

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

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19 **Running title:** A Rare Exception to Haldane’s Rule: Are X Chromosomes Key to Hybrid
20 Incompatibilities?

21

22 **Word count:** 5,525 (excluding references, tables and figures)

23

24 **Data archive location:** Data will be archived on Dryad if the manuscript is accepted.

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.

145

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.

165

166 **Cross Design**

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

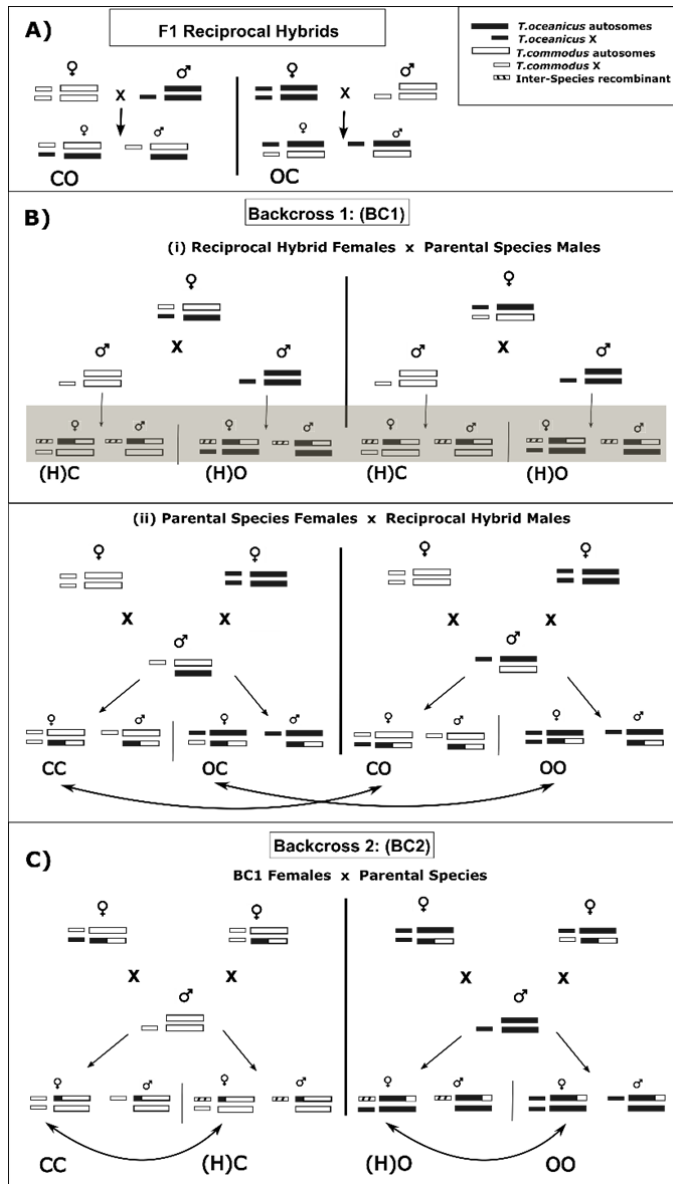
168 Penultimate instar juveniles were separated into single-sex boxes to ensure virginity. For
169 crosses, virgin adult males and females ca. 10-20 days past eclosion were paired together in
170 smaller boxes (7 x 5 cm). Approximately 20 pairs per cross type were used (Fig. 2).

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 ± 27.75) compared to the reciprocal cross (OC, 239.56 ± 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 ± 21.8) than the OC cross (155.04 ± 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 $\alpha < 0.05$.

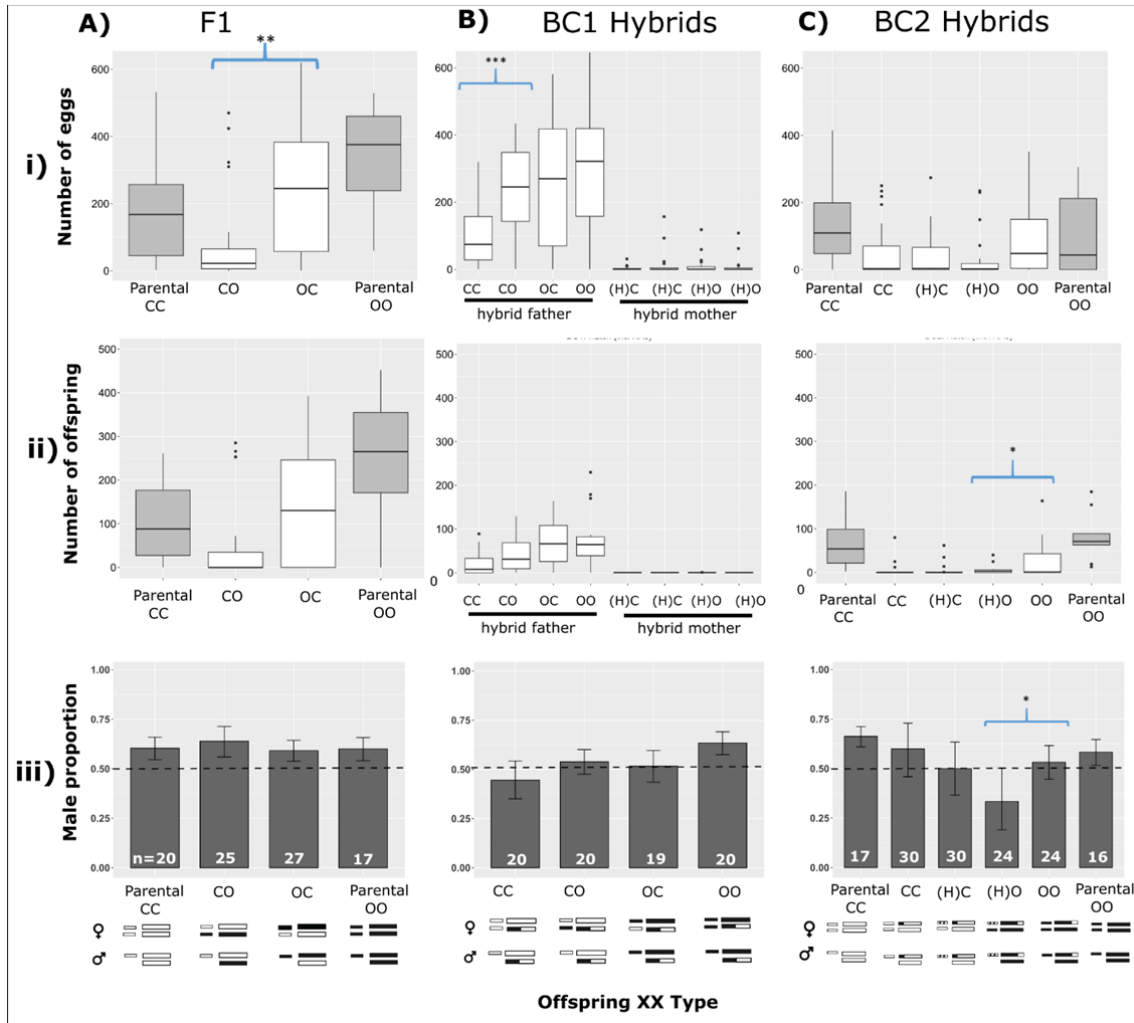
Response Variable	Model & Predictors	Model Components	Pr(> X^2)	Main comparisons	Df	Estimate	Std. Error	Z value	Pr(> Z)
Eggs	Negative binomial								
	Female XX	-	0.002	OC - CO	3,80	-1.014	0.314	-3.226	0.007
	Female weight	-	0.029		1,79				
Offspring	ZINB								
	Female XX	negbin	0.022	OC - CO	11,73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

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293

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 ± 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 ± 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 ± 8.37 vs. CC 22.5 ± 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.

332

333

334

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

342

343

344

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 ± 22.9 vs. (H)O, 33.13 ± 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 ± 10 vs. (H)O, 6.92 ± 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.

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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)
Eggs	ZANB								
	Female XX	negbin	0.125	CC vs. (H)C	13,128	0.168	0.402	0.418	0.992
		Binomial	0.011	CC vs. (H)C	13,128	-0.135	0.519	-0.259	0.999
		negbin		OO vs. (H)O	13,128	0.662	0.416	1.593	0.434
		Binomial		OO vs. (H)O	13,128	1.168	0.648	1.801	0.301
Offspring	ZINB								
	Female XX	negbin	0.011	CC vs. (H)C	13,128	0.344	0.824	0.417	0.991
		Binomial	<0.0001	CC vs. (H)C	13,128	0.342	0.817	0.418	0.991
		negbin		OO vs. (H)O	13,128	1.479	0.500	2.957	0.017
		Binomial		OO vs. (H)O	13,128	-0.579	0.629	-0.920	0.856
Hatching Success	Quasi-Binomial								
	Female XX	-	< 0.001	CC vs. (H)C	5, 85	0.253	0.737	0.344	0.999
	Female weight	-	0.004		1, 84				
	Female XX	-		OO vs. (H)O	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

552 **ACKNOWLEDGEMENTS**

553 We are grateful to the following people for helpful assistance in the field: K Holmes, S
554 Blanksby, M Higgle, G Jones, T Ly, R Ollerynshaw. S Vardy and the Westman family
555 generously provided hospitality and assistance and we appreciate their enthusiasm for our
556 research. We thank four anonymous reviewers and the editor for constructive feedback on the
557 manuscript. D Forbes, A Grant and M McGunnigle helped in the lab. Funding was provided
558 by NERC (NE/G014906/1, NE/L011255/1, NE/I027800/1). Additional funding from the
559 Orthopterists' Society to PM is also gratefully acknowledged.

560

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

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