Variable dosage compensation is associated with female consequences of an X-linked, male-beneficial mutation

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Abstract

Recent theory has suggested that dosage compensation mediates sexual antagonism over X-linked genes. This process relies on the assumption that dosage compensation scales phenotypic effects between the sexes, which is largely untested. We evaluated this by quantifying transcriptome variation associated with a recently arisen, male-beneficial, X-linked mutation across tissues of the field cricket *Teleogryllus oceanicus*, and testing the relationship between the completeness of dosage compensation and female phenotypic effects at the level of gene expression. Dosage compensation in *T. oceanicus* was variable across tissues but usually incomplete, such that relative expression of X-linked genes was typically greater in females. Supporting the assumption that dosage compensation scales phenotypic effects between the sexes, we found tissues with incomplete dosage compensation tended to show female-skewed effects of the X-linked allele. In gonads, where expression of X-linked genes was most strongly female-biased, ovaries-limited genes were much more likely to be X-linked than were testes-limited genes, supporting the view that incomplete dosage compensation favours feminisation of the X. Our results support the expectation that sex chromosome dosage compensation scales phenotypic effects of X-linked genes between sexes, substantiating a key assumption underlying the theoretical role of dosage compensation in determining the dynamics of sexual antagonism on the X.

Keywords: dosage compensation, sexual antagonism, sex chromosomes, *Teleogryllus oceanicus*

Introduction

The X chromosome is widely predicted to be a hotspot for genes with sexually antagonistic fitness effects in XX/XY and XX/XO systems [1–3]. The role of allelic dominance in mediating these effects has been well studied, indicating sexual antagonism on the X should usually favour the spread of female-beneficial variants (as females transmit twice as many copies to the next generation), unless recessive, in which case male-beneficial variants can more readily invade [4–7]. These predictions could explain why genes with male-biased expression often appear underrepresented on X chromosomes [8–11]. However, a less commonly considered factor affecting predictions of sexual antagonism on sex chromosomes is dosage compensation, i.e., whether males and females exhibit differences in expression of X-linked relative to autosomal genes [12,13]. Recent theory has shown that the presence and completeness of dosage compensation is likely to play an important role in mediating sexual antagonism on the X [7,14], but the assumptions underlying this hypothetical role have rarely been addressed in empirical work.

If the sexes are equal in their expression of X-linked genes relative to autosomal genes – for example, through up-regulation in heterogametic males [15] – then phenotypic consequences of X-linked alleles are expected to be of similar magnitude in hemizygous males and homozygous females [4,7,14]. This view has empirical support from gene translocation experiments in species with complete or near-complete dosage compensation [16,17]. In contrast, if there is lower relative expression of X-linked genes in males, e.g., in species where dosage compensation is absent or incomplete [18], then X-linked variants will tend to have greater phenotypic effects and thus more prominent fitness consequences in homozygous females [7]. In this case, X-linked variants are more likely to experience selection favouring female fitness [19]. Thus, the likelihood of alleles with sexually

antagonistic fitness effects spreading is predicted to be affected by the completeness of dosage compensation (Box 1). An important first step testing this idea is to establish whether sex-differences in phenotypic effects scale with the extent of dosage compensation.

Opportunities to test this expectation are rare, because most phenotypes have complex, polygenic architectures [6] and allelic variants with sex-specific fitness effects are difficult to detect. We capitalised on a system of Hawaiian field crickets (*Teleogryllus oceanicus*) in which adaptive male song loss has recently evolved, providing the opportunity to test how effects of an X-linked mutation with sex-specific fitness consequences relate to patterns of dosage compensation. In *T. oceanicus*, adaptive silence is caused by altered male wing venation (the 'flatwing' phenotype – other silencing phenotypes have also been observed [20]), which precludes the production of song. On the island of Kauai, development of this male phenotype is caused by an X-linked allele, *flatwing* [21]. Song loss protects males from an acoustically orienting parasitoid fly whose larvae are lethal endoparasites of *T. oceanicus* adults [22], whereas females do not have differentiated wings and cannot sing.

The sex-determination system of *T. oceanicus* is XO, so males carry one copy of the X chromosome (and thus *flatwing* locus) and females two, but the *flatwing* allele does not have obvious phenotypic consequences for female wing morphology. One might therefore expect *flatwing* to have little if any effect on female gene expression or associated phenotypes, however, this does not appear to be the case. Recent reports indicate that the *flatwing* mutation has pleiotropic or otherwise linked consequences for female gene expression [23], and for female life history traits (reduced reproductive investment, increased rate of mating failure, increased somatic mass index, growth rate) [24–26]. While there is therefore evidence that

85 *flatwing* has sexually antagonistic fitness effects in at least some contexts, such as
86 reproductive investment, we can confidently infer only that it is under strong sex-
87 biased selection, providing considerable fitness benefits to males via sex-limited
88 phenotypic effects on wing morphology (Zuk et al. [22] found <1% of flatwing males
89 harboured lethal endoparasitic larvae, versus >30% of normal-wing males). Female
90 fitness effects are likely to be minor in comparison. A potential explanation, then, for
91 the surprising magnitude of *flatwing*-associated gene expression effects in females is
92 that incomplete dosage compensation causes greater female expression of X-linked
93 genes. In the case of *flatwing*, this would be unlikely to have impeded its spread, due
94 to dramatic fitness benefits in males. However, in the context of less strongly selected
95 X-linked alleles with sexually antagonistic fitness effects, such a role for dosage
96 compensation could have an important influence by increasing the magnitude of
97 phenotypic effect in females relative to males. Given its location on the X-
98 chromosome and male-specific fitness benefits, *flatwing* affords a useful opportunity
99 to test this role.

100 We used multiple RNAseq datasets to measure gene expression in *normal-*
101 *wing* and *flatwing* genotypes, within each sex and across five tissues. By comparing
102 gene expression effects of the *flatwing* allele in different sexes, we could test for
103 differences in its phenotypic effects at the transcriptome level. Specifically, by
104 quantifying differences in expression between morph genotypes within each sex, and
105 the relative expression of X-linked to autosomal genes, we were able to test the
106 presumed association between female expression effects of a male-beneficial X-
107 linked allele, and the completeness of dosage compensation. Based on previous
108 findings of large gene expression effects of *flatwing* in female gonad and somatic
109 tissues [23,24], we predicted that XX females would show greater relative expression
110 of X-linked genes compared with XO males, i.e., incomplete dosage compensation

(**prediction 1**), though the extent would likely vary across tissues [11,12]. Next, we predicted that tissues exhibiting greater female-skew in gene expression effects of carrying the *flatwing* allele would be those with less complete dosage compensation (**prediction 2**). Such a pattern would support the interpretation that substantial female consequences of the male-beneficial *flatwing* allele are a consequence of incomplete dosage compensation, and that this should be an important parameter in predicting the spread of sexually antagonistic variants. Given the theoretical relationship between dosage compensation and spread of sexually antagonistic variants (Box 1), our final prediction was that tissues in which dosage compensation was least evident, i.e., female-biased expression of X-linked genes most pronounced, would exhibit feminisation of the X-chromosome [9]. Following our results from prediction 1, which reported little or no evidence of dosage compensation in sexually dimorphic gonads, we tested this by asking whether ovaries-limited genes were overrepresented on the X (**prediction 3**).

Methods

RNA-seq datasets

We analysed previously published RNA-seq data collected from pure-breeding *normal-wing* and *flatwing* cricket lines across a range of tissues: neural tissue at 7 days post-adulthood (N=48 libraries, 6 replicates per sex*genotype) [23]; neural, thoracic and gonad tissue at ca. 14 days post-adulthood (N=36 libraries, 3 replicates per tissue*sex*genotype) [24]; and developing wingbuds (N=12 libraries, 3 replicates per sex*genotype) [27]. All cricket populations used in the above studies were derived from a single sample of a wild population in Wailua, Kauai [25, Supporting Information]. All males were hemizygous for their respective morph genotype (*flatwing* vs. *normal-wing*), while females were homozygous. Flatwing and normal-

wing lines from the individual studies also shared recent ancestry, having been derived from mixed lab populations, so replicate lines should on average differ at the causative *flatwing* locus/loci, and closely linked loci. The use of replicate lines of each morph enabled us to associate differences in gene expression with differences in morph genotype, and minimise the possibility experimental confounds due to differing background effects [23,24,28].

As well as being from different experimental conditions, neural data from [23] and [24] were collected at different ages (7d and ca. 14d post-adult eclosion), so were treated as different samples, henceforth designated *neural_7d* and *neural_14d*, respectively. In the study from which neural_7d samples were collected [23], crickets had also been subjected to different social acoustic regimes during rearing, which interacted significantly with sex and morph in affecting gene expression. These acoustic regimes included a 'silent' treatment, in which conspecific song is absent (as in the wild Kauai population), and a 'song' treatment where crickets were exposed to high levels of playback of normal-wing male *T. oceanicus* song models meant to mimic a population dense with calling males. For our purposes of calculating 'baseline' differences in gene expression between *Nw* and *Fw* genotypes, specifically addressing the pattern of greater effects in females, we used RNA-seq data from the silent treatment. Details of RNA extraction and sequencing procedures are given in the respective publications, and all RNA-seq data is publicly available (Table S1).

Alignment and quantification of RNA-seq data

Trimmed RNA libraries from each of the samples were aligned to the *T. oceanicus* genome assembled by [29] using HISAT2 [30], then individual transcriptome assemblies were generated using Stringtie [31], restricting transcript quantification to the annotated gene set (N=19,157) provided by [29]. For each dataset, a single

reference transcriptome was created by merging individual transcriptomes, used by Stringtie to quantify individual gene expression for consensus transcripts. Estimated gene counts were used as input in R for further analysis, and TMM normalisation of library counts peformed for libraries from each of the tissues [32].

Quantifying differential gene expression associated with flatwing

Differential expression analysis was performed in the EdgeR package in R v3.4.1 [32]. After filtering genes not expressed at >1 count per million in at least 3 samples for each tissue, differential gene expression between *Nw* and *Fw* lines was analysed by constructing negative binomial generalised linear models for each tissue separately, and performing pairwise contrasts for 'genotype' (i.e. *normal-wing* [*Nw*] – *flatwing* [*Fw*]) in each sex. Significance was tested using likelihood ratio tests, with an initial false discovery rate adjusted significance threshold of FDR<0.05, and corroborating our results using a more robust threshold of FDR<0.01. This more stringent criterion was used to check that the heightened likelihood of false-positives in datasets with fewer replicates per group did not influence our results, and that results were consistent across different significant thresholds. To further check that differences in replicate size, which was greater in the neural_7d tissue dataset than in others, did not affect our results, we iteratively subsampled three libraries from each of the six replicates for tissue*sex*genotype in this dataset, and evaluated whether results were consistent (we found that they were; Supporting Information).

Identification of differentially expressed (DE) genes is influenced by variance among samples within groups (which should be smaller than the variance between groups), thus fewer differentially expressed genes might be reported between groups of samples with greater within-group variance. Although we expected that variance should be similar across genotypes and sexes within each dataset, we calculated the

biological coefficient of variance (i.e. variance within morph genotypes; calculated as the square root of common dispersion [32]) for each sex-by-tissue combination, to check this did not skew our results. The results showed no trend for lower variance in females (Table S2).

Our primary focus in testing the effect of the *flatwing* locus was on the number of DE genes. However, the magnitude of expression differences among DE genes is an important factor to take into account, so we also summed absolute $\log_2$-fold changes across all differentially expressed genes, and visually compared this across sexes and tissues as an approximation of total effect on gene expression irrespective of the genes that were expressed. We did not perform statistical comparisons between summed log-fold changes, as this would be confounded by differences in the contribution of individual genes. Instead, this approach helped ensure that comparing numbers of DE genes did not inadvertently mask contrasting variation in the magnitude of fold-changes.

Quantifying dosage compensation of X-linked genes

Two common approaches to test for dosage compensation in RNA-seq data are: 1) to statistically compare differences in expression of X and autosomal genes for each sex separately, then contrast differences between sexes (requiring two comparisons); and 2) to compare female:male (F:M) expression ratios between X and autosomal genes [12]. Because we found X-linked genes were consistently expressed more highly than autosomal genes when pooled across linkage groups (Figs S1,2), which complicated comparison of X:A expression ratios (as females did not exhibit a 1:1 ratio for null comparison), we used the approach of comparing F:M ratios between X and autosomal genes to quantify dosage compensation (as autosomal genes showed an F:M expression ratio approximately centred around zero). This also more directly

addresses our hypothesis that X-linked genes are more highly expressed in females. Note, however, that the two approaches produced results with similar interpretation (Fig. S1). In calculating F:M expression ratios across genes, we first normalized gene counts, then excluded zero counts and genes expressed in just one sex. After averaging expression across replicates within each sex, F:M expression ratios were compared between X and autosomal genes using Wilcoxon rank sum tests. We found no indication that relative expression of X-linked to autosomal genes differed between *flatwing*/*normal-wing* genotypes of each sex. We therefore pooled normal-wing and flatwing samples within each sex when calculating differences between tissues in the degree of dosage compensation.

Testing feminisation of the X in the gonads

The above analyses revealed greater relative expression of X-linked genes in female ovaries with respect to male testes, so we tested whether putatively female-beneficial genes – those with ovaries-limited expression – were disproportionately X-linked. Such a pattern could support the view that female-beneficial variants on the X chromosome are favoured when dosage compensation is absent or incomplete. We defined genes with sex-limited gonad expression as those expressed >1 count per million in all six samples of one sex, and <1 count per million in all six samples of the opposite sex. Too few genes showed sex-limited expression in somatic tissues to perform the same comparison.

Results

Expression levels of X-linked genes were female-biased but varied across tissues

We found variable degrees of incomplete dosage compensation across tissues and morph genotypes of *T. oceanicus*. Relative expression of X-linked genes was typically

241 greater in females than males, supporting our first prediction (Fig. 1a). *T. oceanicus*
242 thus represents another of a growing list of species which show incomplete sex
243 chromosome dosage compensation [18]. However, this pattern varied across tissues,
244 with sexually dimorphic gonads showing the most extensive female-biased
245 expression (with median expression of X-linked genes 1.98 times greater in females;
246 Fig. 1a). This is consistent with previous work suggesting differences in expression of
247 X-linked genes are strongly exaggerated in gonads, with dosage compensating
248 mechanisms often appearing absent [12,33]. Neural tissue at 7d, in contrast, did not
249 show significant sex differences in expression of X-linked relative to autosomal genes
250 (Fig. 1a). The remaining somatic tissues showed patterns of female-biased X-dosage,
251 albeit much smaller than observed gonads, with median X-expression 1.05 times that
252 of males (Fig. 1a). The X-chromosome also showed a general trend for heightened
253 expression compared with autosomes (Fig. S1), due to low expression of genes in
254 some autosomal linkage groups (Fig. S2). As in previous studies [34,35], we found
255 that the magnitude female-biased X-expression tended to increase with greater
256 average expression level in tissues showing incomplete dosage compensation (Fig.
257 S3).
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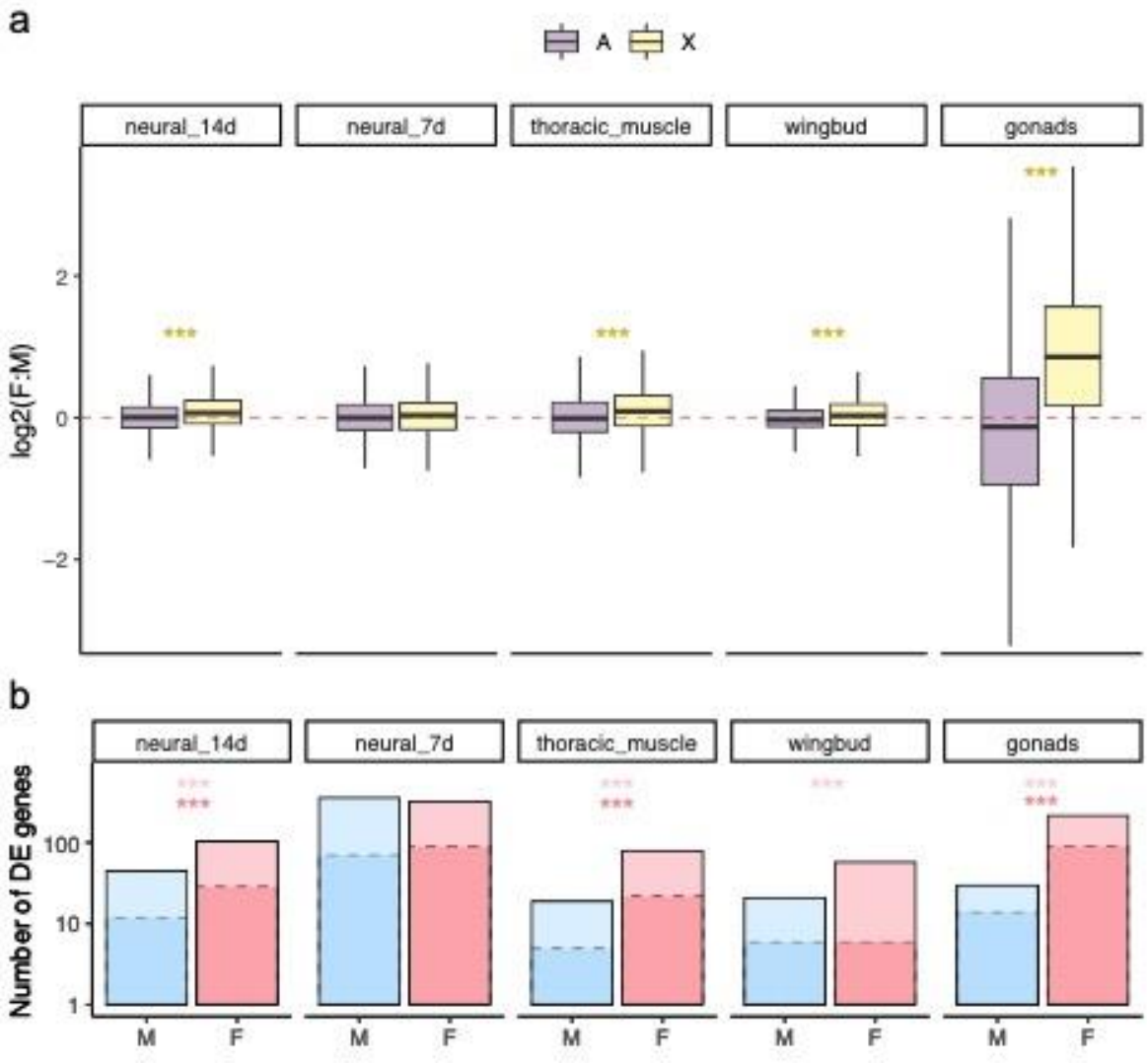


Figure 1. Variable dosage compensation and differential expression associated with *flatwing* across *T. oceanicus* tissues. (a) Females showed greater expression (log_2(F:M) > 0) of X-linked relative to autosomal genes in all tissues but neural tissue at 7 days. Asterisks denote significantly female-biased expression of X-linked relative to autosomal genes, from Wilcoxon rank sum tests. Boxplots show medians and interquartile range, with outliers not shown. The dashed red line illustrates the null expectation of equal expression. **(b)** Females also showed a greater number of DE genes between *flatwing* and *normal-wing* genotypes than males, in all but neural tissue at 7d. Dashed lines and darker shading within bars illustrate the number of DE genes at FDR<0.01, while the full height of the bars illustrates DE gene numbers at FDR<0.05. Light red asterisks denote differences between sexes among genes DE at FDR<0.05, darker red asterisks those DE at FDR<0.01, from Pearson's chi-squared tests. Note the log_{10} y-axis scale. **P<0.01, ***P< 0.001.

Female-biased gene expression effects of flatwing

Females tended to show a greater number of DE genes between morph genotypes compared with males. This large apparent effect of *flatwing* in females is consistent

277 with previous results and analyses, and was apparent in all tissues but neural tissue
278 at 7d in which there was no apparent difference. In wingbuds the above pattern was
279 apparent for DE genes with an FDR < 0.05, but not among those DE at the more
280 stringent FDR < 0.01 threshold (Fig. 1b). A possible explanation for this incongruity
281 is that there were relatively few DE genes in this tissue. Summed absolute log-fold
282 changes across all DE genes showed a similar pattern of female-biased effect (Fig.
283 S4). Across both sexes, a large majority (N=1,251 of 1,407 located in the linkage map)
284 of genes DE between morph genotypes were autosomal, with X-linked genes showing
285 no disproportionate overrepresentation compared with autosomal genes (X_1^2=2.475,
286 P=0.116). However, the pattern of large gene expression effects in females was
287 nevertheless evident among these X-linked genes, and marginally heightened relative
288 to that of autosomes (Fig S5). Though local patterns of dosage compensation are
289 difficult to infer given the presence of genes with sex-biased expression, female-
290 biased expression appeared relatively evenly distributed across the X (Fig S6).
291 Next, we found that female-biased expression of X-linked genes was positively
292 associated with the magnitude of female-biased differential expression between
293 *flatwing* and *normal-wing* lines, a pattern that is in line with our second prediction.
294 Across the five tissues, female-biased X-dosage was positively correlated with
295 female-biased expression effects of carrying *flatwing*, and the relationship
296 approached statistical significance at P<0.05 (Spearman's rho=0.9, P=0.083 at both
297 FDR<0.05 and FDR<0.01 DE thresholds; Fig. S7). This apparent association was
298 most strikingly illustrated by the gonads, where the magnitude of female-biased X-
299 expression and female-biased expression effects associated with *flatwing* were
300 greatest.
301
302 *Feminisation of the X-chromosome in gonads*

As expression of X-linked genes in gonad tissues was considerably greater in females compared with males, we tested whether ovaries-beneficial genes were overrepresented on the X-chromosome (Box 1). Consistent with our third prediction, we found that genes showing ovaries-limited expression were considerably more likely than testes-limited genes to be X-linked (X_1^2=91.19, P<0.001) (Fig. 2).

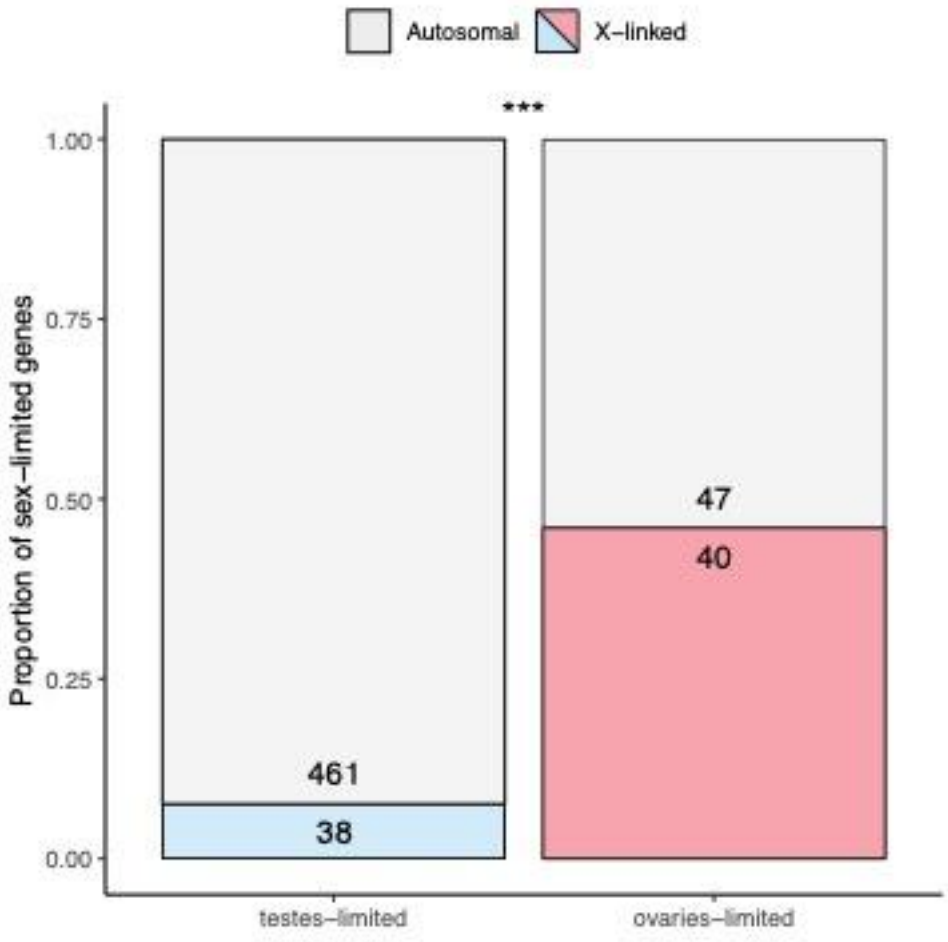


Figure 2. Female ovary-specific genes were much more likely to be X-linked compared with testes-specific genes. Numbers within bar segments indicate the total number of genes in each category, and asterisks indicate significant (P<0.001) differences in proportions of X-linked genes.

Discussion

Theoretical studies have proposed that the degree of sex chromosome dosage compensation will influence the prevalence and dynamics of sexual antagonism on

318 the X chromosome, traditionally viewed as a hotspot for genomic conflict between
319 sexes [4], by scaling effects of X-linked alleles between sexes [7,14]. We identified
320 variable dosage compensation across tissues in *T. oceanicus*, and, consistent with the
321 above expectation, found evidence that this variation was associated with hitherto
322 surprising female consequences of an X-linked, male-beneficial allele. Tissues with
323 incomplete dosage compensation tended to exhibit female-skewed gene expression
324 effects of the X-linked *flatwing* allele, despite the fact it exerts striking
325 morphological consequences only in male wings. These findings support the view
326 that, by scaling phenotypic effects of X-linked alleles, the extent of dosage
327 compensation plays an important role in mediating the potential for sexual
328 antagonism.

329 Our findings also add *T. oceanicus* to the growing list of species in which
330 global dosage compensation is typically absent or incomplete [18,36]. At a minimum,
331 dosage compensation in *T. oceanicus* is not uniform across tissues, as sex differences
332 in the expression of X to autosomal genes were evident in all tissues we studied
333 except neural tissue at 7 days post-eclosion. Early studies of dosage compensation in
334 crickets concluded that dosage compensation was likely complete, either through X-
335 inactivation (*Gryllotalpa orientalis*) or through hypertranscription of the male X
336 (*Acheta domesticus*) [37]. However, these inferences were drawn either through
337 indirect cytogenetic observations, or through studies of single, putatively X-linked
338 genes. Our analysis is the first in Orthoptera to investigate dosage compensation in
339 both a global and cross-tissue manner. Given the diversity of sex chromosome
340 systems more generally in orthopterans [38], it would be interesting to examine
341 whether these differences are the product of different methodologies, or whether
342 there is true biological variation in dosage compensation mechanisms and
343 completeness across the clade.

344 The cross-tissue variation in dosage compensation that we observed is an
345 emerging theme in sex chromosome research [11,36] and has practical implications
346 additional to its hypothetical consequences for sexual antagonism. It has been
347 argued, for instance, that conclusions from studies of dosage compensation using
348 whole-body gene expression data are confounded by not accounting for differences
349 across tissues; in particular, the strong distinction in patterns of X:autosomal
350 expression between somatic and gonad tissues which we also observed [12]. While
351 the differences we observe across somatic tissues were smaller, they could still have
352 consequences for studies of dosage compensation and gene expression which do not
353 separate out tissues. Such variation could influence detectable patterns of sex-biased
354 gene expression [11,39], particularly in organisms for which autosomal and sex-
355 linked genes have not been identified, and, if unaccounted for, could create problems
356 for library normalisation procedures typically used in gene expression analyses [40].
357 Our findings support the expectation that the degree of sex chromosome
358 dosage compensation influences the magnitude of gene expression effects of X-
359 linked variants between the sexes (Box 1). The general pattern we observe of
360 incomplete dosage compensation is perhaps surprising given *flatwing's* male-specific
361 fitness benefits, and the predictions laid out in Box 1, which indicate incomplete
362 dosage compensation should favour the spread of female-beneficial variants.
363 However, in Hawaiian populations, selection for *flatwing* in males is so strong that
364 even if there were substantial female fitness costs, these would be strongly
365 outweighed by male benefit [22]. In other words, the extent of dosage compensation
366 is unlikely to have played an important role in the spread of *flatwing*. It is
367 nevertheless worth noting that developing wing tissue showed near-complete dosage
368 compensation (Figs 1, S3,S7) despite statistically significant female-biased X-
369 expression when compared with autosomes, for it is this tissue which ultimately

370 gives rise to the altered wing morphology that silences males, and upon which
371 natural selection acts in Hawaiian cricket populations.
372 Our observation that ovaries-limited genes were disproportionately X-linked
373 compared with testes-limited genes highlights how female-beneficial genes might
374 accumulate on the X [9], particularly when they are expressed in tissues with absent
375 or incomplete dosage compensation [11,14] (Box 1). However, it is difficult to
376 disentangle cause and effect: in the crickets, do large sex-differences in X-expression
377 in gonads favour the subsequent evolution of female-beneficial genes on the X, or
378 does the presence of female-beneficial genes on the X drive the evolution of
379 incomplete dosage compensation to enhance their effects? Another possibility is that
380 there are upper limits on the extent of hyper-expression of X-linked genes in males
381 which could inhibit the spread of male-biased genes on the X [41], although these
382 limits appear unlikely to explain the pattern we observed in gonads given the
383 apparent lack of dosage compensating mechanisms. Fully understanding the
384 consequences of incomplete dosage compensation for the spread of sexually
385 antagonistic variation demands further research, for example, in a context where
386 researchers can manipulate expression of, and selection on, sexually antagonistic loci
387 on the X.
388 Finally, while we have focussed on the consequences for sexual antagonism,
389 other evolutionary processes may also be modulated by variable and incomplete
390 dosage compensation. Previous theoretical and empirical work has shown that
391 dosage compensation may alter the relative rate of evolution on the autosomes and
392 sex chromosomes (the faster X effect) [5,42–44], with less complete dosage
393 compensation reducing the rate of adaptive substitution on the X chromosome
394 relative to the autosomes [5]. In contrast, certain dosage compensation mechanisms,
395 such as X-inactivation, might increase the rate of adaptive substitution on the X [42].

Thus, in *T. oceanicus,* we should expect the strongest faster-X effect among genes expressed in neural tissue, and the weakest – if any at all – in gonads. More generally, the incomplete but variable patterns of dosage compensation, and the surprisingly large X chromosome, suggest that *T. oceanicus* and Orthoptera more widely may prove fertile ground for investigation of various aspects of sex chromosome evolution.

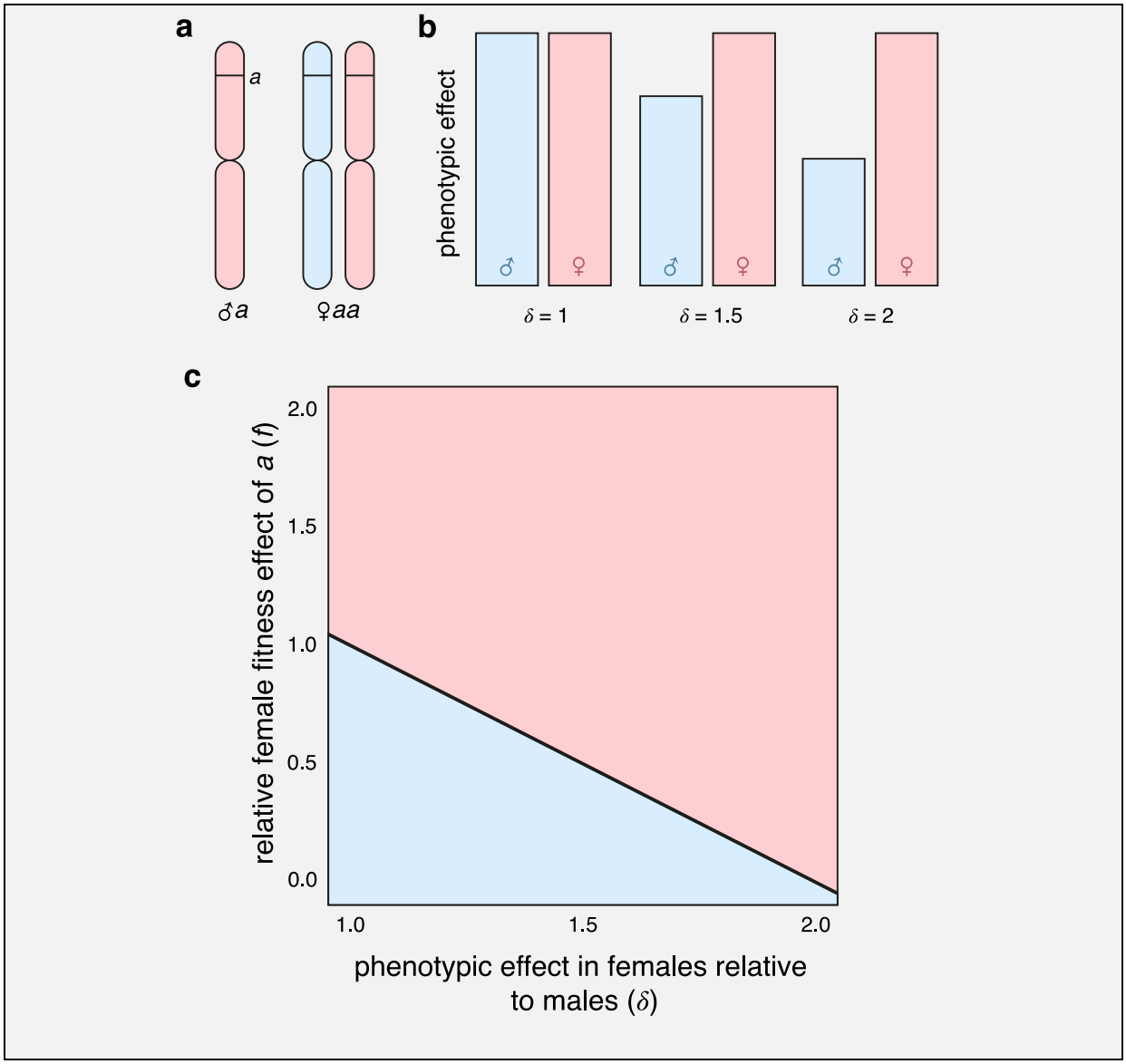
Box 1: The role of dosage compensation in sexual antagonism

Here we illustrate the hypothetical contribution of dosage compensation to patterns of sexual antagonism associated with an additive, X-linked allele, based on existing theory [7,14].

Panel (a) describes an XO system of sex determination, in which males carry one copy of the X and females two; similar dynamics are expected with XX/XY systems of sex determination. With respect to genotype at a given X-linked locus, males carry one copy of an additive allele (here, *a*) whereas females carry two (*aa*). Colours indicate chromosome parental origin (blue = paternal, pink = maternal). There is a 2:1 greater maternal contribution of X chromosomes to the next generation.

Panel (b) shows how differences in the phenotypic effects of carrying *a* or *aa* genotypes are expected to be affected by global differences in expression of X-linked loci, i.e. chromosome-wide differences in expression affecting all genes on the X more-or-less equivalently, or *dosage compensation*. Here, δ represents the magnitude of phenotypic consequence of the specific locus in *aa* females relative to *a* males, which is influenced by the extent of dosage compensation. Three plausible scenarios are illustrated: complete dosage compensation (δ=1), incomplete dosage compensation ($1>\delta<2$; for illustration δ=1.5), and no dosage compensation (δ=2). We illustrate dosage compensation occuring via up-regulation of the X in males, though this could also occur through down-regulation or X-inactivation in females. The less complete dosage compensation is, i.e., as $\delta \rightarrow 2$, the greater the expected phenotypic consequences in homozygous females of the additive allele, *a*, relative to hemizygous males. Therefore, if all else is equal, X-linked alleles should be under female-biased selection when dosage compensation is absent or incomplete.

Panel (c) then illustrates how δ is expected to influence the likelihood that the *a* allele will invade the X chromosome [7,14]. Here, *f* is the marginal benefit to cost ratio of *aa* females relative to *a* males, with fitness effects equal when *f*=1. Blue and pink shaded regions indicate conditions under which male- and female-beneficial antagonistic variants are more likely to invade, respectively. If dosage compensation is incomplete, then $\delta > 1$, and selection will tend to favour female-beneficial variants. Note that our study primarily concerns the patterns in a) and b); panel c) is included to illustrate how these patterns are predicted to influence evolutionary dynamics of sexual antagonism.


a
a
♂a
♀aa
b
phenotypic effect
♂
♀
δ = 1
δ = 1.5
δ = 2
c
relative female fitness effect of a (f)
2.0
1.5
1.0
0.5
0.0
1.0
1.5
2.0
phenotypic effect in females relative to males (δ)

434

435

Author contributions

JGR conceived the study and designed experiments with input from TJH and NWB, JGR performed analyses, and all authors participated in manuscript writing led by JGR.

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Data accessibility

All RNA-seq data is publicly available (accession numbers: PRJEB40088, PRJNA344019, PRJEB27211), with details of source publications provided in Table S1. R scripts are available as Electronic Supplementary Material.

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