanni-1* lineage (Wilkinson *et al.*, 2014), makes them an ideal system for additional comparative studies on the evolution of natural segregation distorter systems. Particularly, additional genomewide investigation of the *T. whitei* X chromosome may explain why we found no evidence of differentiation between SR and ST males in our population level screen. This goal of understanding how and why sex-ratio distorters evolve in populations has become increasingly important in the light of recent interest and growing concern over release of synthetic segregation distorter genes as mechanisms of population control in pest species (Lindholm *et al.*, 2016; Unckless *et al.*, 2016). Continued study in this and other systems exhibiting segregation distortion has potential to not only aid in understanding the evolution of natural distorters but also help predict the evolutionary outcomes of releasing synthetic distorters into the wild.

Data repository

Sequence data is available on NCBI (MF495499-MF495673), and phenotype and genotype data and sequence alignments are available on DRYAD.

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References

Andolfatto, P., Depaulis, F. & Navarro, A. 2001. Inversion polymorphisms and nucleotide variability in Drosophila. *Genet. Res.* **77**: 1–8.

Babcock, C.S. & Anderson, W.W. 1996. Molecular evolution of the sex-ratio inversion complex in *Drosophila pseudoobscura*: analysis of the esterase-5 gene region. *Mol. Biol. Evol.* **13**: 297–308.

Baker, R.H., Wilkinson, G.S. & DeSalle, R. 2001. The phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diopsidae). *Syst. Biol.* **50**: 1–20.

Baker, R.H. & Wilkinson, G.S. 2010. Comparative genomic hybridization (CGH) reveals a neo-X chromosome and biased gene movement in stalk-eyed flies (genus Teleopsis). *PLoS Genet.* **6**: e1001121.

Barton, N.H. & Charlesworth, B. 1998. Why sex and recombination? *Science* **281**: 1986–1990.

Bastide, H., Cazemajor, M., Ogereau, D., Derome, N., Hospital, F. & Montchamp-Moreau, C. 2011. Rapid rise and fall of selfish sex-ratio X chromosomes in *Drosophila simulans*:

spatiotemporal analysis of phenotypic and molecular data. *Mol. Biol. Evol.* **28**: 2461–2470.

Beckenbach, A.T. 1996. Selection and the "sex-ratio" polymorphism in natural populations of *Drosophila pseudoobscura*. *Evolution* **50**: 787–794.

Birge, L.M., Pitts, M.L., Richard, B.H. & Wilkinson, G.S. 2010. Length polymorphism and head shape association among genes with polyglutamine repeats in the stalk-eyed fly, *Teleopsis dalmanni*. *BMC Evol. Biol.* **10**: 227.

Burt, A. & Trivers, R. 2006. *Genes in Conflict*. Harvard University Press, Cambridge, MA.

Carvalho, A.B. & Vaz, S.C. 1999. Are Drosophila SR drive chromosomes always balanced? *Heredity* **83**: 221–228.

Carvalho, A.B., Vaz, S.C. & Klaczko, L.B. 1997. Polymorphism for Y-linked suppressors of sex-ratio in two natural populations of *Drosophila mediopunctata*. *Genetics* **146**: 891–902.

Christianson, S.J., Swallow, J.G. & Wilkinson, G.S. 2005. Rapid evolution of postzygotic reproductive isolation in stalk-eyed flies. *Evolution* **59**: 849–857.

Christianson, S.J., Brand, C.L. & Wilkinson, G.S. 2011. Reduced polymorphism associated with X chromosome meiotic drive in the stalk-eyed fly *Teleopsis dalmanni*. *PLoS One* **6**: e27254.

Cocquet, J., Ellis, P.J.I., Mahadevaiah, S.K., Affara, N.A., Vaiman, D. & Burgoyne, P.S. 2012. A genetic basis for a postmeiotic X versus Y chromosome intragenomic conflict in the mouse. *PLoS Genet.* **8**: e1002900.

Cotton, A.J., Földvári, M., Cotton, S. & Pomiankowski, A. 2014. Male eyespan size is associated with meiotic drive in wild stalk-eyed flies (*Teleopsis dalmanni*). *Heredity* **112**: 363–369.

Curtsinger, J.W. & Feldman, M.W. 1980. Experimental and theoretical analysis of the "sex-ratio" polymorphism in *Drosophila pseudoobscura*. *Genetics* **94**: 445–466.

Dobzhansky, T. & Sturtevant, A.H. 1938. Inversions in the chromosomes of *Drosophila pseudoobscura*. *Genetics* **23**: 28–64.

Dyer, K.A. 2012. Local selection underlies the geographic distribution of sex-ratio drive in *Drosophila neotestacea*. *Evolution* **66**: 973–984.

Dyer, K.A., Charlesworth, B. & Jaenike, J. 2007. Chromosome-wide linkage disequilibrium as a consequence of meiotic drive. *Proc. Natl. Acad. Sci.* **104**: 1587–1592.

Dyer, K.A., Bray, M.J. & Lopez, S.J. 2013. Genomic conflict drives patterns of X-linked population structure in *Drosophila neotestacea*. *Mol. Ecol.* **22**: 157–169.

Edwards, A. 1961. The population genetics of "sex-ratio" in *Drosophila pseudoobscura*. *Heredity* **16**: 291–304.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol. Ecol.* **14**: 2611–2620.

Fouvry, L., Ogereau, D., Berger, A., Gavory, F., Montchamp-Moreau, C. & McKim, K.S. 2011. Sequence analysis of the segmental duplication responsible for Paris sex-ratio drive in *Drosophila simulans*. *G3 (Bethesda)* **1**: 401–410.

Francis, R.M. 2017. POPHELPER: an R package and web app to analyse and visualize population structure. *Mol. Ecol. Resour.* **17**: 27–32.

Frank, S.A. 1991. Divergence of meiotic drive-suppression systems as an explanation for sex- biased hybrid sterility and inviability. *Evolution* **45**: 262–267.

Haig, D. & Grafen, A. 1991. Genetic scrambling as a defence against meiotic drive. *J. Theor. Biol.* **153**: 531–558.

Hamilton, W.D. 1967. Extraordinary sex ratios. A sex-ratio theory for sex linkage and inbreeding has new implications in cytogenetics and entomology. *Science* **156**: 477–488.

Hartl, D.L. 1975. Modifier theory and meiotic drive. *Theor. Popul. Biol.* **7**: 168–174.

Hurst, L.D. & Pomiankowski, A. 1991. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. *Genetics* **128**: 841–858.

Jaenike, J. 1996. Sex-ratio meiotic drive in the *Drosophila quinaria* group. *Am. Nat.* **148**: 237–254.

Jaenike, J. 2001. Sex chromosome meiotic drive. *Annu. Rev. Ecol. Syst.* **32**: 25–49.

Johns, P.M., Wolfenbarger, L.L. & Wilkinson, G.S. 2005. Genetic linkage between a sexually selected trait and X chromosome meiotic drive. *Proc. R. Soc. B Biol. Sci.* **272**: 2097–2103.

Kingan, S.B., Garrigan, D. & Hartl, D.L. 2010. Recurrent selection on the winters sex-ratio genes in *Drosophila simulans*. *Genetics* **184**: 253–265.

Kovacevic, M. & Schaeffer, S.W. 2000. Molecular population genetics of X-linked genes in *Drosophila pseudoobscura*. *Genetics* **156**: 155–172.

Lindholm, A.K., Dyer, K.A., Firman, R.C., Fishman, L., Forstmeier, W., Holman, L. *et al.* 2016. The ecology and evolutionary dynamics of meiotic drive. *Trends Ecol. Evol.* **31**: 315–326.

Lorch, P.D., Wilkinson, G.S. & Reillo, P.R. 1993. Copulation duration and sperm precedence in the stalk-eyed fly Cyrtodiopsis whitei (Diptera: Diopsidae). *Behav. Ecol. Sociobiol.* **32**: 303–311.

Machado, C.A., Haselkorn, T.S. & Noor, M.A.F. 2007. Evaluation of the genomic extent of effects of fixed inversion differences on intraspecific variation and interspecific gene flow in *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* **175**: 1289–1306.

Maddison, W.P. & Maddison, D.R. 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2. http://mesquiteproject.org.

McDermott, S.R. & Noor, M.A.F. 2010. The role of meiotic drive in hybrid male sterility. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**: 1265–1272.

Meiklejohn, C.D. & Tao, Y. 2010. Genetic conflict and sex chromosome evolution. *Trends Ecol. Evol.* **25**: 215–223.

Merçot, H., Atlan, A., Jacques, M. & Montchamp-Moreau, C. 1995. Sex-ratio distortion in *Drosophila simulans*: co-occurence of a meiotic drive and a suppressor of drive. *J. Evol. Biol.* **8**: 283–300.

Montchamp-Moreau, C., Ginhoux, V. & Atlan, A. 2001. The Y chromosomes of *Drosophila simulans* are highly polymorphic for their ability to suppress sex-ratio drive. *Evolution* **55**: 728–737.

Montchamp-Moreau, C., Ogereau, D., Chaminade, N., Colard, A. & Aulard, S. 2006. Organization of the sex-ratio meiotic drive region in *Drosophila simulans*. *Genetics* **174**: 1365–1371.

Nam, K., Munch, K., Hobolth, A., Dutheil, J.Y., Veeramah, K.R., Woerner, A.E. *et al.* 2015. Extreme selective sweeps independently targeted the X chromosomes of the great apes. *Proc. Natl. Acad. Sci.* **112**: 6413–6418.

Novitski, E. 1947. Genetic analysis of an anomalous sex ratio condition in *Drosophila affinis*. *Genetics* **32**: 526–534.

Patten, M.M. 2014. Meiotic drive influences the outcome of sexually antagonistic selection at a linked locus. *J. Evol. Biol.* **27**: 2360–2370.

Phadnis, N. 2011. Genetic architecture of male sterility and segregation distortion in *Drosophila pseudoobscura* Bogota–USA hybrids. *Genetics* **189**: 1001–1009.

Phadnis, N. & Orr, H.A. 2009. A single gene causes both male sterility and segregation distortion in Drosophila hybrids. *Science* **323**: 376–379.

Pieper, K.E. & Dyer, K.A. 2016. Occasional recombination of a selfish X-chromosome may permit its persistence at high frequencies in the wild. *J. Evol. Biol.* **29**: 2229–2241.

Presgraves, D. 2008. Drive and sperm: the evolution and genetics of male meiotic drive. In: *Sperm Evolution* (T.R. Birkhead, D.J. Hosken & S. Pitnick, eds.), pp. 471–506. Elsevier Ltd, Burlington.

Presgraves, D.C., Severance, E. & Willrinson, G.S. 1997. Sex chromosome meiotic drive in stalk-eyed flies. *Genetics* **147**: 1169–1180.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Reed, F.A., Reeves, R.G. & Aquadro, C.F. 2005. Evidence of susceptibility and resistance to cryptic X-linked meiotic drive in natural populations of *Drosophila melanogaster*. *Evolution* **59**: 1280–1291.

Reinhardt, J.A., Brand, C.L., Paczolt, K.A., Johns, P.M., Baker, R.H. & Wilkinson, G.S. 2014. Meiotic drive impacts expression and evolution of X-linked genes in stalk-eyed flies. *PLoS Genet.* **10**: e1004362.

Rose, E.G., Brand, C.L. & Wilkinson, G.S. 2014. Rapid evolution of asymmetric reproductive incompatibilities in stalk-eyed flies. *Evolution* **68**: 384–396.

Rydzewski, W.T., Carioscia, S.A., Liévano, G., Lynch, V.D. & Patten, M.M. 2016. Sexual antagonism and meiotic drive cause stable linkage disequilibrium and favour reduced recombination on the X chromosome. *J. Evol. Biol.* **29**: 1247–1256.

Shimodaira, H. & Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* **17**: 1246–1247.

Swallow, J.G., Wallace, L.E., Christianson, S.J., Johns, P.M. & Wilkinson, G.S. 2005. Genetic divergence does not predict change in ornament expression among populations of stalk-eyed flies. *Mol. Ecol.* **14**: 3787–3800.

Tao, Y., Araripe, L., Kingan, S.B., Ke, Y., Xiao, H. & Hartl, D.L. 2007a. A sex-ratio meiotic drive system in *Drosophila simulans*. II: an X-linked distorter. *PLoS Biol.* **5**: e293.

Tao, Y., Masly, J.P., Araripe, L., Ke, Y. & Hartl, D.L. 2007b. A sex-ratio meiotic drive system in *Drosophila simulans*. I: an autosomal suppressor. *PLoS Biol.* **5**: e292.

Taylor, J.E. & Jaenike, J. 2002. Sperm competition and the dynamics of X chromosome drive: stability and extinction. *Genetics* **160**: 1721–1731.

Ubeda, F., Patten, M.M. & Wild, G. 2014. On the origin of sex chromosomes from meiotic drive. *Proc. R. Soc. B Biol. Sci.* **282**: 20141932.

Unckless, R. & Clark, A. 2015. Driven to extinction: on the probability of evolutionary rescue from sex-ratio meiotic drive. *bioRxiv*: 18820; https://doi.org/10.1101/018820.

Unckless, R.L., Clark, A.G. & Messer, P.W. 2016. Evolution of resistance against CRISPR/Cas9 gene drive. *Genetics* **116**: 197285.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. *et al.* 2012. Primer3—new capabilities and interfaces. *Nucleic Acids Res.* **40**: e115.

Van Ooijen, J.W. 2006. *JoinMap® 4, Software for the Calculation of Genetic Linkage Maps in Experimental Populations*, Vol. 33, pp. 10–1371. Kyazma BV, Wagening.

Wallace, B. 1948. Studies on "sex-ratio" in *Drosophila pseudoobscura*. I. Selection and "sex- ratio". *Evolution* **2**: 189–217.

Wilkinson, G.S. & Sanchez, M.I. 2001. Sperm development, age and sex chromosome meiotic drive in the stalk-eyed fly, Cyrtodiopsis whitei. *Heredity* **87**: 17–24.

Wilkinson, G.S., Swallow, J.G., Christensen, S.J. & Madden, K. 2003. Phylogeography of sex ratio and multiple mating in stalk-eyed flies from Southeast Asia. *Genetica* **117**: 37–46.

Wilkinson, G.S., Johns, P.M., Kelleher, E.S., Muscedere, M.L. & Lorsong, A. 2006. Fitness effects of X chromosome drive in the stalk-eyed fly, Cyrtodiopsis dalmanni. *J. Evol. Biol.* **19**: 1851–1860.

Wilkinson, G.S., Christianson, S.J., Brand, C.L., Ru, G. & Shell, W. 2014. Haldane's rule is linked to extraordinary sex ratios and sperm length in stalk-eyed flies. *Genetics* **198**: 1167–1181.

Wright, T.F., Johns, P.M., Walters, J.R., Lerner, A.P., Swallow, J.G. & Wilkinson, G.S. 2004. Microsatellite variation among divergent populations of stalk-eyed flies, genus Cyrtodiopsis. *Genet. Res.* **84**: 27–40.

Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. *Curr. Zool.* **61**: 854–865.

Yang, Z. & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci.* **107**: 9264–9269.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Indel variation in fragments generated using 'Ed-seq2' primers.

Figure S2 Maximum Likelihood phylogeny generated using Mitochondrial Data.

Figure S3 Maximum Likelihood phylogeny generated using combined data from an X linked gene (*wingless*) from current and previous analysis.

Figure S4 STRUCTURE cluster assignments for X-linked markers in *Teleopsis whitei*.

Table S1 Numbers of individuals in each population and generation screened and used in STRUCTURE analyses (shown parenthetically).

Table S2 Sequences of primers used to obtain nuclear and mitochondrial sequences.

Table S3 Randomly permuted trees used in CONSEL analysis.

Table S4 Summary of genotypes from recombination studies.

Data deposited at Dryad: https://doi.org/10.5061/dryad.04pj7

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