

1 **A Rare Exception to Haldane’s Rule: Are X Chromosomes Key to**  
2 **Hybrid Incompatibilities?**

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25 *Abstract*

26 The prevalence of Haldane's rule suggests that sex chromosomes commonly play a key role  
27 in reproductive barriers and speciation. However, the majority of research on Haldane's rule  
28 has been conducted in species with conventional sex determination systems (XY and ZW)  
29 and exceptions to the rule have been understudied. Here we test the role of X-linked  
30 incompatibilities in a rare exception to Haldane's rule for female sterility in field cricket  
31 sister species (*Teleogryllus oceanicus* and *T. commodus*). Both have an XO sex determination  
32 system. Using three generations of crosses, we introgressed X chromosomes from each  
33 species onto different, mixed genomic backgrounds to test predictions about the fertility and  
34 viability of each cross type. We predicted that females with two different species X  
35 chromosomes would suffer reduced fertility and viability compared to females with two  
36 parental X chromosomes. However, we found no strong support for such X-linked  
37 incompatibilities. Our results preclude X-X incompatibilities and instead support an  
38 interchromosomal epistatic basis to hybrid female sterility. We discuss the broader  
39 implications of these findings, principally whether deviations from Haldane's rule might be  
40 more prevalent in species without dimorphic sex chromosomes.

41

42 **Key words:** sex chromosomes, large X effect, dominance, female sterility, *Teleogryllus*

43

44 *Introduction*

45 Haldane's rule is one of very few generalizations in evolutionary biology. It predicts that in  
46 crosses between closely related species, if either sex of the offspring suffers disproportionate  
47 fitness costs, such as reduced fertility or viability, it will be the heterogametic sex (Haldane,  
48 1922). It is a widespread phenomenon, observed across a broad range of taxa, irrespective of  
49 whether males or females are heterogametic (e.g. mammals, birds, reptiles, amphibians, fish,  
50 insects, nematodes and the plant genus *Silene* (Coyne & Orr., 2004; Brothers & Delph, 2010;  
51 Schilthuizen et al., 2011; Delph & Demuth, 2016). The pervasiveness of the rule indicates  
52 that sex chromosomes might commonly play a key role in the establishment of postzygotic  
53 reproductive barriers and by extension, speciation (Presgraves, 2008; Qvarnström & Bailey,  
54 2009; Johnson & Lachance, 2012; Phillips & Edmands, 2012). However, the majority of  
55 research on Haldane's rule has been conducted in species with conventional sex  
56 determination systems (e.g. XY and ZW). Exceptions to the rule, although rare, do occur but  
57 have been understudied (Turelli & Orr, 1995; Laurie, 1997; Malone & Michalak, 2008;  
58 Watson & Demuth, 2012). Atypical sex determination systems and exceptions to Haldane's  
59 rule provide unique opportunities to test the generality of proposed genetic explanations.  
60 Here, we test the importance of X chromosome incompatibilities in a rare deviation from  
61 Haldane's rule for female sterility, which occurs in both cross directions, in an XO sex  
62 determination system.

63

64 The general consensus from published research is that Haldane's rule results from a  
65 composite of evolutionary processes (Coyne & Orr, 2004). This is unsurprising considering  
66 that fertility and viability largely represent distinct functional pathways (Orr, 1993b; Wu &  
67 Davis, 1993). Three of the most consistent genetic theories proposed to explain the ubiquity

68 of Haldane's rule (which are not mutually exclusive) are the dominance theory, faster male  
69 theory and the faster X theory (Coyne & Orr, 2004). The **dominance theory** (Muller, 1942;  
70 Orr, 1993a; Turelli & Orr, 1995) proposes that the heterogametic sex suffers disproportionate  
71 fitness effects because all X (or Z)-linked loci involved in incompatible interactions with  
72 other loci are expressed. In contrast, the homogametic sex will only be affected by dominant  
73 or co-dominant incompatibilities as recessive X-linked incompatibility loci will be masked by  
74 the other X chromosome. Therefore, a key assumption of the dominance theory is that X-  
75 linked incompatibility loci contributing to the manifestation of Haldane's rule should be  
76 predominantly recessive. The dominance theory appears to be the most common underlying  
77 cause of Haldane's rule, as it has the most empirical support and can explain both sterility  
78 and inviability irrespective of which sex is heterogametic (Davies & Pomiankowski, 1995;  
79 Coyne & Orr, 2004). The **faster male theory** (Wu & Davis, 1993) suggests that hybrid  
80 sterility is more prevalent in heterogametic males due to sex differences in the rate of  
81 evolution of sterility loci arising from stronger sexual selection in males. In addition,  
82 spermatogenesis has been suggested to be especially prone to hybrid dysfunction (Wu &  
83 Davis, 1993; Presgraves, 2008; Malone & Michalak, 2008). There is good empirical support  
84 for the faster male theory from introgression experiments in mosquitoes (Presgraves & Orr  
85 1998) and *Drosophila* (Coyne & Orr, 2004; Masly & Presgraves, 2007), and gene expression  
86 studies in *Drosophila* (Michalak & Noor, 2003; Ranz et al. 2004). However, the faster male  
87 theory fails to explain Haldane's rule in female heterogametic taxa, despite the fact that many  
88 groups such as Lepidoptera obey Haldane's rule for sterility (Presgraves, 2002). The **faster X**  
89 **theory** copes with this because it argues that X chromosomes disproportionately accumulate  
90 hybrid incompatibilities, as recessive loci that increase fitness in the heterogametic sex would  
91 accumulate more readily on the X chromosome as they are immediately exposed to selection  
92 (Charlesworth et al. 1987). Such a pattern could partly reflect ascertainment bias from

93 underestimating autosomal effects in backcross designs (Wu & Davis 1993; Hollocher & Wu  
94 1996), although genome-wide introgression studies in *Drosophila* controlling for this  
95 potential bias have identified a higher density of hybrid male sterility factors on the X  
96 chromosome compared to the autosomes (Masly & Presgraves, 2007). The faster X theory  
97 favours the occurrence of Haldane's rule in both male and female heterogametic species but  
98 has the weakest empirical support out of the three main theories. Overall, these prominent  
99 genetic models all predict that X-linked incompatibilities play a central role in Haldane's  
100 rule.

101

102 Unusual sex determination systems and taxa that disobey Haldane's rule provide important  
103 opportunities to test the generality of these genetic models, to identify less well recognized  
104 processes, and to disentangle their relative contributions to Haldane's rule (Malone &  
105 Michalak, 2008; Koevoets & Beukeboom, 2009; Schilthuizen et al., 2011). Traditionally,  
106 species with XO systems have been understudied, and the species pairs which have been  
107 examined have been found to conform to Haldane's rule (Virdee & Hewitt, 1992; Baird &  
108 Yen, 2000; Baird, 2002; Woodruff et al., 2010; Kozłowska et al., 2012). Recently,  
109 *Caenorhabditis* nematodes (XO sex determination system) have emerged as a useful system  
110 for studying postzygotic reproductive barriers. Hybridization studies have revealed that some  
111 of the species pairs exhibit Haldane's rule (Baird, 2002; Dey et al., 2014; Bundus et al.,  
112 2015). However, the diversity of reproductive modes, with many of the *Caenorhabditis*  
113 species pairs examined involving gonochoristic (male/female) (Dey et al. (2014)) and  
114 androdioecious (male/ hermaphrodite) partners may make them difficult to compare to  
115 dioecious taxa. Although the three main genetic models should still apply in XO taxa, the  
116 absence of dimorphic sex chromosomes might reduce the likelihood that Haldane's rule will

117 manifest (Johnson, 2010). An obvious distinction is the absence of Y chromosomes, which  
118 have been found to play an important role in male sterility in some species of *Drosophila* but  
119 not others (Coyne 1985; Turelli & Orr 2000). Additionally, the potential for meiotic drive or  
120 genomic conflict, which have been argued to contribute to Haldane's rule for sterility, might  
121 be reduced in taxa with monomorphic sex chromosomes (Coyne et al., 1991; Frank, 1991;  
122 Tao et al., 2001; Johnson, 2010; McDermott & Noor, 2010; Meiklejohn & Tao, 2010).

123

124 As with most Orthopterans, the two closely related Australian field cricket species  
125 (*Teleogryllus oceanicus* and *T. commodus*) have an XX-XO sex determination system, yet  
126 they provide an intriguing rare exception to Haldane's rule for sterility (Hogan & Fontana,  
127 1973). As males of this species are heterogametic (XO - they inherit a single X chromosome  
128 from their mother) and females are homogametic (XX - they inherit an X from each parent),  
129 Haldane's rule predicts that hybrid males should suffer disproportionate negative fitness  
130 effects. However, early studies reported that reciprocal F1 hybrid females experienced  
131 disproportionate sterility compared to hybrid males (Hogan & Fontana, 1973). Reasons for  
132 this exception to Haldane's rule are not clear. Neither the dominance theory nor faster male  
133 evolution are viable explanations for this case of sex-biased effects. Both *T. oceanicus* and *T.*  
134 *commodus* share the same diploid number of chromosomes ( $2n = 26 + XO, XX$ ), but differ in  
135 the frequency of chiasmata and structural rearrangements, especially on the X (Fontana &  
136 Hogan, 1969; Hogan & Fontana, 1973). As a result of these differences, one possibility is that  
137 X-X interactions during alignment and crossing over might be disrupted, resulting in meiotic  
138 dysfunction and thus hybrid female sterility. Furthermore, the X chromosome accounts for a  
139 large proportion of these species' genomes (ca. 30% in the diploid female: genome size is ca.  
140 4.8gb for a diploid female, 0.8 gb for a single X chromosome; K Klappert; unpublished

141 data/pers comm), increasing the potential for X-linked incompatibilities. Hogan & Fontana  
142 (1973) reported that hybrid females had degenerate ovaries and laid few eggs, suggesting a  
143 combination of incompatibilities targeting both somatic and germ line cells in the female  
144 reproductive system.

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146 In this experiment we tested whether interactions between X chromosomes might explain  
147 female sterility and inviability in *T. commodus* and *T. oceanicus*. We introgressed X  
148 chromosomes from either species onto recombinant autosomal backgrounds over three  
149 generations of crosses, and tested the fertility and viability of the different cross types. We  
150 predicted that females inheriting two different X chromosomes on an averaged autosomal  
151 background (i.e. sharing a similar proportion of autosomal material from both species) would  
152 be less viable and suffer reduced fertility compared to females with two pure parental species  
153 X chromosomes.

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155

## 156 *Methods*

### 157 **Maintenance and Rearing**

158 We established laboratory populations from the offspring of ca. 35 wild caught females from  
159 each of two allopatric Australian populations (*T. commodus* – near Moss Vale, NSW and *T.*  
160 *oceanicus* near Townsville, QLD). Colonies were bred in the lab for at least three generations  
161 before the experiment began. Stock crickets were housed in 16-L plastic boxes of ca. 80  
162 individuals in a 25 °C temperature-controlled room on a 12:12 light:dark cycle. They were

163 provided twice weekly with Burgess Excel “Junior and Dwarf” rabbit food and cotton wool  
164 pads for drinking water and supplied with cardboard egg cartons for shelter.

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### 166 **Cross Design**

167 The experimental design was similar across the three generations of crosses (Fig. 1).

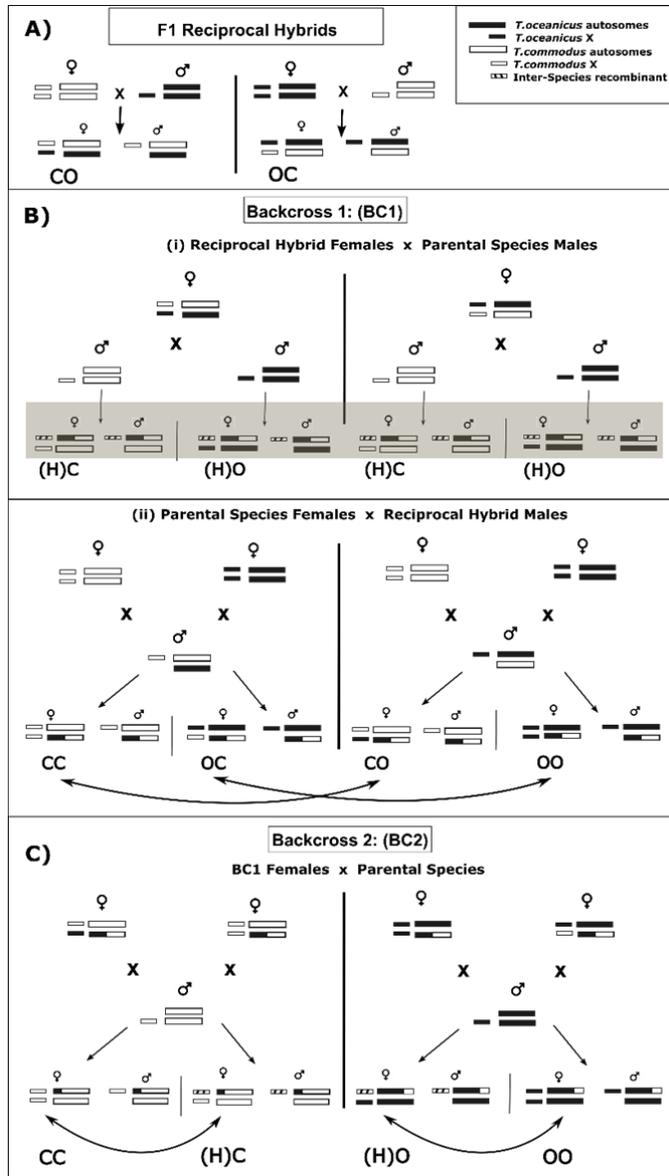
168 Penultimate instar juveniles were separated into single-sex boxes to ensure virginity. For  
169 crosses, virgin adult males and females ca. 10-20 days past eclosion were paired together in  
170 smaller boxes (7 x 5 cm). Approximately 20 pairs per cross type were used (Fig. 2).

171 Females oviposited in moist cotton pads; these egg pads were collected every three to four  
172 days and mating pairs were kept together for a ten day period. Eggs were counted by  
173 examining the egg pads with a magnifying glass. The collected egg pads were monitored  
174 every 3-4 days, to prevent desiccation and to check for hatchlings. Newly hatched offspring  
175 were provisioned with food and cardboard shelter. Egg pads were retained for 2-3 weeks and  
176 the final hatchling count was conducted ca. 3 weeks after the final egg pad was removed. Sex  
177 ratios were estimated once the hatchlings reached the penultimate instar juvenile stage (ca. 2  
178 months) which is within days of adult sexual maturity.

179

180 In the first generation crosses (**F1**), which comprised heterospecific and conspecific pairs, we  
181 investigated whether the species obey Haldane’s rule for inviability and whether  
182 unidirectional or bidirectional incompatibilities exist between them. The cross types were  
183 classified by two letter codes, indicating the female offspring sex chromosome type. The first  
184 letter indicates the maternal species identity and the second the paternal species identity (C =  
185 *T. commodus*; O = *T. oceanicus*) (Fig. 1). In the second generation (**BC1**), reciprocal F1  
186 hybrid females and males were backcrossed to both parental species to test whether the  
187 species obey Haldane’s rule for sterility and if X-linked incompatibilities contribute to

188 offspring inviability. The key comparisons were between backcross types in which female  
189 offspring shared, on average, the same autosomal background (~75:25% species  
190 combination) but differed in their compliment of X chromosomes (Fig. 1B). We predicted  
191 that cross types in which females inherited two different species Xs would produce fewer  
192 hatchlings and a higher proportion of males due to X-linked incompatibilities, compared to  
193 crosses in which females inherited two of the same species Xs. In the third generation (**BC2**),  
194 female offspring from BC1 were backcrossed to their maternal species to test directly  
195 whether X-linked incompatibilities contribute to female sterility. The key comparisons were  
196 again between groups which on average shared the same autosomal background  
197 (~87.5:12.5% species combination expected) but differed in their sex chromosome  
198 compliment; either inheriting two pure species X chromosomes or one pure and the other an  
199 inter-species recombinant X (Fig. 1C).



200

201 **Figure 1.** Schematic of the cross design. Letters below the crosses indicate X chromosome  
 202 compositions of the female offspring (e.g. ‘CO’, ‘CC’, etc.) [A] **F1 Reciprocal Hybrids:**  
 203 Reciprocal inter-species crosses [B] **Backcross 1 (BC1):** Reciprocal F1 hybrid females (i)  
 204 and males (ii) backcrossed to both parental species. Female hybrid crosses are highlighted in  
 205 grey as we did not expect any offspring. Striped X chromosomes represent inter-species X  
 206 recombinants. Arrows indicate the key comparisons, in which females either share or differ in  
 207 their X chromosome compliment. [C] **Backcross 2 (BC2):** BC1 females backcrossed to their  
 208 maternal species. The arrows indicate group comparisons. (H) indicates an inter-species  
 209 recombinant X. Control crosses, of pure species pairs, were also carried out for the F1 and  
 210 BC2 generations but are omitted for clarity.

211 **Statistical analysis**

212 We used binomial tests to assess whether sex ratios differed from the predicted mean of 0.5  
213 within each cross type, and whether the sex ratios differed between the main groups of  
214 interest. Generalized linear models (GLM) were fitted to test whether the X chromosome  
215 compliment of females predicted their fertility, as would be expected if X-linked  
216 incompatibilities make a significant contribution to female fertility. All statistical analyses  
217 were performed in R (Version 3.1.3).

218

219 Our analyses focused on two types of data that reflect different processes: we compared the  
220 proportion of pairs exhibiting any response (a binary measure) among different cross types,  
221 and we also examined differences in the magnitude of any responses (a continuous measure)  
222 among cross types. For example, our response variables included (i) the proportion of pairs  
223 that produced eggs, (ii) the proportion that produced offspring, (iii) egg numbers, (iv)  
224 offspring numbers, and (v) hatchling success rate (offspring/eggs). In each case, the main  
225 predictor of interest was female offspring XX type which was fitted as a fixed effect. Female  
226 weight was fitted as a covariate. The decision to include or remove variables from models  
227 was made based on comparison of the model fit using ANOVAs and chi squared distributions  
228 (or  $F$  test for quasi likelihood models). Models were compared using the Akaike information  
229 criterion (AIC), and models with the lowest AIC were considered the best fit.

230

231 The count data were heavily overdispersed ( $\theta > 20$ ), so we examined if quasi-binomial,  
232 quasi-poisson and negative binomial regression models fitted better using the “MASS”  
233 package (Venables & Ripley, 2002). In some cases the models were still overdispersed, so  
234 zero adjusted models were fitted. These allowed us to account for the excess of zeros and

235 distinguish two different biological processes; whether females laid eggs, and if they did,  
236 how many hatched. There are two types of zero adjusted models which differ in the treatment  
237 of zeros: zero inflated and zero altered (Zuur et al., 2012). Zeros in egg counts can be treated  
238 as arising from a single process, either females laid eggs or did not lay eggs, and therefore we  
239 used zero altered models for egg counts (specifically zero altered negative binomial (ZANB)  
240 models fitted best). The zero altered negative binomial (ZANB) model employs two  
241 components, the positive (i.e. non-zero) data follows a truncated negative binomial  
242 distribution (negbin) while all the zero data is modelled together (binomial). However, an  
243 offspring count of zero could occur when females lay no eggs, or when females laid eggs but  
244 none hatched. Therefore, we used zero inflated models for offspring counts (specifically zero  
245 inflated negative binomial (ZINB) models fitted best). Zero inflated models assume there are  
246 two processes generating the zeros in the data and models these two processes separately, a  
247 poisson GLM for the count data and a binomial GLM for the occurrence of zeros. The  
248 package “pscl” was used to fit zero adjusted models (Zeileis et al. 2008). To test for  
249 differences between the groups of interest, Tukey pairwise comparisons were fitted with the  
250 “multcomp” package (glht function; Hothorn et al., 2008).

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## 259 *Results*

### 260 **F1 generation**

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#### 262 **Asymmetric production of F1 hybrids**

263 Reproductive success was strongly asymmetric. Crosses between *T. commodus* females and  
264 *T. oceanicus* males (CO) had lower fertility compared to the reverse cross (OC). (Fig. 2A,  
265 Table 1). Nearly all females laid eggs, but the number of eggs was markedly lower for CO  
266 crosses (mean  $\pm$  SE: CO,  $84 \pm 27.75$ ) compared to the reciprocal cross (OC,  $239.56 \pm 34.28$ )  
267 (Negative binomial GLM:  $Z_{3,80} = -3.226$ ,  $P = 0.007$ ; Table 1). There was an excess of zeros  
268 among CO pairs, as only 41% of CO pairs produced offspring compared to 70% for OC  
269 crosses (ZINB binomial:  $Z_{11,73} = 2.426$ ,  $P = 0.053$ ). Females from the CO group also produced  
270 fewer offspring (mean  $\pm$  SE,  $55.73 \pm 21.8$ ) than the OC cross ( $155.04 \pm 29.77$ ), although this  
271 was non-significant (Table 1).

272

273 The asymmetry in reproductive success may be due to maternal effects or sperm-egg  
274 incompatibilities. If X-cytoplasmic interactions contribute to the asymmetry in F1 production,  
275 we predicted hybrid females would suffer disproportionate inviability compared to males as  
276 they inherit an X on a foreign species' cytoplasmic background. However, the absence of  
277 sex-specific inviability indicates this is not the case (Fig. 2Aiii). In line with *T. commodus*  
278 females performing poorly when crossed to a heterospecific, they also had reduced fertility  
279 when paired with a conspecific partner in the F1 generation (Fig. 2Ai-ii; Table S1). They  
280 produced both fewer eggs (Parental CC vs. Parental OO: negative binomial GLM,  $Z_{3,80} =$   
281  $2.374$ ,  $P = 0.082$ ) and fewer offspring (ZINB negbin,  $Z_{3,80} = -3.325$ ,  $P < 0.001$ ). However,  
282 this species difference was not observed in the BC2 generation (Fig. 2Ci - ii, Table S1).

283 **Table 1.** Results from generalized linear models examining egg number, offspring number  
 284 and hatching success in **F1** crosses. Main predictors fitted were the X chromosome  
 285 composition of female offspring (“Female XX”) and female weight. The Zero inflated  
 286 negative binomial model (ZINB) employs two components, a negative binomial count model  
 287 (negbin) and the logit model (binomial) for predicting excess zeros. Significance for fixed  
 288 effects examined using likelihood ratio tests ( $X^2$ ), by comparing a null model with only the  
 289 intercept fitted to a model with the predictor fitted for either the negative binomial (negbin)  
 290 or binomial component. Main comparisons based on Tukey pairwise contrasts. *P* values in  
 291 bold indicate statistical significance at  $\alpha < 0.05$ .

Response Variable	Model & Predictors	Model Components	Pr(>  $X^2$  )	Main comparisons	Df	Estimate	Std. Error	Z value	Pr(> Z )
<b>Eggs</b>	<b>Negative binomial</b>								
	Female XX	-	<b>0.002</b>	<b>OC - CO</b>	3,80	-1.014	0.314	-3.226	<b>0.007</b>
	Female weight	-	<b>0.029</b>		1,79				
<b>Offspring</b>	<b>ZINB</b>								
	Female XX	negbin	<b>0.022</b>	<b>OC - CO</b>	11,73	-0.482	0.354	-1.362	0.455
		Binomial	<b>&lt;0.001</b>	<b>OC - CO</b>	11,73	1.441	0.594	2.426	0.053
<b>Hatching</b>	<b>Quasi-binomial</b>								
	Female XX	-	0.585	<b>CO - OC</b>	3,82	-1.021	0.438	-2.333	0.090

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294 **No evidence of Haldane’s rule for inviability**

295 All four F1 cross types, two intra-specific (parental crosses) and two inter-specific crosses,

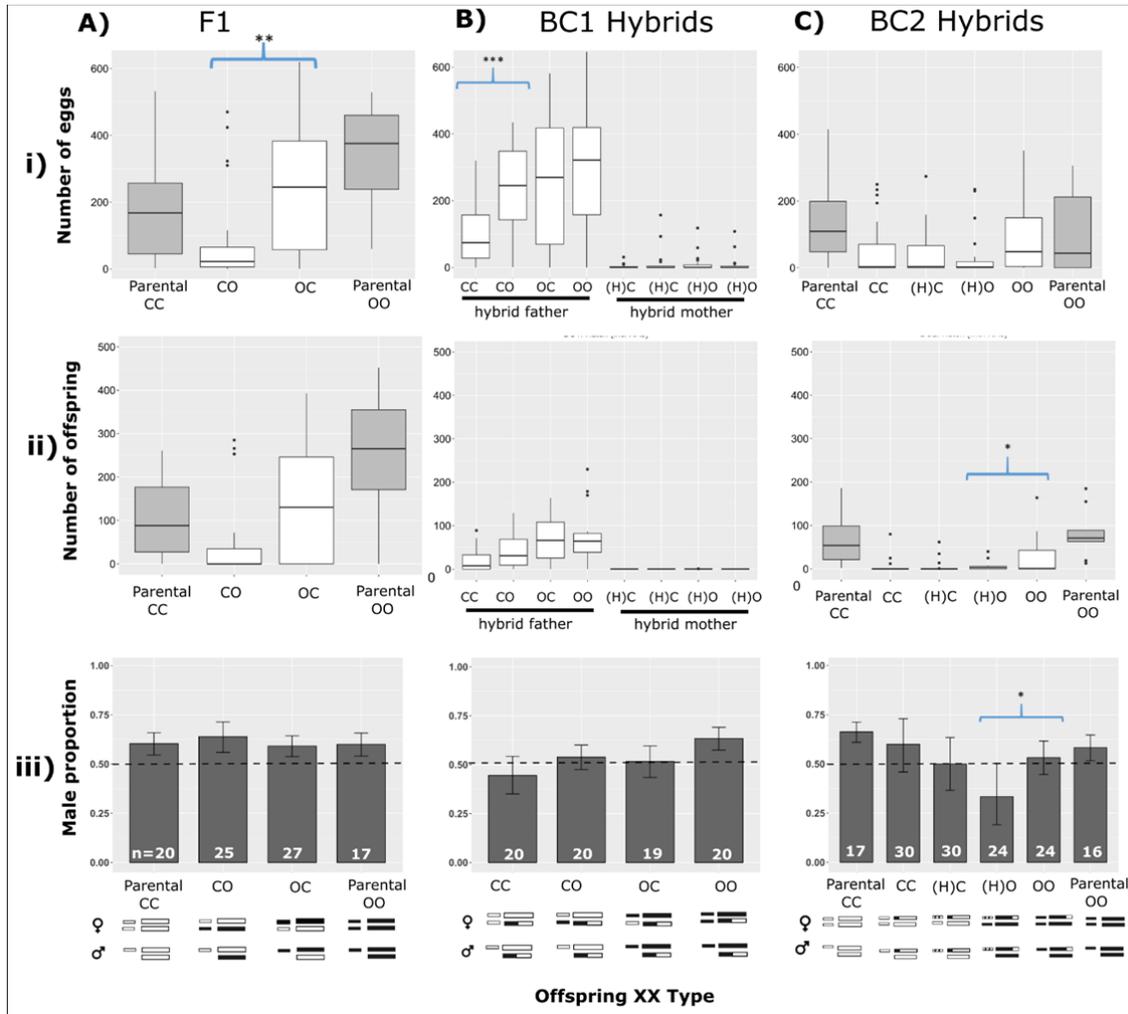
296 had a higher proportion of males than the expected 0.5 sex ratio (Binomial exact test: *P*

297 <0.001) (Fig. 2Aiii). Importantly, there was no differential viability between males and

298 females in the hybrid crosses compared to the parental crosses (Parental CC vs. CO:  $X^2 =$

299 0.418, df = 1, *P* = 0.518; Parental OO vs. OC:  $X^2 = 0.02$ , df = 1, *P* = 0.888). Therefore, there

300 is no evidence for Haldane’s rule for inviability within these species.



302 **Figure 2.** Three generations of crosses: **A)** F1 **B)** BC1 and **C)** BC2, showing for each cross  
 303 type: **i)** numbers of eggs, **ii)** numbers of offspring and **iii)** proportion of female offspring (n =  
 304 number of pairs per cross type. n = 20 for backcrosses with hybrid females). The X axis is  
 305 labelled based on the female offspring XX type, the first letter reflects the maternal species X  
 306 and the second letter the paternal species X (C=*T. commodus*; O=*T. oceanicus*). In BC1 and  
 307 BC2, (H) indicates potential inter-species recombination on the X. Significant comparisons  
 308 are highlighted by brackets ( $P > 0.01 = *$ ,  $P > 0.001 = **$ ,  $P < 0.001 = ***$ ). In the last row, error  
 309 bars indicate 95% confidence intervals (binomial test) for the observed proportions, and  
 310 dashed lines indicate a 50:50 sex ratio expectation.  
 311

312 **BC1 Generation**

313 **Reciprocal exceptions to Haldane's rule for sterility**

314 *T. oceanicus* and *T. commodus* provide a reciprocal exception to Haldane's rule as nearly all  
315 hybrid females were sterile in both directions of the cross (only a single BC1 offspring was  
316 produced from 80 backcrosses), while all four hybrid male backcross types were fertile (Fig.  
317 2Bii). We predicted that hybrid male backcrosses which produced female offspring with  
318 heterospecific X chromosomes would exhibit reduced fertility (BC1: OO vs. OC or CC vs.  
319 CO) due to X-X interactions. We found no support for this hypothesis in either the proportion  
320 of pairs exhibiting a response or in the strength of response (i.e. number of eggs or offspring  
321 per pair) (Table 2). Contrary to the prediction that heterospecific X-X interactions would  
322 reduce fertility, CO pairs (*T. commodus* females paired with male hybrids carrying a *T.*  
323 *oceanicus* X chromosome) produced more eggs (mean  $\pm$  SE: CO  $242.4 \pm 27.36$ ) than the  
324 comparison CC pairs (*T. commodus* females paired with male hybrids carrying a *T.*  
325 *commodus* X chromosome) (mean  $\pm$  SE:  $103.25 \pm 22.29$ ) (ZANB negbin:  $Z_{9,70} = 3.72$ ,  $P$   
326  $<0.001$ ). However, the number of offspring was not significantly different between these two  
327 groups (mean  $\pm$  SE: CO  $40.55 \pm 8.37$  vs. CC  $22.5 \pm 6.89$ ) (ZINB negbin:  $Z_{9,70} = -0.861$ ,  $P =$   
328  $0.389$ ). In the other group comparison, there was no difference between OC and OO pairs in  
329 either the number of eggs or offspring (Table 2). The hatching success rate also did not differ  
330 amongst the groups of interest (Table 2). Overall, we detected no support for X-X  
331 interactions affecting fertility.

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335 **Table 2.** Results from Generalized Linear Models examining egg number, offspring number  
 336 and hatching success in **BC1** crosses. Main predictors fitted were female offspring XX type  
 337 (“Female XX”) (female weight was not significant). Significance for fixed effects was  
 338 examined using likelihood ratio tests ( $\chi^2$ ), by comparing a null model with only the intercept  
 339 fitted to a model with the predictor fitted for either the negative binomial (negbin) or  
 340 binomial component. Main comparisons based on Tukey pairwise contrasts. *P* values in bold  
 341 indicate statistical significance at  $\alpha < 0.05$ .

Response Variable	Model & Predictors	Model Component	Pr(> $\chi^2$ )	Main comparisons	Df	Estimate	Std. Error	Z value	Pr(> Z )
<b>Eggs</b>	<b>ZANB</b>								
	Female XX	negbin	<b>&lt; 0.0001</b>	<b>OO - OC</b>	9,70	0.039	0.228	0.172	0.998
		Binomial	0.47	<b>OO - OC</b>	9,70	0.876	0.934	0.937	0.733
	Female XX	negbin	-	<b>CC - CO</b>	9,70	0.799	0.215	3.721	<b>&lt;0.001</b>
		Binomial	-	<b>CC - CO</b>	9,70	0.747	1.268	0.589	0.915
<b>Offspring</b>	<b>ZINB</b>								
	Female XX	negbin	<b>0.015</b>	<b>OO - OC</b>	9,70	0.085	0.293	0.291	0.989
		Binomial	0.197	<b>OO - OC</b>	9,70	-0.326	0.717	-0.455	0.959
	Female XX	negbin	-	<b>CC - CO</b>	9,70	0.261	0.303	0.861	0.783
		Binomial	-	<b>CC - CO</b>	9,70	-1.545	0.782	-1.975	0.155
<b>Hatching Success</b>	<b>Quasi-binomial</b>								
	Female XX	-	0.189	<b>OO - OC</b>	3,67	0.087	0.323	0.268	0.993
				<b>CC - CO</b>		-0.166	0.354	-0.469	0.966

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### 345 **No X effect on viability**

346 Under a scenario in which X-linked incompatibilities disproportionately affect viability, we  
 347 predicted an excess of males due to female inviability in groups in which females inherited  
 348 two different species Xs. Again, contrary to this prediction, there was a lower proportion of  
 349 females in the OO group than the expected mean of 0.5 (Binomial exact test,  $P < 0.001$ ), and

350 this was significantly lower than the comparison group OC (OO vs. OC groups:  $X^2 = 5.358$ ,  
351  $df=1$ ,  $P = 0.021$ ) (Fig. 2Biii). Comparing the CC vs. CO cross types, there was no sex ratio  
352 bias ( $X^2 = 2.326$ ,  $df=1$ ,  $P = 0.127$ ). Overall, females that inherited two different species X  
353 chromosomes did not exhibit reduced viability.

354

## 355 **BC2 Generation**

### 356 **X-X interactions do not cause female sterility**

357 We predicted that females with a mixed species compliment of X chromosomes would suffer  
358 reduced fertility compared to females with conspecific X chromosomes. There was no  
359 difference between the CC vs. (H)C groups in either the number of eggs produced (ZANB  
360 negbin:  $Z_{13,128} = -0.418$ ,  $P = 0.992$ ) or the number of offspring (ZINB negbin:  $Z_{13,128} = 0.417$ ,  
361  $P = 0.991$ ; Fig. 2Cii, Table S1). In line with our prediction, there was a marginal difference in  
362 fertility between OO vs. (H)O groups. OO females appeared to produce more eggs (mean  $\pm$   
363 SE: OO,  $92.5 \pm 22.9$  vs. (H)O,  $33.13 \pm 14.2$ ), although this was not significant (ZANB  
364 negbin:  $Z_{13,128} = -1.593$ ,  $P = 0.434$ , Table 3). However, OO pairs produced more offspring  
365 than the corresponding (H)O group (mean  $\pm$  SE: OO,  $28.68 \pm 10$  vs. (H)O,  $6.92 \pm 3.34$ )  
366 (ZINB negbin:  $Z_{13,128} = 2.957$ ,  $P = 0.017$ ; Table 3), which was consistent with our prediction  
367 that females with a mixed species compliment of X chromosomes will suffer reduced  
368 fertility. Although the proportion of parental OO pairs (control crosses) that produced eggs  
369 was surprisingly low (0.56) (Table S1), all parental pairs that produced eggs resulted in  
370 hatchlings, compared to a range of only 19% - 63% for the backcrosses.

371

372

373

374 **Table 3.** Results from Generalized Linear Models examining egg number, offspring number  
375 and hatching success in **BC2** crosses. Main predictors fitted were female offspring XX type  
376 (“Female XX”) and female weight. Significance for fixed effects examined using likelihood  
377 ratio tests ( $X^2$ ), by comparing a null model with only the intercept fitted to a model with the  
378 predictor fitted for either the negative binomial (negbin) or binomial component. Main  
379 comparisons based on Tukey pairwise contrasts. *P* values in bold indicate statistical  
380 significance at  $\alpha < 0.05$ .

Response Variable	Model & Predictors	Model Component	Pr(>  $X^2$  )	Main comparisons	Df	Estimate	Std. Error	Z value	Pr(> Z )
<b>Eggs</b>	<b>ZANB</b>								
	Female XX	negbin	0.125	<b>CC vs. (H)C</b>	13,128	0.168	0.402	0.418	0.992
		Binomial	<b>0.011</b>	<b>CC vs. (H)C</b>	13,128	-0.135	0.519	-0.259	0.999
		negbin		<b>OO vs. (H)O</b>	13,128	0.662	0.416	1.593	0.434
		Binomial		<b>OO vs. (H)O</b>	13,128	1.168	0.648	1.801	0.301
<b>Offspring</b>	<b>ZINB</b>								
	Female XX	negbin	<b>0.011</b>	<b>CC vs. (H)C</b>	13,128	0.344	0.824	0.417	0.991
		Binomial	<b>&lt;0.0001</b>	<b>CC vs. (H)C</b>	13,128	0.342	0.817	0.418	0.991
		negbin		<b>OO vs. (H)O</b>	13,128	1.479	0.500	2.957	<b>0.017</b>
		Binomial		<b>OO vs. (H)O</b>	13,128	-0.579	0.629	-0.920	0.856
<b>Hatching Success</b>	<b>Quasi-Binomial</b>								
	Female XX	-	<b>&lt; 0.001</b>	<b>CC vs. (H)C</b>	5, 85	0.253	0.737	0.344	0.999
	Female weight	-	<b>0.004</b>		1, 84				
	Female XX	-		<b>OO vs. (H)O</b>	5, 85	0.125	0.553	0.225	1.000

381

382

### 383 **Limited role for X chromosomes in inviability**

384 Sex ratio data showed a higher proportion of females in the (H)O group compared to the OO  
385 group (Binomial test;  $X^2=4.059$ ,  $df=1$ ,  $P=0.044$ ) indicating that (H)O males may suffer  
386 disproportionate inviability (Fig. 2Ciii). In this cross, males potentially inherit an interspecies  
387 recombinant X, which is hemizygous and could therefore expose them to an elevated  
388 likelihood of epistatic incompatibilities involving recessive X substitutions (e.g. X-autosomal  
389 incompatibilities). Comparisons between CC & (H)C revealed no significant sex ratio  
390 difference (Binomial test;  $X^2=0.772$ ,  $df=1$ ,  $P=0.38$ ). Both parental species crosses showed a

391 reduction of females from the expected mean of 0.5, particularly in the parental CC crosses  
392 (Fig. 2Ciii).

393

394

### 395 *Discussion*

396

397 Two important empirical findings in evolutionary biology, Haldane's rule and the large X  
398 effect, are so consistent that they have been thought to be nearly universal (Coyne & Orr,  
399 1989; Coyne & Orr, 2004). Both suggest that X chromosomes play a key role in the  
400 establishment of post-zygotic barriers between species (Coyne & Orr, 1989; Masly &  
401 Presgraves, 2007; Presgraves, 2010; Johnson & Lachance, 2012; Phillips & Edmands, 2012).  
402 However, most research on the genetic basis of reproductive isolation has focused on male  
403 sterility and on male heterogametic species, as opposed to female fertility (though see Orr &  
404 Coyne, 1989; Davis et al., 1994; Hollocher & Wu, 1996; Watson & Demuth, 2012; Suzuki &  
405 Nachman, 2015). Rare cases in which homogametic females suffer disproportionate effects of  
406 hybridization provide an important opportunity to investigate the genetic basis of female  
407 sterility and processes that may counter Haldane's rule. Crosses between *T. oceanicus* and *T.*  
408 *commodus* provide one such remarkably rare exception to Haldane's rule – female hybrids  
409 were almost uniformly sterile in our experiment, and out of 80 backcrosses with reciprocal  
410 hybrid females only a single offspring hatched. A considerable number of hybrid females,  
411 derived from numerous different cross types, produced eggs, indicating that not all ovaries  
412 are degenerate (Fig. 2Bi). This observation suggests a complex genetic basis for hybrid  
413 female sterility, in which certain hybrid genic combinations may occasionally result in fertile  
414 hybrid females in natural populations (Virdee & Hewitt, 1994).

415

416 *Asymmetrical reproductive isolation*

417 Asymmetrical genetic incompatibilities are a common observation among animal and plant  
418 hybridizations (Turelli & Moyle, 2007). They are believed to principally arise from negative  
419 epistasis between autosomal or sex-linked loci and uniparentally inherited maternal factors  
420 (e.g. mitochondrial DNA, cytoplasmic background) (Turelli & Orr, 2000; Turelli & Moyle,  
421 2007; but see Bundus et al., 2015). We found a clear asymmetry in genetic compatibility. *T.*  
422 *commodus* females mated to *T. oceanicus* males produced far fewer eggs and offspring than  
423 the reciprocal cross (Fig. 2A). In other words, hybridisation was more successful when the  
424 mother was *T. oceanicus*. This unidirectional incompatibility appears to manifest at a very  
425 early stage, as egg laying was disrupted.

426 Maternal effects (or cyto-nuclear incompatibilities) may lead to exceptions to  
427 Haldane's rule for inviability if incompatibility loci are sex linked, as hybrid females inherit  
428 one of their X chromosomes on a different species' cytoplasmic background. However, we  
429 did not detect any sex-specific inviability in comparisons between the F1 hybrid and parental  
430 species crosses (Fig. 2Aiii). Instead, sperm-egg incompatibilities or autosomal-cytoplasmic  
431 interactions, rather than X-cytoplasmic interactions, might be responsible for the  
432 asymmetrical reduction in fertility. If species differ in the degree of sperm competitiveness,  
433 asymmetric gametic isolation may occur (Martín-Coello et al., 2009). Females of both  
434 *Teleogryllus* species mate multiply in natural populations, and paternity is highly skewed,  
435 more so in *T. oceanicus* than *T. commodus* (Simmons & Beveridge, 2010). Heterospecific  
436 crosses with *T. oceanicus* males may therefore be predicted to have higher mating success  
437 compared to the reciprocal cross. However, this was not the case; heterospecific crosses with  
438 *T. oceanicus* males had reduced fertility compared to the reverse cross. Overall, Haldane's  
439 rule does not manifest for any inviability patterns in crosses between these species.

440

441 Contrary to a previous report, which found a 1:1 sex ratio for pure-species crosses (Hogan &  
442 Fontana, 1973), we found a male biased sex ratio for both intraspecific and interspecific  
443 crosses. This discrepancy between the studies could have arisen due to population  
444 differences. The previous cytogenetic (Fontana & Hogan, 1969) and hybridization work  
445 (Hogan & Fontana, 1973) was conducted on laboratory populations of *T. oceanicus* collected  
446 from Ayr, northern Queensland (ca. 90km from where we sampled our study population in  
447 Townsville), and *T. commodus* from Melbourne, southern Victoria (ca. 750km from where  
448 we sampled our study population in Moss Vale, New South Wales). In general, populations  
449 within a species can show a high degree of variation for genetic incompatibilities (Cutter,  
450 2012) with other species, including X-chromosome inversions, endosymbiont strains or  
451 infection rates (e.g. *Wolbachia* (Telschow et al. 2005)) that alter sex ratios. However, the  
452 latter mechanisms usually result in female bias. In addition, differences in environmental  
453 conditions, such as temperature, or differential fertilization of nullo-X sperm may alter sex  
454 ratios (Wade et al., 1999; Bundus et al., 2015).

455

#### 456 *X-linked incompatibilities*

457 What is the genetic cause of the deviation from Haldane's rule for sterility in Australian  
458 *Teleogryllus*, and can it inform us more broadly about hybrid incompatibilities? Maternal  
459 effects (and cyto-nuclear incompatibilities) have previously been implicated in deviations  
460 from Haldane's rule for inviability (Sawamura et al., 1993; Sawamura, 1996; Abe et al.,  
461 2005) but not sterility (Orr & Irving, 2001). Early developmental stages are predicted to be  
462 especially sensitive to maternal effects (Mousseau, 1991), however little is known about  
463 maternal effects on adult reproductive traits. Disruption to early developmental stages could  
464 influence later reproductive output. However, we do not believe this explains hybrid female  
465 sterility in our study system, because maternal effects often exhibit asymmetrical effects and

466 are not necessarily expected to influence both directions of the cross equally (Turelli &  
467 Moyle, 2007). Also, if maternal effects played a role in female sterility, we would predict  
468 backcrosses with hybrid males to be more compatible with their maternal species, which was  
469 not the case.

470

471 Laurie (1997) highlighted two factors that might promote exceptions to Haldane's rule with  
472 respect to female hybrid sterility, and which affect both directions of a cross equally: X-X  
473 incompatibilities and dominant X-autosomal interactions. Both depend on X interactions, but  
474 our results yielded negligible support for the former. We hypothesized that reciprocal hybrid  
475 female sterility had a shared basis, namely due to chromosomal rather than genic interactions,  
476 in particular X-X interactions leading to meiotic dysfunction. Only one of our comparisons  
477 was consistent with X-linked incompatibilities reducing female fertility; a higher number of  
478 offspring produced from OO vs. (H)O groups in BC2 (Fig. 2Cii, Table 3). However, there  
479 was no detectable difference between the CC vs. (H)C groups in BC2 (Fig. 2Cii, Table 3).  
480 Furthermore, among the BC1 crosses the CO pairs produced more eggs on average than CC  
481 pairs (Fig. 2B, Table 2). This pattern also refutes our prediction. If X-X incompatibilities  
482 were primarily responsible for the sterility of F1 hybrid females, we expected to observe a  
483 clear reduction in fertility for crosses in which females inherited two different X  
484 chromosomes. Instead, our results are more consistent with an epistatic origin of the  
485 incompatibilities due to Dobzhansky-Muller incompatibilities (Dobzhansky, 1937; Muller,  
486 1942; Maheshwari & Barbash, 2011). This could be autosomal-autosomal or could still  
487 involve the X chromosome if these were dominant X-A interactions. We cannot  
488 unambiguously distinguish these, but the fact that there are large differences between similar  
489 genotypes that differ in the source of the X and A chromosomes, rather than the proportion of

490 interspecies material (e.g. CC versus OO in BC1, Fig. 2C), suggests that specific X-A  
491 interactions may contribute to lower female fertility.  
492  
493 The lack of a large X effect on female sterility might be explained by the fact that theory  
494 predicts a disproportionate accumulation of male but not female fertility loci on the X  
495 chromosome in male heterogametic species (Charlesworth et al. 1987). The loci underlying  
496 female fertility may be just as likely to accumulate on the autosomes as on the X (Masly &  
497 Presgraves, 2007), so X-linked loci that affect male fertility would need to have pleiotropic  
498 effects in hybrid females to produce a large X effect on female fertility (Coyne & Orr, 1989;  
499 Presgraves, 2008). Introgression studies examining the large X effect in *Drosophila* have  
500 provided mixed results; some support the view that male and female sterility loci are  
501 qualitatively different (Wu & Davis, 1993; Coyne & Orr, 2004), while others have detected X  
502 effects on both male and female sterility (Orr, 1987; Orr & Coyne, 1989). In this study we did  
503 not test the effect of X introgression on the fertility of both sexes, but the absence of evidence  
504 for a large X effect in females supports the view that X chromosomes do not play a  
505 pronounced role in female sterility.

506

#### 507 *XO sex determination system*

508 As exceptions to Haldane's rule are extremely rare, particularly in both directions of a cross,  
509 could deviations for female sterility be caused by a peculiarity of XO sex determination  
510 systems? While the main genetic models underlying Haldane's rule should apply to XO  
511 systems, the absence of dimorphic sex chromosomes might relax the operation of some less  
512 well recognized processes that could contribute to Haldane's rule (e.g. meiotic drive, Y-  
513 incompatibilities). Previous hybridization studies in XO taxa suggest they generally obey  
514 Haldane's rule (Ohmachi & Masaki, 1964; Mantovani & Scali, 1992; Virdee & Hewitt,

1992; Baird & Yen, 2000; Baird, 2002; Woodruff et al., 2010; Kozłowska et al., 2012).  
However, only two previous reciprocal exceptions to Haldane's rule have been described, one  
for inviability in an XO species (Spence, 1990) and the other for male sterility in a female  
heterogametic species (Malone & Michalak, 2008). The later exception can be explained  
under existing theory and has been experimentally shown to be due to faster male evolution  
(Malone & Michalak, 2008), which would not explain the exception to Haldane's rule in our  
study system. The former case occurs in the Heteropteran pondskater *Limnoporous spp* which  
has an XO sex determination system (Spence 1990). Spence (1990) found that in crosses  
between *Limnoporus notabilis* and *L. dissortis*, F1 hybrid females suffer disproportionate  
inviability compared to male hybrids. Applying a backcross design similar to that used in our  
study, Spence (1990) tested whether the presence of two different species X chromosomes  
contributed to hybrid inviability. However, his results differed from ours, because he detected  
a large X effect on female inviability. Considering that XO species represent a relatively  
small fraction of the species examined in hybridization studies, yet exhibit two remarkably  
rare exceptions to Haldane's rule (*Limnoporous spp* – female inviability; *Teleogryllus spp* –  
female sterility), future research would benefit from investigating why Haldane's rule might  
be less prevalent in systems which lack dimorphic sex chromosomes.

532

### 533 *Conclusions*

534 *T. commodus* and *T. oceanicus* provide a rare exception to Haldane's rule for sterility, but not  
535 viability. Unexpectedly, we found negligible support for X-linked incompatibilities  
536 contributing to hybrid female sterility. This lack of support is surprising given the size of the  
537 X chromosomes in these species; when in single copy in males, the X chromosome represents  
538 approximately 20% of the diploid male genome, and when in two copies in females it  
539 represents approximately 30% of the diploid female genome (K Klappert; unpublished

540 data/pers comm). Even though no large X effect was detected in our study it does not rule out  
541 the potential for X-linked incompatibilities. However, the low fitness seen in backcross  
542 offspring, irrespective of their XX identity, suggests that partially dominant autosomal loci  
543 may supersede X-linked interactions in disrupting female fertility. Our results also revealed a  
544 clear asymmetry in fertility in reciprocal F1 crosses, with greater viability when hybrids were  
545 derived from *T. oceanicus* mothers, indicating that maternal effects (e.g. autosomal-  
546 cytoplasmic interactions) or sperm-egg incompatibilities might play an important role in  
547 reproductive barriers and asymmetric introgression between these species. Whether this rare  
548 exception to Haldane's rule represents a more general pattern of deviation from the rule in  
549 systems without dimorphic sex chromosomes (e.g. XO systems, haplodiploid) remains to be  
550 determined.

551

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560

561 **Data archive location:** Data will be archived on Dryad when the manuscript is accepted.

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