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3   **The *Drosophila simulans* Y chromosome interacts with the**

4   **autosomess to influence male fitness.**

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20   **ABSTRACT**

21   The Y chromosome should degenerate because it cannot recombine. However, male limited

22   transmission increases selection efficiency for male benefit alleles on the Y, and therefore Y-

23   chromosomes should contribute significantly to variation in male-fitness. This means that

24   although the *Drosophila* Y chromosome is small and gene-poor, Y-linked genes are vital for

25   male fertility in *D. melanogaster* and the Y chromosome has large male-fitness effects. It is

26   unclear if the same pattern is seen in the closely related *D. simulans*. We backcrossed Y

27   chromosomes from 3 geographic locations into 5 genetic backgrounds and found strong Y

28   and genetic background effects on male fertility. There was a significant Y-background

29   interaction, indicating substantial epistasis between the Y and autosomal genes that

30   impacted on male fertility. This supports accumulating evidence that interactions between

31   the Y chromosome and the autosomes are key determinants of male fitness.

32

33   **KEY-WORDS**

34   *Drosophila simulans, ebony,* fertility, Y chromosome.

35

36   **INTRODUCTION**

37   Over time, the Y chromosome should degenerate because it does not recombine.

38   Recombination breaks linkage disequilibria between unfavourable gene combinations that

39   can slow evolution and facilitate genetic hitchhiking, and also protects against mutation

40   accumulation, which can ultimately destroy the information content of functional genes

41   (Charlesworth & Charlesworth, 2000). In the absence of recombination therefore, the Y

42   chromosome should degenerate as mutations accumulate. In keeping with this idea, both

43   human and fruit fly X chromosomes contain thousands of functional genes, whereas the Y

44   chromosome only contains a few dozen (Carvalho, 2002). *Drosophila melanogaster* males

45   that lack these genes are viable but infertile (Bridges, 1916), suggesting that the Y

46   chromosome only has limited, but important, phenotypic effects. In some species, the Y

47   chromosome has disappeared altogether (Just *et al.*, 2002).

48   Despite these observations, Y chromosome degeneration is not inevitable. Sequence

49   data from humans and rhesus macaques reveals that although Y chromosome decay was

50   initially rapid, degeneration has been negligible for the past ca. 25 million years, leaving a

51   suite of stable ancestral genes on the Y (Hughes *et al.*, 2012). Maintenance of Y-linked genes

52   is largely due to sex-specific purifying selection. The Y chromosome is transmitted from

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

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

80

81 **Materials and Methods**

82 *Animals and Husbandry*

83   All experimental flies, and flies used to create backcrossed animals, were housed at
84   25°C on a 12/12 hour light/dark cycle. We used flies collected from three locations, Athens
85   (Greece), Crete (Greece) and Tuncurry (Australia). Previous work shows that *D.*
86   *melanogaster* (Lemos *et al.*, 2008) and *D. simulans* (Kopp *et al.*, 2006) from different
87   populations have polymorphisms on the Y. Accordingly, we assume that flies from each
88   population have divergent Y chromosomes. From the Athens population, multiple isolines
89   were established from gravid females and maintained for four years by full-sib meeting
90   before the start of this study. We backcrossed each Y chromosome into each of five of these
91   isolines for four generations, which theoretically homogenises ca. 94% of the background so
92   any consistent non-isoline genetic variation that remained should be Y-linked.

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

106

107   *Fitness Assay*

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

113   success of focal males. Fitness was assessed for 360 males (N = 3 replicates from each of 120

114   backcrossed lines). Focal males were housed with their *ebony* mates and *ebony* competitors

115   for 24 hours, before females were removed and placed in egg-laying vials for seven days.

116   After females were removed, all vials were checked daily for eclosion of offspring. Once

117   eclosions began, the vial was left for seven more days to allow all eggs to hatch. Offspring

118   were anaesthetized using CO_2 and the number of wild-type (*wt*) and *ebony* offspring and the

119   *wt/ebony* proportion were all recorded. A schematic of this assay is given in Fig S2 in the SI.

120

121   *Statistics*

122   This experiment was designed such that we could compare the proportion of

123   offspring sired by wild-type males that carried divergent Y chromosomes, expressed in

124   different genetic backgrounds, when in competition with *ebony* males. However, production

125   of *ebony* offspring was uniformly very low across Y backgrounds, with only 47 experimental

126   females producing any *ebony* offspring. Given this, variance in total wild-type offspring

127   production is likely a better reflection of how the Y chromosome affects male fitness.

128   All analyses were conducted using R version 3.3.3. (R core development team.,

129   2013). To determine the effect of Y chromosome and genetic background on male

130   reproductive success we used the function "glmer" in the lme4 package (Bates & Maechler,

131   2009), and the optimisation method "bobyqa". Because mating assays were conducted over

132   three different blocks separated in time, block was included as a random effect. Moreover,

133   three flies were assayed from each of the eight males from each population that were used

134   to backcross into every genetic background and so the identity of the male used to establish

135   each backcross was given as a random effect, nested within Y chromosome. Total offspring

136   production was the response variable, and Y chromosome and genetic background, and the

137   interaction between them, were the explanatory variables. Because these are count data we

138   used a *Poisson* error structure, however, data were over-dispersed and so an individual level

139   random effect was incorporated into the model to account for this. The effects of each

140   explanatory variable were determined by backwards model simplification, where terms

141   were excluded at a level of *P > 0.05.* Results of model comparison are presented. *Post hoc*

142   tests were carried out using the function LSmeans (Lenth, 2014).

143

Results

145   Total offspring production was influenced by the interaction between genetic
146 background and Y chromosome ($\chi^2_{11,19}$ = 30.58, *P* = 0.0002) (Figures 1 and 2). *Post hoc*
147 testing of the interaction (Table S2) showed that when backcrossed into genetic
148 backgrounds 1 and 2, all populations had similar fertility (all P > 0.13). However, in genetic
149 background 3, males from the Australian population were significantly less fertile than
150 males from the Crete population (*P* = 0.024) but not the Athens population (*P* = 0.098). In
151 genetic background 4, Australian flies were less fertile than males from both Greek
152 populations (*P* < 0.0001), although the Greek flies did not differ significantly from one
153 another (*P* = 0.854). The same was true in genetic background 5, however, these effects
154 were slightly weaker (Australia vs. Athens *P* = 0.044 ; Australia vs. Crete *P* = 0.0003). To
155 better understand the effects of genetic background on male fertility, data for each Y
156 chromosome were analysed separately using the same error structure as in the full model
157 (random effects = block, individual and male ID). In this case, there was no significant effect
158 of background on fertility in males from Crete ($\chi^2_{8,4}$ = 2.98, *P* =0.561), but genetic
159 background had a significant effect on fertility in males from Athens ($\chi^2_{8,4}$ = 12.90, *P* = *P* =
160 0.012) and Australia ($\chi^2_{8,4}$ = 45.92, *P* = <0.001).

161

Discussion

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

173 <mark>originating from Australia. Because the isolines that the Y chromosomes were regressed into</mark>
174 <mark>also originate from Greece, this may indicate co-evolution between the Y and the</mark>
175 <mark>autosomes.</mark>

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

190 While genetic background interacted with the Y chromosome to affect male fertility,
191 these effects were relatively weak: in three out of the five genetic backgrounds males from
192 Crete had the greatest fertility and males from the Australian population had the lowest. In
193 the two remaining genetic backgrounds, fitness ranks changed but absolute fertility was
194 statistically indistinguishable among the three Y chromosome populations. The similar
195 fertility of males from Crete and Athens populations may indicate less variation between Y
196 chromosomes in Greek males, relative to Australian flies. Analysis of polymorphisms in
197 different populations of *D. simulans* shows that there is often little geographic structure, or
198 variation, in populations outside of East Africa (Dean & Ballard 2004; Hamblin & Veuille
199 1999). This is because the species spread out of Africa relatively recently, and did so rapidly
200 with considerable genetic draft (Schlenke & Begun, 2004). Despite this, there is considerable
201 polymorphism on the *D. simulans* Y chromosome (Montchamp-Moreau *et al.*, 2001; Kopp *et*
202 *al.*, 2006), and this may reflect environmental variation. For example, 50% of the differences
203 in thermal tolerance between natural *D. melanogaster* populations are due to the Y (David

204   *et al.*, 2005). Given this, males originating from Greek populations may have experienced

205   similar selection on the Y, relative to flies from Australia. Moreover, because the isolines

206   that the Y chromosomes were regressed into also originate from Greece, Y-background

207   matching is likely to be better in Crete and Athens males, than between Australian Ys and

208   Greek autosomess. While this idea requires testing, the similarity in reproductive success of

209   Greek populations and the poor fertility of Australian populations, further highlights the

210   importance of interactions between the Y chromosome and the genetic background that

211   chromosome is expressed in.

212   The overall effect of background on fitness is consistent with earlier work showing

213   that in *D. simulans* male fitness components such as attractiveness (Taylor *et al.*, 2007;

214   Ingleby *et al.*, 2013b) and sperm competitiveness (Hosken *et al.*, 2008) are heritable, and

215   even when elements of male attractiveness show genotype-by-environment interactions,

216   overall attractiveness transfers across environments (Ingleby *et al.*, 2013b). The heritability

217   of such fitness determining traits is an important prerequisite for female choice for indirect

218   genetic benefits. However despite the background effect, fitness was non-transitivity across

219   some Y-background combinations, which is consistent with the disruption of co-adapted

220   gene complexes caused by our backcrossing, and in some ways reflects background epistasis

221   identified for other genetic elements showing sex-limited transmission (e.g. mito-nuclear

222   epistasis (Arnqvist *et al.*, 2010).

223   We did not determine whether the fitness effects of the Y chromosome were

224   mediated by male attractiveness (i.e. the chromosome affects how well males attract

225   females) or by sperm production/function. However, the clear role of *D. melanogaster* Y-

226   genes in spermatogenesis (Lemos *et al.*, 2008), and the disrupted sperm production in *D.*

227   *simulans* males with a heterospecific Y chromosome (Sackton *et al.*, 2011), suggest that

228   reduced sperm function is a likely candidate of the fitness effects we see here. We hoped

229   however, that our competitive mating assay would offer some preliminary insight into this.

230   We know that *D. simulans* females tend to prefer wild-type mates over *ebony* mutants

231   (Sharma *et al.*, 2010). If females had a reduced preference for one genetic background-Y

232   chromosome wild-type combination then this would have been revealed by the increased

233   production of *ebony* progeny. However, the low numbers of *ebony* offspring suggests that

234   females generally preferred wild-type males, which is consistent with previous findings

235 (Sharma *et al.*, 2010). So while sperm performance impacts are the most likely candidate for

236 the Y-background fitness effects we document, further dissecting the mechanism by which

237 the Y chromosome affects male fitness in *D. simulans* is warranted.

238

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323 **Figure 1.** Mean offspring production for males with divergent Y-chromosome, in different

324 genetic backgrounds, when mated to an *ebony* female in the presence of a competing *ebony*

325 male. Each point represents a different genetic background where genetic background 1 =

326 open circle, 2 = open triangle, 3 = filled square, 4 = filled circle, 5 = filled triangle. Error bars

327 represent standard errors around the mean.

328

329 **Figure 2.** Offspring production by males from each different Y chromosome (Australian

330 Population – broad dashed lines; Crete – solid line, Athens – narrow dotted line), ranked

331 from worse (low) to best (high) for each of the 5 genetic backgrounds. Crossing over

332 between these lines indicates an interaction effect between Y chromosome and genetic

333 background on male fertility i.e. the rank order of fertility changes as a function of genetic

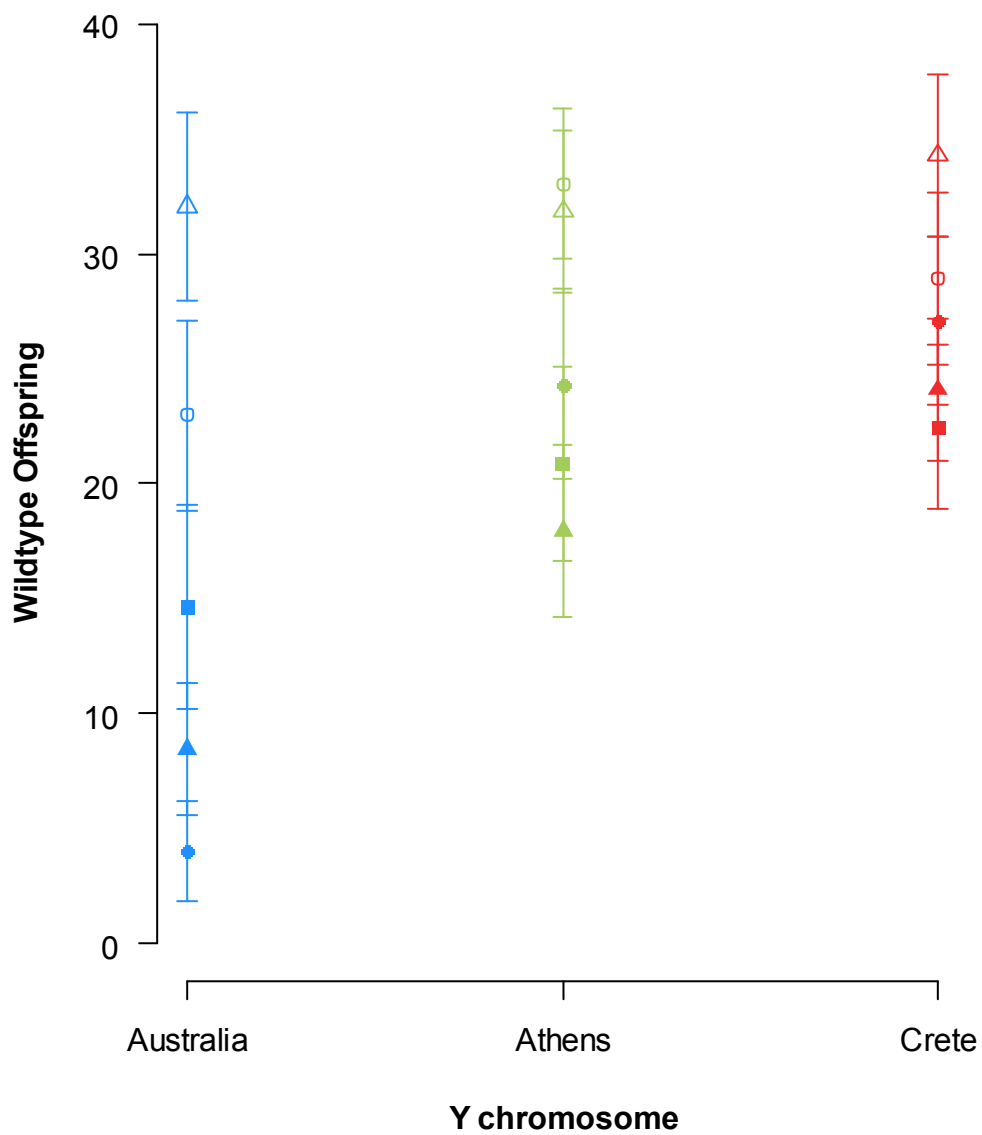334 background.

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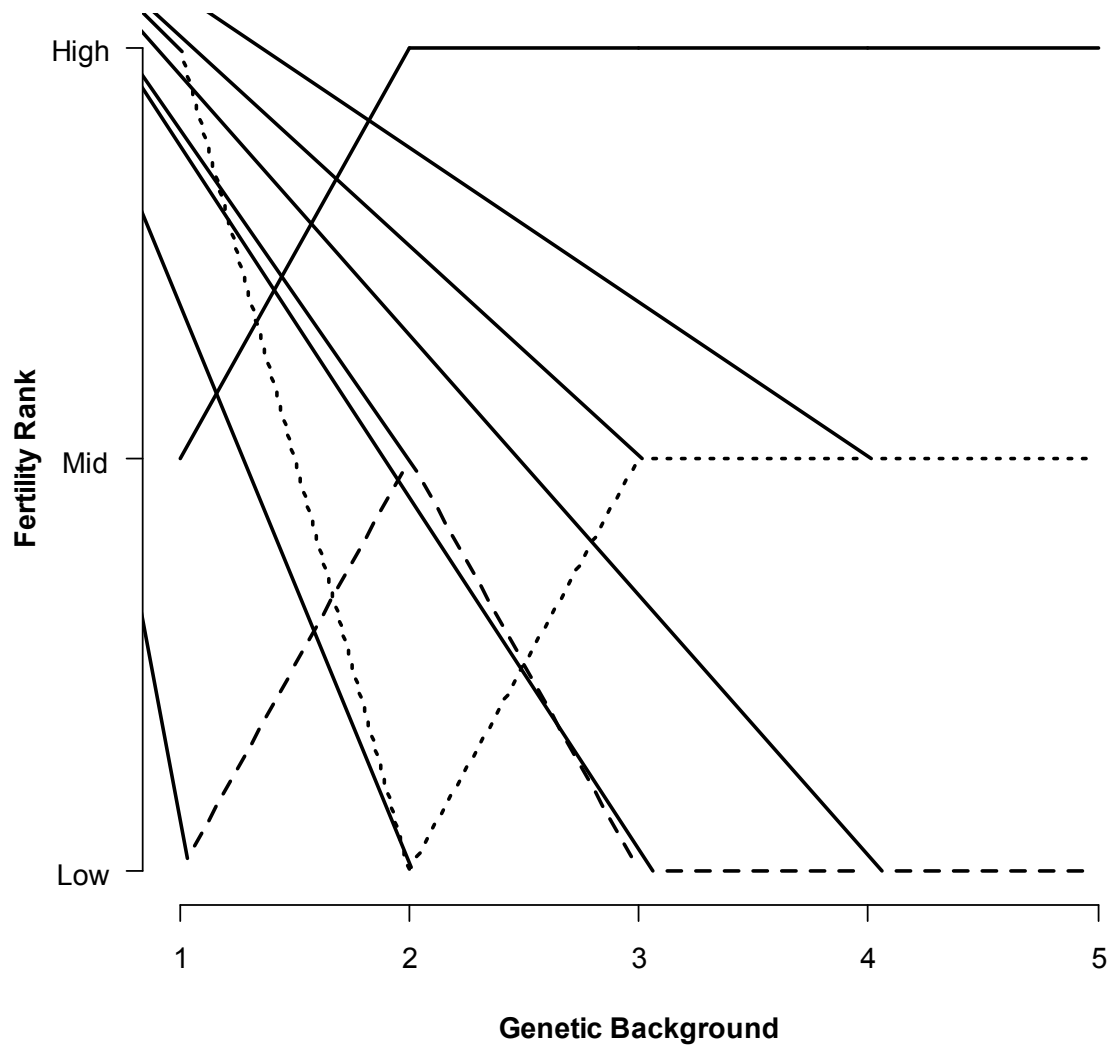
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