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The *Drosophila simulans* Y chromosome interacts with the autosomess to influence male fitness.

C. Ruth Archer[1†], Robert M. Stephens[1†], Manmohan D. Sharma[1], & David J. Hosken[1*]

[1] Science and Engineering Research Support Facility Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Penryn, UK.

[†] These authors contributed equally to this work.

* corresponding author

Author email:

r.matt.stephens@gmail.com

M.D.Sharma@exeter.ac.uk

C.Archer@exeter.ac.uk

D.J.Hosken@exeter.ac.uk

ABSTRACT

The Y chromosome should degenerate because it cannot recombine. However, male limited transmission increases selection efficiency for male benefit alleles on the Y, and therefore Y-chromosomes should contribute significantly to variation in male-fitness. This means that

although the *Drosophila* Y chromosome is small and gene-poor, Y-linked genes are vital for male fertility in *D. melanogaster* and the Y chromosome has large male-fitness effects. It is unclear if the same pattern is seen in the closely related *D. simulans*. We backcrossed Y chromosomes from 3 geographic locations into 5 genetic backgrounds and found strong Y and genetic background effects on male fertility. There was a significant Y-background interaction, indicating substantial epistasis between the Y and autosomal genes that impacted on male fertility. This supports accumulating evidence that interactions between the Y chromosome and the autosomes are key determinants of male fitness.

KEY-WORDS

Drosophila simulans, ebony, fertility, Y chromosome.

INTRODUCTION

Over time, the Y chromosome should degenerate because it does not recombine. Recombination breaks linkage disequilibria between unfavourable gene combinations that can slow evolution and facilitate genetic hitchhiking, and also protects against mutation accumulation, which can ultimately destroy the information content of functional genes (Charlesworth & Charlesworth, 2000). In the absence of recombination therefore, the Y chromosome should degenerate as mutations accumulate. In keeping with this idea, both human and fruit fly X chromosomes contain thousands of functional genes, whereas the Y chromosome only contains a few dozen (Carvalho, 2002). *Drosophila melanogaster* males that lack these genes are viable but infertile (Bridges, 1916), suggesting that the Y chromosome only has limited, but important, phenotypic effects. In some species, the Y chromosome has disappeared altogether (Just *et al.*, 2002).

Despite these observations, Y chromosome degeneration is not inevitable. Sequence data from humans and rhesus macaques reveals that although Y chromosome decay was initially rapid, degeneration has been negligible for the past ca. 25 million years, leaving a suite of stable ancestral genes on the Y (Hughes *et al.*, 2012). Maintenance of Y-linked genes is largely due to sex-specific purifying selection. The Y chromosome is transmitted from

fathers to sons and this increases the efficiency of selection for male benefit alleles, meaning that these should accumulate on the Y chromosome (Charlesworth & Charlesworth, 2000). In support of this, ca. 25% of ancestral genes surviving on human Y chromosomes have diverged from their X homologues and now play important roles in male reproductive development or sperm production (Bellott *et al.*, 2014). Similarly, the 13 protein encoding genes located on the *D. melanogaster* Y chromosome are only expressed during sperm production (Carvalho *et al.*, 2009). The effects of the Y chromosome however, are seen across the genome, as the Y chromosome affects the expression of genes across the autosomes (*D. melanogaster* - Lemos *et al.*, 2010) and so may have large phenotypic effects. In turn, polymorphisms on the Y chromosome are a major determinant of male reproductive success (Chippindale & Rice, 2001) and lifespan (Griffin *et al.*, 2015) in *D. melanogaster*. However, our understanding of how Y chromosomes affect overall fitness is not especially well characterised outside of *D. melanogaster.*

To improve our understanding of the fitness effects of the Y chromosome, we assessed the fitness of *D. simulans* males originating from 3 geographic locations - Athens (Greece), Crete (Greece) and Tuncurry (Australia). Elements of sexual fitness including male fertility, have been extensively studied in these populations (Hosken *et al.*, 2008; Taylor *et al.*, 2008; Okada *et al.*, 2011; Ingleby *et al.*, 2013a; b). Y-polymorphisms exist between *Drosophila* populations and these influence the expression of hundreds of genes (Lemos *et al.*, 2008). To assess the fitness effects of divergent Y chromosomes, the Y needs to be separated from its original genetic background (Chippindale & Rice, 2001). We achieved this by backcrossing males from each of the three geographic locations into five tester backgrounds (different isolines), for four generations, such that any variation in fitness above that attributable to isoline must largely originate from the Y chromosome or linked genes (ca. 94% of the original background is lost after four generations of backcrossing). We then assayed the reproductive success of males with different Y chromosomes, in each genetic background, when paired with a virgin female and competing male.

Materials and Methods

Animals and Husbandry

All experimental flies, and flies used to create backcrossed animals, were housed at 25°C on a 12/12 hour light/dark cycle. We used flies collected from three locations, Athens (Greece), Crete (Greece) and Tuncurry (Australia). Previous work shows that *D. melanogaster* (Lemos *et al.*, 2008) and *D. simulans* (Kopp *et al.*, 2006) from different populations have polymorphisms on the Y. Accordingly, we assume that flies from each population have divergent Y chromosomes. From the Athens population, multiple isolines were established from gravid females and maintained for four years by full-sib meeting before the start of this study. We backcrossed each Y chromosome into each of five of these isolines for four generations, which theoretically homogenises ca. 94% of the background so any consistent non-isoline genetic variation that remained should be Y-linked.

To backcross the Y chromosomes into each background, virgin females were collected from each isoline. Each of eight females from a single isoline, was paired with one of eight males from a single population (Crete, Athens or Australia) in a vial (100ml) containing fly food (Jazz Mix) and watched to confirm mating. Once copulation was completed, females were removed and placed in egg-laying vials with excess Jazz mix food. Each male was then moved to a vial with a female from another of the five isolines and watched until copulation was completed. This was repeated until each male had mated with a female from each of the five isolines. This means that, from each of three populations, each of eight males were mated to females from five different isolines, to create 120 backcrosses (3 x 8 x 5). Mated females were left on egg-laying vials for three days. For each subsequent backcross, a male was sampled from each of the egg-laying vials and mated to a two day old virgin female from the appropriate initial isoline. A schematic of this mating regime is given in Fig S1 of the SI.

Fitness Assay

To assay male fitness, backcrossed males were placed in a vial containing Jazz Mix food, and a sexually mature virgin female and male e*bony D. simulans* (3-5 days old). *ebony* is a recessive mutant that affects fly colour, meaning that readily identifiable mutants are only produced when an *ebony* female mates with an *ebony* male. Pairing focal male flies with *ebony* males provides a standardised way of comparing the fertilization/reproductive

success of focal males. Fitness was assessed for 360 males (N = 3 replicates from each of 120 backcrossed lines). Focal males were housed with their *ebony* mates and *ebony* competitors for 24 hours, before females were removed and placed in egg-laying vials for seven days. After females were removed, all vials were checked daily for eclosion of offspring. Once eclosions began, the vial was left for seven more days to allow all eggs to hatch. Offspring were anaesthetized using CO_2 and the number of wild-type (*wt*) and *ebony* offspring and the *wt*/*ebony* proportion were all recorded. A schematic of this assay is given in Fig S2 in the SI.

Statistics

This experiment was designed such that we could compare the proportion of offspring sired by wild-type males that carried divergent Y chromosomes, expressed in different genetic backgrounds, when in competition with *ebony* males. However, production of *ebony* offspring was uniformly very low across Y backgrounds, with only 47 experimental females producing any *ebony* offspring. Given this, variance in total wild-type offspring production is likely a better reflection of how the Y chromosome affects male fitness.

All analyses were conducted using R version 3.3.3. (R core development team., 2013). To determine the effect of Y chromosome and genetic background on male reproductive success we used the function "glmer" in the lme4 package (Bates & Maechler, 2009), and the optimisation method "bobyqa". Because mating assays were conducted over three different blocks separated in time, block was included as a random effect. Moreover, three flies were assayed from each of the eight males from each population that were used to backcross into every genetic background and so the identity of the male used to establish each backcross was given as a random effect, nested within Y chromosome. Total offspring production was the response variable, and Y chromosome and genetic background, and the interaction between them, were the explanatory variables. Because these are count data we used a *Poisson* error structure, however, data were over-dispersed and so an individual level random effect was incorporated into the model to account for this. The effects of each explanatory variable were determined by backwards model simplification, where terms were excluded at a level of $P > 0.05$. Results of model comparison are presented. *Post hoc* tests were carried out using the function LSmeans (Lenth, 2014).

Results

Total offspring production was influenced by the interaction between genetic background and Y chromosome ($\chi^2_{11,19}$ = 30.58, *P* = 0.0002) (Figures 1 and 2). *Post hoc* testing of the interaction (Table S2) showed that when backcrossed into genetic backgrounds 1 and 2, all populations had similar fertility (all P > 0.13). However, in genetic background 3, males from the Australian population were significantly less fertile than males from the Crete population (*P* = 0.024) but not the Athens population (*P* = 0.098). In genetic background 4, Australian flies were less fertile than males from both Greek populations (*P* < 0.0001), although the Greek flies did not differ significantly from one another (*P* = 0.854). The same was true in genetic background 5, however, these effects were slightly weaker (Australia vs. Athens *P* = 0.044 ; Australia vs. Crete *P* = 0.0003). To better understand the effects of genetic background on male fertility, data for each Y chromosome were analysed separately using the same error structure as in the full model (random effects = block, individual and male ID). In this case, there was no significant effect of background on fertility in males from Crete ($\chi^2_{8,4}$ = 2.98, *P* =0.561), but genetic background had a significant effect on fertility in males from Athens ($\chi^2_{8,4}$ = 12.90, *P = P =* 0.012) and Australia ($\chi^2_{8,4}$ = 45.92, *P* = <0.001).

Discussion

Given that the Y chromosome cannot recombine, mutation and drift should lead to the progressive loss of genes on this chromosome (Charlesworth & Charlesworth, 2000). The genes that remain on the Y may have strong effects on male fitness because male limited transmission means that selection favours the accumulation of male benefit alleles on the Y, and these will subsequently be under strong purifying selection (Bachtrog, 2004). In keeping with this, polymorphisms on this chromosome have pronounced effects on male fitness in *D. melanogaster* (Griffin *et al.*, 2015). Here, we find that in *D. simulans,* the Y chromosome also has a large effect on male reproductive success but these effects depend on the genetic background in which the Y was expressed, indicating Y-autosomal epistasis. Moreover, males originating from Greece had greater reproductive success than males

originating from Australia. Because the isolines that the Y chromosomes were regressed into also originate from Greece, this may indicate co-evolution between the Y and the autosomes.

Epistasis was manifest as changes in the rank order of Y-fertility effects depending on the genetic background in which the Y was expressed. In other words, the rank order of reproductive success of different Y chromosomes varied across genetic backgrounds. This is consistent with findings in *D. melanogaster,* where genetic background also had a strong effect on the fertility of males with different Y chromosomes (Chippindale & Rice, 2001). These results probably reflect the well documented effects of the Y on gene expression across the genome (Lemos *et al.*, 2008, 2010). For example, when Y chromosomes from *D. sechellia* were introgressed into *D. simulans*, 2 to 3% of genes in the genome showed disruptions in their expression and these were largely involved in sperm production (Sackton *et al.*, 2011). In turn, males whose autosomess and Y chromosome originated from different species were similarly attractive to females, but had reduced sperm competitive ability and reproductive success (Sackton *et al.*, 2011). If there is widespread Y-background epistasis influencing sperm production, then mismatches between the Y chromosome and the rest of the genome will generally reduce male reproductive success.

While genetic background interacted with the Y chromosome to affect male fertility, these effects were relatively weak: in three out of the five genetic backgrounds males from Crete had the greatest fertility and males from the Australian population had the lowest. In the two remaining genetic backgrounds, fitness ranks changed but absolute fertility was statistically indistinguishable among the three Y chromosome populations. The similar fertility of males from Crete and Athens populations may indicate less variation between Y chromosomes in Greek males, relative to Australian flies. Analysis of polymorphisms in different populations of *D. simulans* shows that there is often little geographic structure, or variation, in populations outside of East Africa (Dean & Ballard 2004; Hamblin & Veuille 1999). This is because the species spread out of Africa relatively recently, and did so rapidly with considerable genetic draft (Schlenke & Begun, 2004). Despite this, there is considerable polymorphism on the *D. simulans* Y chromosome (Montchamp-Moreau *et al.*, 2001; Kopp *et al.*, 2006), and this may reflect environmental variation. For example, 50% of the differences in thermal tolerance between natural *D. melanogaster* populations are due to the Y (David

et al., 2005). Given this, males originating from Greek populations may have experienced similar selection on the Y, relative to flies from Australia. Moreover, because the isolines that the Y chromosomes were regressed into also originate from Greece, Y-background matching is likely to be better in Crete and Athens males, than between Australian Ys and Greek autosomess. While this idea requires testing, the similarity in reproductive success of Greek populations and the poor fertility of Australian populations, further highlights the importance of interactions between the Y chromosome and the genetic background that chromosome is expressed in.

The overall effect of background on fitness is consistent with earlier work showing that in *D. simulans* male fitness components such as attractiveness (Taylor *et al.*, 2007; Ingleby *et al.*, 2013b) and sperm competitiveness (Hosken *et al.*, 2008) are heritable, and even when elements of male attractiveness show genotype-by-environment interactions, overall attractiveness transfers across environments (Ingleby *et al.*, 2013b). The heritability of such fitness determining traits is an important prerequisite for female choice for indirect genetic benefits. However despite the background effect, fitness was non-transitivity across some Y-background combinations, which is consistent with the disruption of co-adapted gene complexes caused by our backcrossing, and in some ways reflects background epistasis identified for other genetic elements showing sex-limited transmission (e.g. mito-nuclear epistasis (Arnqvist *et al.*, 2010).

We did not determine whether the fitness effects of the Y chromosome were mediated by male attractiveness (i.e. the chromosome affects how well males attract females) or by sperm production/function. However, the clear role of *D. melanogaster* Y-genes in spermatogenesis (Lemos *et al.*, 2008), and the disrupted sperm production in *D. simulans* males with a heterospecific Y chromosome (Sackton *et al.*, 2011), suggest that reduced sperm function is a likely candidate of the fitness effects we see here. We hoped however, that our competitive mating assay would offer some preliminary insight into this. We know that *D. simulans* females tend to prefer wild-type mates over *ebony* mutants (Sharma *et al.*, 2010). If females had a reduced preference for one genetic background-Y chromosome wild-type combination then this would have been revealed by the increased production of *ebony* progeny. However, the low numbers of *ebony* offspring suggests that females generally preferred wild-type males, which is consistent with previous findings

(Sharma *et al.*, 2010). So while sperm performance impacts are the most likely candidate for the Y-background fitness effects we document, further dissecting the mechanism by which the Y chromosome affects male fitness in *D. simulans* is warranted.

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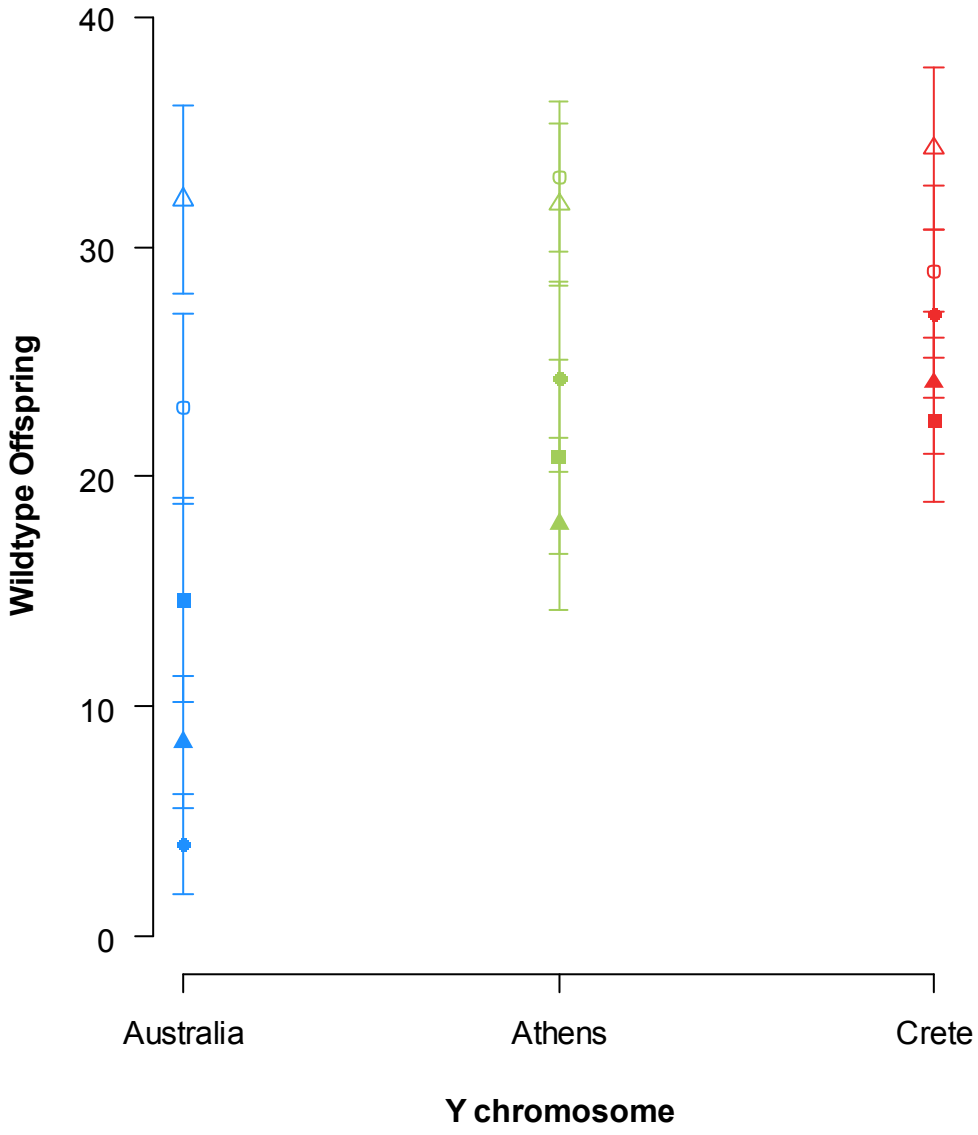
Figure 1. Mean offspring production for males with divergent Y-chromosome, in different genetic backgrounds, when mated to an *ebony* female in the presence of a competing *ebony* male. Each point represents a different genetic background where genetic background 1 = open circle, 2 = open triangle, 3 = filled square, 4 = filled circle, 5 = filled triangle. Error bars represent standard errors around the mean.

Figure 2. Offspring production by males from each different Y chromosome (Australian Population – broad dashed lines; Crete – solid line, Athens – narrow dotted line), ranked from worse (low) to best (high) for each of the 5 genetic backgrounds. Crossing over between these lines indicates an interaction effect between Y chromosome and genetic background on male fertility i.e. the rank order of fertility changes as a function of genetic background.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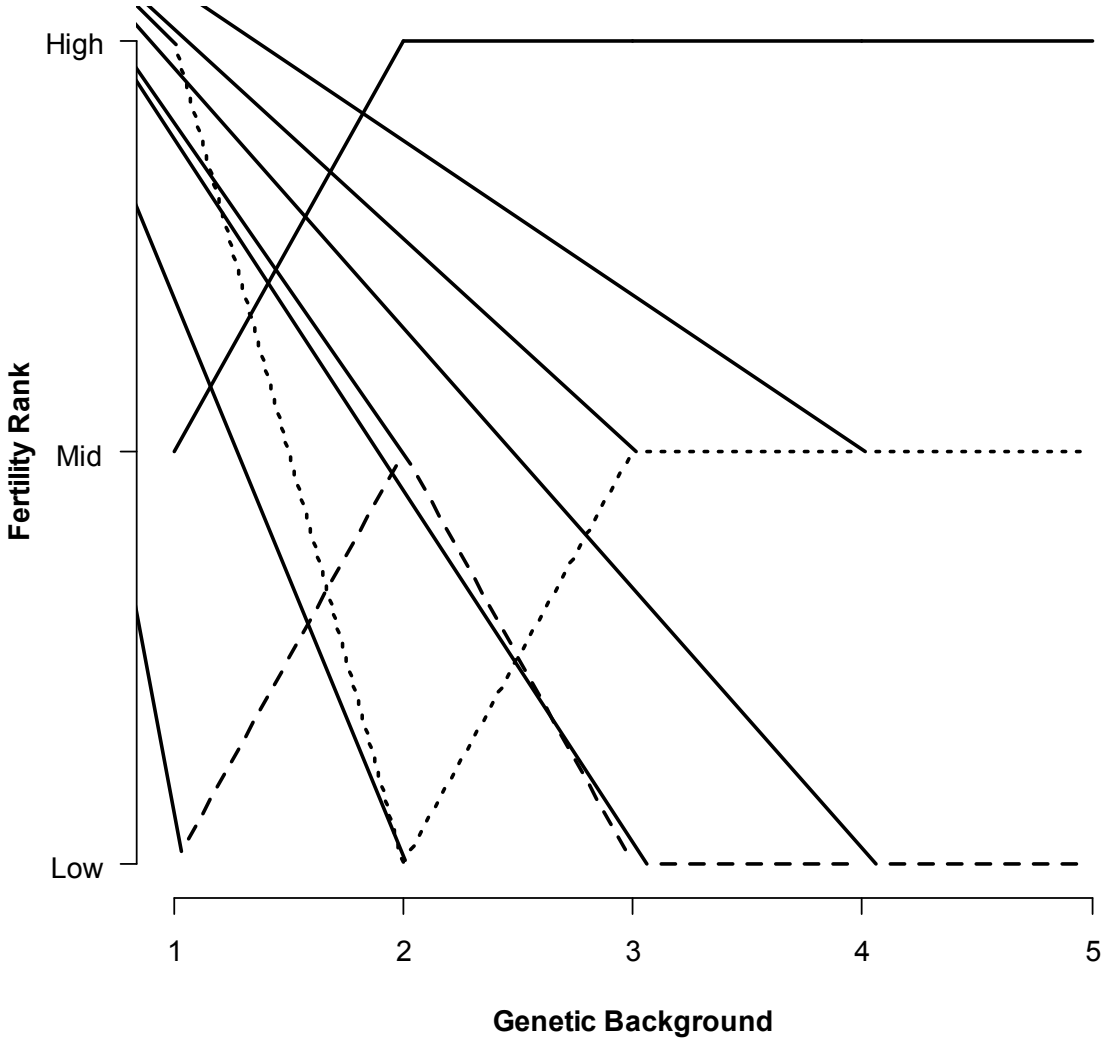

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