JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12708

Within-population Y-linked genetic variation for lifespan in *Drosophila melanogaster*

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Keywords:

intralocus sexual conflict;
longevity;
sex chromosomes;
sexual dimorphism;
Y chromosome.

Abstract

The view that the Y chromosome is of little importance for phenotypic evolution stems from early studies of *Drosophila melanogaster*. This species' Y chromosome contains only 13 protein-coding genes, is almost entirely heterochromatic and is not necessary for male viability. Population genetic theory further suggests that non-neutral variation can only be maintained at the Y chromosome under special circumstances. Yet, recent studies suggest that the *D. melanogaster* Y chromosome *trans*-regulates hundreds to thousands of X and autosomal genes. This finding suggests that the Y chromosome may play a far more active role in adaptive evolution than has previously been assumed. To evaluate the potential for the Y chromosome to contribute to phenotypic evolution from standing genetic variation, we test for Y-linked variation in lifespan within a population of *D. melanogaster*. Assessing variation for lifespan provides a powerful test because lifespan (i) shows sexual dimorphism, which the Y is primarily predicted to contribute to, (ii) is influenced by many genes, which provides the Y with many potential regulatory targets and (iii) is sensitive to heterochromatin remodelling, a mechanism through which the Y chromosome is believed to regulate gene expression. Our results show a small but significant effect of the Y chromosome and thus suggest that the Y chromosome has the potential to respond to selection from standing genetic variation. Despite its small effect size, Y-linked variation may still be important, in particular when evolution of sexual dimorphism is genetically constrained elsewhere in the genome.

Introduction

The potential for adaptive evolution of phenotypic traits through the Y chromosome is currently being re-evaluated (Mank, 2012). Once a pair of neo-Y and neo-X chromosomes stops recombining, the Y chromosome becomes exposed to a range of degenerative processes (Charlesworth & Charlesworth, 2000; Bachtrog, 2013). These include Müller's ratchet, Hill–Robertson interference, background selection and genetic hitchhiking (Charlesworth & Charlesworth, 2000; Kaiser & Charlesworth, 2010). In concert with the small effective population size of the Y, these processes act to decrease the efficacy of selection, which eventually should result in a gradual shut down, and later loss, of genes on the Y chromosome (Rice, 1996; Bachtrog, 2005; Zhou & Bachtrog, 2012). Population genetic models also predict that the Y chromosome can only maintain non-neutral genetic variation under very special circumstances (Clark, 1987, 1990). According to theory, a mature Y chromosome should hence have a very limited capacity to maintain standing genetic variation for phenotypic traits.

In accordance with above scenario, the Y chromosome of *Drosophila melanogaster* features just 13 protein-coding genes (Carvalho *et al.*, 2001; Carvalho & Clark,

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2005; Koerich *et al.*, 2008; Vibranovski *et al.*, 2008; Krsticevic *et al.*, 2010), which all exhibit very low levels of nucleotide polymorphism within populations (Zurovcova & Eanes, 1999; Larracuente & Clark, 2013). The Y chromosome is, furthermore, completely heterochromatic (densely packed DNA which typically suppresses expression) (Hoskins *et al.*, 2002), and although males which lack a Y chromosome (XO) are infertile, they are viable and only have minor changes to their phenotype (Bridges, 1916). For these reasons, the *D. melanogaster* Y chromosome was long considered a genetic desert, with the exception of its importance for fertility (Francisco & Lemos, 2014).

Despite both theory and the above empirical observations suggesting that the *D. melanogaster* Y chromosome should have a very limited potential to contribute to adaptive evolution, there is evidence that suggests the opposite. The chromosome has remained large and constitutes as much as 13% of the male genome (Hoskins *et al.*, 2002), and although the vast majority of the chromosome is made up of seemingly nonfunctional repetitive DNA and transposable elements (Hoskins *et al.*, 2002), this class of DNA actually displays substantial molecular variation (Lyckegaard & Clark, 1989; Clark, 1990). Over the last decades, a few studies of *D. melanogaster*, and its close relatives, have also suggested that the Y chromosome harbours genetic variation for phenotypic traits including geotaxis (Stoltenberg & Hirsch, 1997), suppression of X-linked gametic drive (Carvalho *et al.*, 1997; Montchamp-Moreau *et al.*, 2001; Branco *et al.*, 2013), courtship song (Huttunen & Aspi, 2003), thermal sensitivity (Rohmer *et al.*, 2004) and fitness (Chippindale & Rice, 2001). There are also a few findings in other taxa which point to an effect of the Y or W chromosome (the equivalent of the Y in ZW sex determination systems) on colour traits (e.g. Lindholm *et al.*, 2004; Postma *et al.*, 2011; Evans *et al.*, 2014). None of these findings were, however, able to fully challenge the perception that the Y is a largely inert chromosome.

Recent findings have, however, strongly called into question the long-held view of the Y as a passive chromosome. In a study of Y chromosomes collected from multiple globally dispersed populations of *D. melanogaster*, Lemos *et al.* (2008) showed that the Y chromosome affects the expression of hundreds, potentially thousands, of genes spread throughout the genome. This finding has now been thoroughly replicated by a number of studies (Paredes & Maggert, 2009; Jiang *et al.*, 2010; Lemos *et al.*, 2010; Paredes *et al.*, 2011; Sackton *et al.*, 2011). Because the Y chromosome is only inherited from father to son, it is predicted to primarily affect genes and traits which are sex-limited or show sexual dimorphism. The fact that the set of genes which the Y chromosome regulates is enriched for testis-specific genes supports the hypothesis that the Y chromosome's gene regulatory effect is adaptive (Lemos *et al.*, 2008; Jiang *et al.*, 2010; Sackton *et al.*, 2011).

The finding that the Y chromosome has a substantial capacity to regulate gene expression warrants further investigations into its effect on phenotypic traits. Of particular interest are those which show sexual dimorphism, as the Y chromosome masculinizes the transcriptome (Lemos *et al.*, 2008). Lifespan shows sexual dimorphism in many species (Maklakov & Lummaa, 2013), including *D. melanogaster* (e.g. Lehtovaara *et al.*, 2013). In addition to being sexually dimorphic, there are also other aspects of lifespan which suggest it should be a good candidate trait to assess for Y-linked genetic effects. First, lifespan is a life-history trait and therefore is presumably affected by a large number of genes. This should provide the Y chromosome with ample targets for gene regulation, despite likely having a limited set of mechanisms through which it can regulate expression (Sackton & Hartl, 2013; Francisco & Lemos, 2014). The Y chromosome is furthermore seemingly enriched for variation affecting metabolism and mitochondrial function (Lemos *et al.*, 2008, 2010; Paredes *et al.*, 2011; Sackton *et al.*, 2011), which should have links to lifespan (Balaban *et al.*, 2005). In addition, it has been shown that lifespan is sensitive to modulations of the heterochromatin landscape (Larson *et al.*, 2012), which is the main mechanism through which the Y chromosome is believed to exert its gene regulatory effect (Sackton & Hartl, 2013; Francisco & Lemos, 2014).

Here we assess the influence of the Y chromosome on within-population genetic variation for lifespan in *D. melanogaster*. To accomplish this, we cloned and amplified a set of Y chromosomes, which we expressed in a common genetic background. This allowed us to measure the effect of the Y chromosome independent from all other genomic components. We detect a small, yet statistically significant, effect of the Y chromosome. Our study thus shows that the Y chromosome does contribute to phenotypic variation and that it has the potential to influence the evolution of sexual dimorphism from standing genetic variation, but only to a limited extent because the estimated variance is small.

Materials and methods

Y chromosome substitution lines

We studied within-population genetic variation in lifespan among a set of 33 Y chromosomes, all derived from the Drosophila Genetic Reference Panel (DGRP). The DGRP lines were created through 20 generations of sister–brother mating from a set of flies collected in 2003 from Raleigh, North Carolina (Mackay *et al.*, 2012). The flies were kept under standard conditions throughout the experiment (12:12 light–dark cycle, 60% humidity, 25 °C and on a standard yeast–sugar diet). By a series of backcrosses (Fig. 1), each of the focal Y chromosomes was placed in a common homozygous genetic background from the same population (DGRP-486,

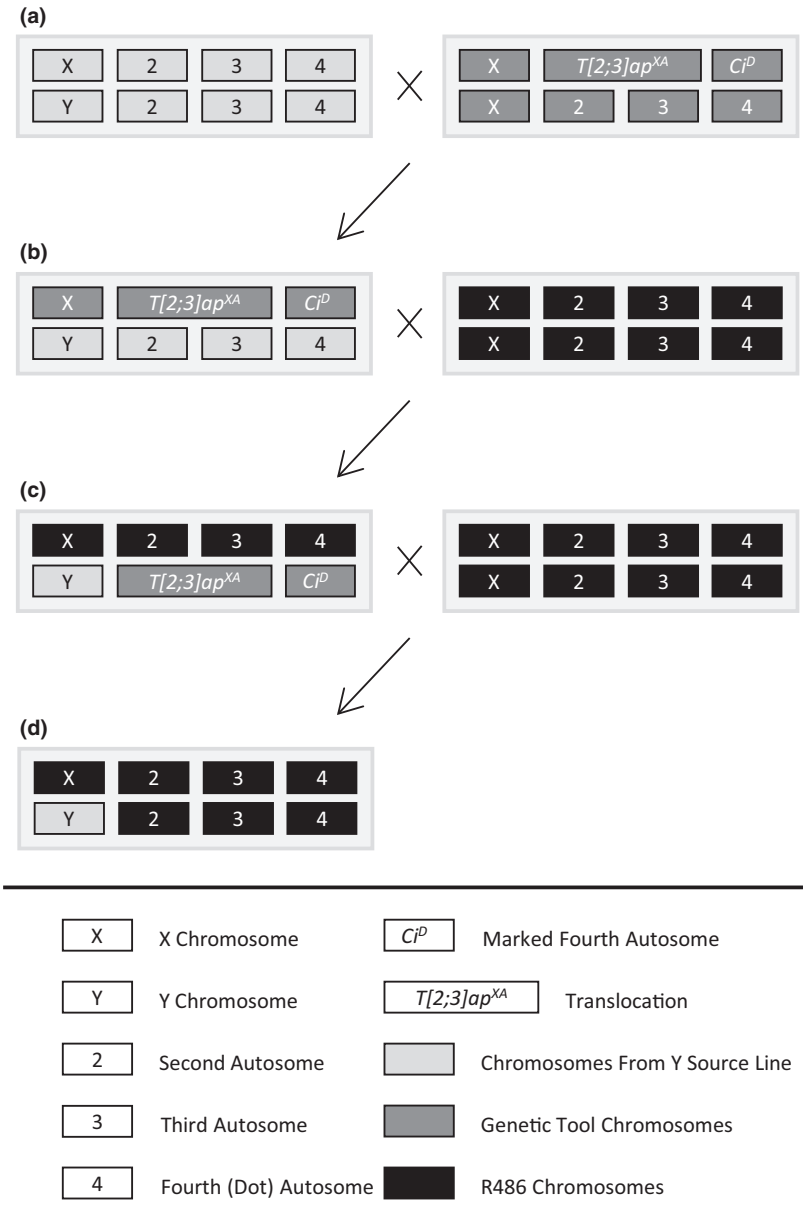

Fig. 1 Crossing scheme to produce Y chromosome substitution lines. (a) Males from each of the 33 source Drosophila Genetic Reference Panel (DGRP) lines were separately crossed to virgin females carrying a dominant marked translocation between the second and third autosomes ($T[2;3]ap^{XA}$), and a dominant marked fourth chromosome (Ci^D). (b) Sons from the above cross, carrying the marked chromosomes, were subsequently mated with virgin females from a randomly selected, completely homozygous, DGRP line (DGRP-486, Bloomington Stock Number 25195). (c) From the above cross, sons carrying the marked chromosomes were crossed to virgin females from the DGRP-486 homozygous stock. (d) Sons emerging from the last cross, not carrying any of the marked chromosomes, had a Y chromosome from one of the focal lines, placed in the homozygous DGRP-486 genetic background. Lifespan was studied for males with this genotype, and lines of these males were maintained by mating to virgin DGRP-486 females.

Bloomington Stock Number 25195). In this way, studied lines only differed genetically with respect to their Y chromosome, and any variation among lines thus has to be linked to this chromosome.

Lifespan assay

Focal males were produced by pairing 20 males from each Y-line with 40 virgin DGRP-486 females, in multiple vials over three consecutive blocks. Vials were trimmed to contain approximately 150 eggs, to standardize larval competition. Ten days after oviposition, we collected multiple vials of 30 males per line, under a light CO_2 anaesthesia (< 4 min of exposure). Males were housed without females, as we have shown in a previous experiment that housing males with other males or females only have a limited effect on average lifespan (~ 10%) and have no detectable effect on the magnitude of genetic variation (Lehtovaara *et al.*, 2013). Experimental males were transferred without anaesthesia to fresh food on day 1, 2 and 5, and every 2 days thereafter, until all flies had died. At each transfer, we scored deaths and discarded dead flies. On average, we assayed the lifespan of 411 (SD = 81) focal males per line and 29.7 (SD = 1.5) flies per vial.

Statistical analysis

Variation in lifespan was analysed using mixed-effects models fitted by Markov chain Monte Carlo (MCMC)

sampling, using the MCMCglmm package (Hadfield, 2010) in R version 3.1.2 (R Core Team, 2014). A random-effects model assuming Gaussian error distributions was used with lifespan as the response variable, block as a fixed effect, and vial and line (DGRP line of origin) as random effects. Parameter-expanded priors, suited for estimation of variances which are expected to be small, were used to estimate variances for the random effects, with the prior defined as prior variance (V) of 1, a belief parameter (nu) of 1, prior mean (alpha.mu) of 0 and prior covariance (alpha.V) of 1000 (Hadfield, 2010; J. Hadfield, personal communication). A weak prior was used for the residual variance where V = 1 and nu = 0.002 (Hadfield, 2010). Results were robust to alternative values of V and nu. Two independent MCMC chains were run for 500 000 iterations, with a burn-in of 100 000 iterations and a thinning interval of 100 iterations. Further, to ensure that the line variance estimate represents a true signal, rather than an artefact introduced by the sampling algorithm when estimating variances near zero, we randomized each vial's assignment with respect to Y-line and generated 100 additional chains, one for each of 100 independent randomizations. The posterior distributions of line variance were then compared to the original distributions. This processing confirmed that we had detected a true signal of the Y chromosome (see Results), because the observed data do not stack values at zero, whereas the randomized do (Fig. 2). Furthermore, results were confirmed using restricted maximum likelihood (REML) in the R package lme4 (Bates *et al.*, 2015) and are reported with standard deviations and *P*-values. Convergence was checked visually for each parameter and replicate MCMC chain. From the MCMC chains, we extracted mean lifespan and estimates for each variance component, as well as standard errors and 95% credible intervals for each estimate.

Results

Mean male lifespan across all 33 Y lines was estimated to 66.85 days (± 0.31 SE, 95% CI [66.23–67.50], Fig. 3). The variance explained by Y-line (variance among Y chromosomes) was 0.65 (± 0.37 SE, 95% CI [0.09–1.52], Fig. 2), and the total phenotypic variance was estimated to 153.97 (± 1.93 SE, 95% CI [150.21–157.73]). The vial variance was 4.42 (± 0.66 SE, 95% CI [3.21–5.81]), and block variance was 0.89 (± 0.27 SE, 95% CI [0.42–1.47], estimated as the variance in the predicted values of the fixed effect). Variance among Y chromosomes therefore explained 0.4% (± 0.2% SE, 95% CI [0.2–1.0%]) of the total phenotypic variance in male lifespan. The genetic and phenotypic coefficients of variation were 0.012 (± 0.004 SE, 95% CI [0.005–0.019]) and 0.190 (± 0.001 SE, 95% CI [0.187–0.193]), respectively. Using REML, we show similar levels of genetic (0.58 ± 0.76, $P = 0.009$), vial (4.35 ± 2.09, $P < 0.001$) and phenotypic variance.

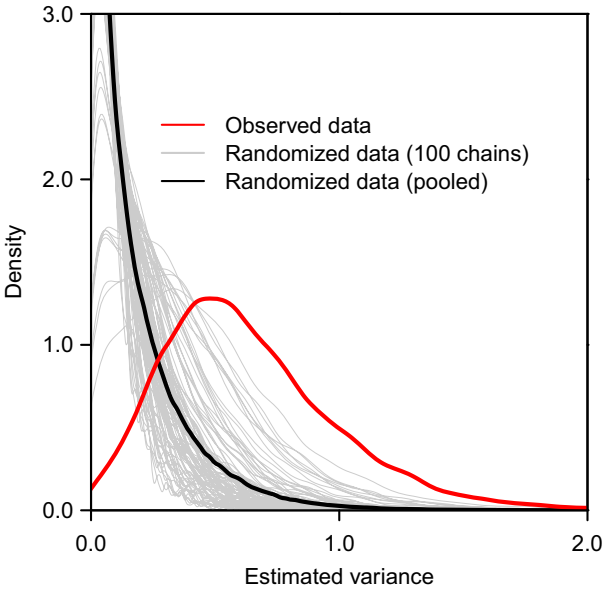

Fig. 2 Plots of the posterior distributions for estimates of line variance, obtained from the analyses with the observed data (red) and the randomized vial data (black/grey), see Materials and Methods for details. Randomization of vial label causes lower, and often zero, estimates of variance, whereas the observed data produce variance estimates which are higher and do not stack against estimates of zero variance.

Two earlier assays of the DGRP lines have measured the total genetic variance for lifespan across the whole genome. They estimate genetic variance to be 93.75 (Ivanov *et al.*, 2015) and 104.34 (Ayroles *et al.*, 2009). Dividing our estimate of the Y-linked genetic variance through these estimates suggests that the Y chromosome explains approximately 0.65% (0.69%, 0.62%) of the total genetic variation, although experimental conditions were not identical.

Discussion

Motivated by the newly discovered large gene regulatory capacity of the Y chromosome (Lemos *et al.*, 2008, 2010; Paredes & Maggert, 2009; Jiang *et al.*, 2010; Paredes *et al.*, 2011; Sackton *et al.*, 2011), and the possibility that the Y chromosome might play a larger role in phenotypic evolution than previously appreciated, we here assessed the Y chromosome's impact on within-population genetic variation for lifespan. In support of the emerging view, we find that the Y chromosome harbours genetic variation for this trait. The effect is small, but suggests that the Y has the potential to contribute to phenotypic evolution from standing genetic variation.

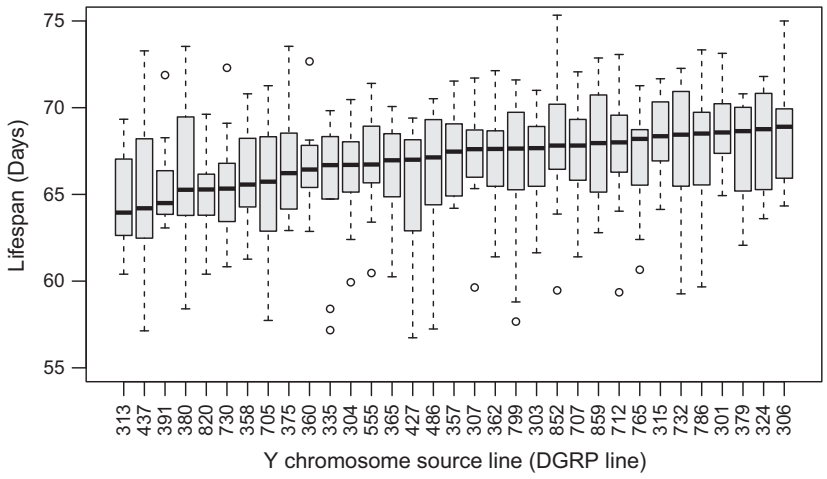

Fig. 3 Boxplot of lifespan in order of shortest to longest lived Y chromosome substitution line, based on median lifespan per vial.

The evolution of sexual dimorphism is constrained by males and females sharing the same genome (Lande, 1980; Bonduriansky & Rowe, 2005; Bonduriansky & Chenoweth, 2009; Poissant *et al.*, 2010; Lewis *et al.*, 2011; Gosden *et al.*, 2012; Griffin *et al.*, 2013; Pennell & Morrow, 2013; Ingleby *et al.*, 2014). This constraint does not, however, concern the Y chromosome, which is free to accumulate male-specific adaptations independently of their effect in females, due to its strict inheritance from father to son. Proof of this principle recently gained support from a study of the W chromosome in chickens, where the expression level of W-linked genes rapidly responded to female-limited selection (Moghadam *et al.*, 2012). From the perspective that evolution of sexual dimorphism in general is constrained, the Y-linked genetic variation found here may thus be important in facilitating evolution of sex differences, despite being small in its effect size. It is also possible that the effect of the Y chromosome detected here have larger pleiotropic effects on other key traits in males.

The small Y-linked effect we report here is not in conflict with the relatively larger effects on gene expression and fertility observed at the between-population/species level (Lemos *et al.*, 2008; Sackton *et al.*, 2011). Population genetic models suggest selected variation should only rarely be maintained at the Y chromosomes (Clark, 1987, 1990), whereas differences at the between-population level can rapidly accumulate through fixation of slightly deleterious mutations, because the Y chromosome does not recombine and has a relatively small effective population size. What probably helps maintain a small amount of variation is that the Y presumably has a larger mutational input than previously thought, where the whole chromosome acts as a single locus determining the amount of heterochromatin at other chromosomes, which should shift the equilibrium frequency towards more variation at mutation–selection–drift balance.

Among the genes that the Y chromosome regulates, those interacting with mitochondrial genes or associated with metabolism are over-represented (Lemos *et al.*, 2008, 2010; Paredes *et al.*, 2011; Sackton *et al.*, 2011). The idea that there is an association between metabolism and lifespan, mediated through the 'rate of living hypothesis', has been around for a long time, but the empirical evidence for this connection is weak at best (Speakman, 2005). More direct evidence has been established for a link between mitochondrial function and lifespan (e.g. James & Ballard, 2003; Trifunovic *et al.*, 2004; Maklakov *et al.*, 2006). This link appears especially strong in males (Camus *et al.*, 2012), presumably because mutations with adverse effects on males, and neutral effects on females, are free to accumulate in mitochondria (Frank & Hurst, 1996; Friberg & Dowling, 2008; Innocenti *et al.*, 2011). To reduce the effect of such male detrimental mutations, males may evolve counter adaptations (Yee *et al.*, 2013; Dean *et al.*, 2015). It is thus not improbable that the Y chromosome plays a role in this context (Rogell *et al.*, 2014) and that this is part of how the Y mediates the variation in male lifespan detected here.

We are only aware of one other study testing for an effect of the Y chromosome on lifespan in *Drosophila*. In this study, males having either a *Drosophila sechellia* or *Drosophila simulans* Y chromosome, placed in a *D. simulans* genetic background, were compared (Johnson *et al.*, 1993). The estimated difference was sizable (14%) but marginally nonsignificant, potentially due to a relatively small sample size. For guppies, a within-population effect has been reported (Brooks, 2000), and in a study between two populations of seed beetle, no effect was detected (Fox *et al.*, 2004).

Our approach of placing Y chromosomes in a standardized genetic background provides a powerful test for Y-linked within-population genetic variation. The drawback with this method is that we are unable to

discern whether the variation is additive, or largely locked into epistatic interactions with the rest of the genome. Previous studies of the *D. melanogaster* Y chromosome have emphasized the prevalence of Y by genetic background interactions, for both gene expression (Jiang *et al.*, 2010) and fitness (Chippindale & Rice, 2001), although theory suggests such should rarely maintain variation (Clark, 1987, 1990). However, the mitochondria, which shares many of its characteristics with the Y chromosome (haploid genome selected exclusively in one sex with small effective population size), only displays interactions with the genetic background for females fitness within a population of *D. melanogaster* (Dowling *et al.*, 2007), whereas the same set of mt-types displayed additive genetic variation for female lifespan (Maklakov *et al.*, 2006).

In conclusion, our study provides support for Y-linked standing genetic variation in lifespan, but the effect is small and required high sample size to detect. Given the facts which are lined up in favour of finding a Y-linked effect on lifespan (see Introduction), it is plausible that the effect on other sexually dimorphic traits is frequently even smaller, but the reverse may apply to male-limited traits on which the Y chromosome may have a larger gene regulatory influence. This may explain why a Y-linked effect on within-population genetic variation only rarely has been reported. Our data nonetheless support that the Y chromosome could have a small but distinct capacity to contribute to phenotypic evolution from standing genetic variation, especially for traits where sex-specific evolution is constrained elsewhere in the genome.

Acknowledgments

We thank Aivars Cīrulis for excellent technical assistance, and Christopher Kimber and two anonymous reviewers for constructive comments on an earlier draft of this study. The study was supported by grants from the Swedish Foundation for Strategic Research, Carl Tryggers Foundation and the Swedish Research Council to UF. HS was supported by the German Research Foundation (DFG; SCHI 1188/1-1).

References

Ayroles, J.F., Carbone, M.A., Stone, E.A., Jordan, K.W., Lyman, R.F., Magwire, M.M. *et al.* 2009. Systems genetics of complex traits in *Drosophila melanogaster*. *Nat. Genet.* **41**: 299–307.

Bachtrog, D. 2005. Sex chromosome evolution: molecular aspects of Y-chromosome degeneration in *Drosophila*. *Genome Res.* **15**: 1393–1401.

Bachtrog, D. 2013. Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. *Nat. Rev. Genet.* **14**: 113–124.

Balaban, R.S., Nemoto, S. & Finkel, T. 2005. Mitochondria, oxidants, and aging. *Cell* **120**: 483–495.

Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *arXiv*::1406.5823.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.* **24**: 280–288.

Bonduriansky, R. & Rowe, L. 2005. Intralocus sexual conflict and the genetic architecture of sexually dimorphic traits in *Prochyliza xanthostoma* (*Diptera*: *Piophilidae*). *Evolution* **59**: 1965–1975.

Branco, A.T., Tao, Y., Hartl, D.L. & Lemos, B. 2013. Natural variation of the Y chromosome suppresses sex ratio distortion and modulates testis-specific gene expression in *Drosophila simulans*. *Heredity* **111**: 8–15.

Bridges, C.B. 1916. Non-disjunction as proof of the chromosome theory of heredity (concluded). *Genetics* **1**: 107–163.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature* **406**: 67–70.

Camus, M.F., Clancy, D.J. & Dowling, D.K. 2012. Mitochondria, maternal inheritance, and male aging. *Curr. Biol.* **22**: 1717–1721.

Carvalho, A.B. & Clark, A.G. 2005. Y chromosome of *D. pseudoobscura* is not homologous to the ancestral *Drosophila* Y. *Science* **307**: 108–110.

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.

Carvalho, A.B., Dobo, B.A., Vibranovski, M.D. & Clark, A.G. 2001. Identification of five new genes on the Y chromosome of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98**: 13225–13230.

Charlesworth, B. & Charlesworth, D. 2000. The degeneration of Y chromosomes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**: 1563–1572.

Chippindale, A.K. & Rice, W.R. 2001. Y chromosome polymorphism is a strong determinant of male fitness in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98**: 5677–5682.

Clark, A.G. 1987. Natural selection and Y-linked polymorphism. *Genetics* **115**: 569–577.

Clark, A.G. 1990. Two tests of Y chromosomal variation in male-fertility of *Drosophila melanogaster*. *Genetics* **125**: 527–534.

Dean, R., Lemos, B. & Dowling, D.K. 2015. Context-dependent effects of Y chromosome and mitochondrial haplotype on male locomotive activity in *Drosophila melanogaster*. *J. Evol. Biol.* **28**: 1861–1871.

Dowling, D.K., Friberg, U., Hailer, F. & Arnqvist, G. 2007. Intergenomic epistasis for fitness: within-population interactions between cytoplasmic and nuclear genes in *Drosophila melanogaster*. *Genetics* **175**: 235–244.

Evans, S.R., Schielzeth, H., Forstmeier, W., Sheldon, B.C. & Husby, A. 2014. Nonautosomal genetic variation in carotenoid coloration. *Am. Nat.* **184**: 374–383.

Fox, C.W., Bush, M.L., Roff, D.A. & Wallin, W.G. 2004. Evolutionary genetics of lifespan and mortality rates in two populations of the seed beetle, *Callosobruchus maculatus*. *Heredity* **92**: 170–181.

Francisco, F.O. & Lemos, B. 2014. How do Y-chromosomes modulate genome-wide epigenetic states: genome folding, chromatin sinks, and gene expression. *J. Genomics* **2**: 94–103.

Frank, S.A. & Hurst, L.D. 1996. Mitochondria and male disease. *Nature* **383**: 224.

Friberg, U. & Dowling, D.K. 2008. No evidence of mitochondrial genetic variation for sperm competition within a population of *Drosophila melanogaster*. *J. Evol. Biol.* **21**: 1798–1807.

Gosden, T.P., Shastri, K.L., Innocenti, P. & Chenoweth, S.F. 2012. The B-matrix harbors significant and sex-specific constraints on the evolution of multicharacter sexual dimorphism. *Evolution* **66**: 2106–2116.

Griffin, R.M., Dean, R., Grace, J.L., Rydén, P. & Friberg, U. 2013. The shared genome is a pervasive constraint on the evolution of sex-biased gene expression. *Mol. Biol. Evol.* **30**: 2168–2176.

Hadfield, J.D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**: 1–22.

Hoskins, R.A., Smith, C.D., Carlson, J.W., Carvalho, A.B., Halpern, A., Kaminker, J.S. *et al.* 2002. Heterochromatic sequences in a *Drosophila* whole-genome shotgun assembly. *Genome Biol.* **3**: research0085.0081–0085.0016.

Huttunen, S. & Aspi, J. 2003. Complex inheritance of male courtship song characters in *Drosophila virilis*. *Behav. Genet.* **33**: 17–24.

Ingleby, F.C., Innocenti, P., Rundle, H.D. & Morrow, E.H. 2014. Between-sex genetic covariance constrains the evolution of sexual dimorphism in *Drosophila melanogaster*. *J. Evol. Biol.* **27**: 1721–1732.

Innocenti, P., Morrow, E.H. & Dowling, D.K. 2011. Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. *Science* **332**: 845–848.

Ivanov, D.K., Escott-Price, V., Ziehm, M., Magwire, M.M., Mackay, T.F.C., Partridge, L. *et al.* 2015. Longevity GWAS using the Drosophila genetic reference panel. *J. Gerontol. A Biol. Sci. Med. Sci.* doi: 10.1093/gerona/glv047

James, A.C. & Ballard, J.W.O. 2003. Mitochondrial genotype affects fitness in *Drosophila simulans*. *Genetics* **164**: 187–194.

Jiang, P.P., Hartl, D.L. & Lemos, B. 2010. Y not a dead end: epistatic interactions between Y linked polymorphisms and genetic background affect global gene expression in *Drosophila melanogaster*. *Genetics* **186**: 109–118.

Johnson, N.A., Hollocher, H., Noonburg, E. & Wu, C.I. 1993. The effects of interspecific Y-chromosome replacements on hybrid sterility within the *Drosophila simulans* clade. *Genetics* **135**: 443–453.

Kaiser, V.B. & Charlesworth, B. 2010. Muller's ratchet and the degeneration of the *Drosophila miranda* neo-Y chromosome. *Genetics* **185**: 339–348.

Koerich, L.B., Wang, X.Y., Clark, A.G. & Carvalho, A.B. 2008. Low conservation of gene content in the *Drosophila* Y chromosome. *Nature* **456**: 949–951.

Krsticevic, F.J., Santos, H.L., Januário, S., Schrago, C.G. & Carvalho, A.B. 2010. Functional copies of the Mst77F gene on the Y chromosome of *Drosophila melanogaster*. *Genetics* **184**: 295–307.

Lande, R. 1980. Sexual dimorphism, sexual selection, and polygenic characters. *Evolution* **34**: 292–305.

Larracuente, A.M. & Clark, A.G. 2013. Surprising differences in the variability of Y chromosomes in African and cosmopolitan populations of *Drosophila melanogaster*. *Genetics* **193**: 201–214.

Larson, K., Yan, S.-J., Tsurumi, A., Liu, J., Zhou, J., Gaur, K. *et al.* 2012. Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. *PLoS Genet.* **8**: e1002473.

Lehtovaara, A., Schielzeth, H., Flis, I. & Friberg, U. 2013. Heritability of life span is largely sex limited in *Drosophila*. *Am. Nat.* **182**: 653–665.

Lemos, B., Araripe, L.O. & Hartl, D.L. 2008. Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences. *Science* **319**: 91–93.

Lemos, B., Branco, A.T. & Hartl, D.L. 2010. Epigenetic effects of polymorphic Y chromosomes modulate chromatin components, immune response, and sexual conflict. *Proc. Natl. Acad. Sci. USA* **107**: 15826–15831.

Lewis, Z., Wedell, N. & Hunt, J. 2011. Evidence for strong intralocus sexual conflict in the Indian Meal Moth, *Plodia interpunctella*. *Evolution* **65**: 2085–2097.

Lindholm, A.K., Brooks, R. & Breden, F. 2004. Extreme polymorphism in a Y-linked sexually selected trait. *Heredity* **92**: 156–162.

Lyckegaard, E.M. & Clark, A.G. 1989. Ribosomal DNA and stellate gene copy number variation on the Y chromosome of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **86**: 1944–1948.

Mackay, T.F.C., Richards, S., Stone, E.A., Barbadilla, A., Ayroles, J.F., Zhu, D.H. *et al.* 2012. The *Drosophila melanogaster* genetic reference panel. *Nature* **482**: 173–178.

Maklakov, A.A. & Lummaa, V. 2013. Evolution of sex differences in lifespan and aging: causes and constraints. *BioEssays* **35**: 717–724.

Maklakov, A.A., Friberg, U., Dowling, D.K. & Arnqvist, G. 2006. Within-population variation in cytoplasmic genes affects female life span and aging in *Drosophila melanogaster*. *Evolution* **60**: 2081–2086.

Mank, J.E. 2012. Small but mighty: the evolutionary dynamics of W and Y sex chromosomes. *Chromosome Res.* **20**: 21–33.

Moghadam, H.K., Pointer, M.A., Wright, A.E., Berlin, S. & Mank, J.E. 2012. W chromosome expression responds to female-specific selection. *Proc. Natl. Acad. Sci. USA* **109**: 8207–8211.

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.

Paredes, S. & Maggert, K.A. 2009. Ribosomal DNA contributes to global chromatin regulation. *Proc. Natl. Acad. Sci. USA* **106**: 17829–17834.

Paredes, S., Branco, A.T., Hartl, D.L., Maggert, K.A. & Lemos, B. 2011. Ribosomal DNA deletions modulate genome-wide gene expression: "rDNA-sensitive" genes and natural variation. *PLoS Genet.* **7**: e1001376.

Pennell, T.M. & Morrow, E.H. 2013. Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecol. Evol.* **3**: 1819–1834.

Poissant, J., Wilson, A.J. & Coltman, D.W. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution* **64**: 97–107.

Postma, E., Spyrou, N., Rollins, L.A. & Brooks, R.C. 2011. Sex-dependent selection differentially shapes genetic variation on and off the guppy Y chromosome. *Evolution* **65**: 2145–2156.

R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rice, W.R. 1996. Evolution of the Y sex chromosome in animals. *Bioscience* **46**: 331–343.

Rogell, B., Dean, R., Lemos, B. & Dowling, D.K. 2014. Mito-nuclear interactions as drivers of gene movement on and off the X-chromosome. *BMC Genom.* **15**: 330.

Rohmer, C., David, J.R., Moreteau, B. & Joly, D. 2004. Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. *J. Exp. Biol.* **207**: 2735–2743.

Sackton, T.B. & Hartl, D.L. 2013. Meta-analysis reveals that genes regulated by the Y chromosome in *Drosophila melanogaster* are preferentially localized to repressive chromatin. *Genome Biol. Evol.* **5**: 255–266.

Sackton, T.B., Montenegro, H., Hartl, D.L. & Lemos, B. 2011. Interspecific Y chromosome introgressions disrupt testis-specific gene expression and male reproductive phenotypes in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **108**: 17046–17051.

Speakman, J.R. 2005. Body size, energy metabolism and lifespan. *J. Exp. Biol.* **208**: 1717–1730.

Stoltenberg, S.F. & Hirsch, J. 1997. Y-chromosome effects on *Drosophila* geotaxis interact with genetic or cytoplasmic background. *Anim. Behav.* **53**: 853–864.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E. *et al.* 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429**: 417–423.

Vibranovski, M.D., Koerich, L.B. & Carvalho, A.B. 2008. Two new Y-linked genes in *Drosophila melanogaster*. *Genetics* **179**: 2325–2327.

Yee, W.K.W., Sutton, K.L. & Dowling, D.K. 2013. *In vivo* male fertility is affected by naturally occurring mitochondrial haplotypes. *Curr. Biol.* **23**: R55–R56.

Zhou, Q. & Bachtrog, D. 2012. Chromosome-wide gene silencing initiates Y degeneration in *Drosophila*. *Curr. Biol.* **22**: 522–525.

Zurovcova, M. & Eanes, W.F. 1999. Lack of nucleotide polymorphism in the Y-linked sperm flagellar dynein gene Dhc-Yh3 of *Drosophila melanogaster* and *D. simulans*. *Genetics* **153**: 1709–1715.

Data deposited at Dryad: doi: 10.5061/dryad.kc33b

Received 10 June 2015; revised 24 July 2015; accepted 24 July 2015