Release from intralocus sexual conflict? Evolved loss of a male sexual trait demasculinises female gene expression

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Abstract

The loss of sexual ornaments is observed across taxa, and pleiotropic effects of such losses provide an opportunity to gain insight into underlying dynamics of sex-biased gene expression and intralocus sexual conflict (IASC). We investigated this in a Hawaiian field cricket, *Teleogryllus oceanicus*, in which an X-linked genotype (*flatwing*) feminises males' wings and eliminates their ability to produce sexually selected songs. We profiled adult gene expression across somatic and reproductive tissues of both sexes. Despite the feminising effect of *flatwing* on male wings, we found no evidence of feminised gene expression in males. Instead, female transcriptomes were more strongly affected by *flatwing* than males', and exhibited demasculinised gene expression. These findings are consistent with a relaxation of IASC constraining female gene expression through loss of a male sexual ornament. In a follow-up experiment we found reduced testes mass in flatwing males, whereas female carriers showed no reduction in egg production. In contrast, female carriers exhibited greater measures of body condition. Our results suggest sex-limited phenotypic expression offers only partial resolution to intralocus sexual conflict, owing to pleiotropic effects of the loci involved. Benefits conferred by release from intralocus conflict could help explain widespread loss of sexual ornaments across taxa.

1. Introduction

Sex-biased gene expression produces striking phenotypic differences in species where the sexes share a substantial portion, if not all, of the same genome [1-4]. Such evolved differences between sexes in gene regulation play an important role in attenuating intralocus sexual conflict (IASC), which arises when sexes are under contrasting selection pressures at shared loci, by achieving phenotypic dimorphism [5-8]. However, it is increasingly recognised that resolution of such conflict is not necessarily complete [9-12], and that IASC can persist even when genes and phenotypes have evolved under contrasting selection pressures to exhibit sex-biased or even sex-limited expression [13,14]. One of the reasons for this is pleiotropy exerted by loci involved in the conflict upon other traits which are not directly under selection (Fig. 1). Sexual trait loci can thus exert spillover effects across sexes and tissues. For example, the enlarged mandibles of male broad-horned flour beetles *Gnatocerus cornutus* are genetically associated with reduced female lifetime fecundity [13] despite their sex-limited expression, illustrating incomplete resolution of associated IASC.

As well as its role in regulating differences between sexes, recent studies have demonstrated that varying degrees of sex-biased gene expression are associated with intra-sexual phenotypic variance, often with fitness-associated effects [15]. Pointer et al. [16] found subordinate males of the wild turkey *Meleagris gallopavo* exhibit feminised patterns of gene expression relative to more ornamented dominant males. Similarly, in the bulb mite *Rhizoglyphus robini*, ‘fighter’ male morphs show exaggerated transcriptional sexual dimorphism compared with unarmoured ‘scrambler’ males [17], and are associated with increased IASC at the population

level [18,19]. A fundamental assumption of sexual selection models is that such elaborated, dimorphic sexual traits should eventually be checked by countervailing natural selection [20-22], but evidence for the involvement of sex-biased pathways of gene expression in naturally-selected adaptations is surprisingly limited, and the consequences for IASC after sexual trait reduction or loss are therefore of key interest.

To explore these consequences, we examined the effects of sexual trait loss on patterns of sex-biased gene expression in the rapidly evolving Hawaiian field cricket, *Teleogryllus oceanicus*. Approximately 15 years ago, male morphs incapable of producing sexual advertisement calls were observed to appear and rapidly spread on multiple Hawaiian islands under natural selection from a phonotactic parasitoid fly, *Ormia ochracea* [23]. Obligate silence is caused by mutation(s) that cause males to develop female-like wing venation, erasing sound-producing structures and protecting them against fatal parasitism. The silent male phenotype, flatwing, segregates as a single-locus variant (*flatwing*) on the X chromosome (sex determination is XX/XO; males and females share all genes), though the exact nature of the mutation(s) is not known [24]. Although it is transmitted on the X, *flatwing's* effects upon wing phenotype appear to be male-limited; female carriers show no readily detectable wing differences. There is evidence for widespread pleiotropic effects of *flatwing* in both sexes [25,26], and males carrying the genotype exhibit more female-like cuticular hydrocarbons [24], in addition to their feminised wing membranes. Given the potential role of pleiotropy in IASC (Fig. 1), we profiled gene expression from a range of non-wing, somatic and gonad tissues of adults from lines that were pure-breeding for *flatwing* or *normal-wing* genotypes. Our aims were

84 to test the role of sex-biased genes in evolved song loss, and explore the latter's
85 consequences for IASC.
86 If *flatwing* widely impacts sex-biased pathways of gene expression, we
87 anticipated one of two patterns among affected loci. Given its feminising effect in
88 male wing tissues, and upon male cuticular hydrocarbons, *flatwing* might be
89 associated with a general increase in female-biased gene expression,
90 demasculinising female carriers and feminising male carriers (Hypothesis 1 in Fig. 1)
91 [19,27]. An alternative, but non-mutually exclusive, scenario is that the loss of the
92 male sexual trait releases female gene expression from pleiotropic IASC-associated
93 constraints, in which case we anticipated up-regulation of female-biased (or down-
94 regulation of male-biased) gene expression (demasculinisation) predominantly
95 affecting females (Hypothesis 2 in Fig. 1). Unexpectedly, we found that female gene
96 expression was much more strongly affected by carrying the *flatwing* genotype than
97 was males', particularly in thoracic muscle and gonad tissues. Gene expression in
98 adult *flatwing* males showed no evidence of being feminised, but we did observe
99 demasculinised gene expression among female carriers consistent with predictions
100 under relaxed IASC. In a follow-up experiment, we found that *flatwing* males had
101 reduced testes mass while *flatwing*-carrier females showed no differences in egg
102 production, but exhibited higher body condition. Our results show that at adult stages,
103 female gene expression is more strongly affected by a genotype responsible for the
104 loss of a male sexual trait. Females also show a pattern of demasculinised gene
105 expression and increased body condition, and analyses of the tissue-specificity of
106 gene expression supported a role for pleiotropy in driving IASC in this system. These
107 findings are consistent with female release from constraints relating to IASC in the

rapid spread of a mutation associated with the loss of a male sexual trait, a phenomenon which may play an important role in the widely observed loss of sexual ornaments [28].

2. Materials and Methods

2.1 Sampling, sequencing and differential expression analysis

Detailed descriptions of all methodologies are provided in the Supporting Methods. Briefly, we collected tissue samples from virgin adults (ca. 3 months from egg stage) from replicate lines breeding pure with respect to each morph genotype (*flatwing* '*FW*', or *normal-wing* '*NW*'). RNA was extracted from three tissues (neural, thoracic, and gonads) of a single male and a single female from each of 6 lines (N=3 lines of each genotype), for a total of 36 samples from 12 individuals. The lines were all bred from the same laboratory population originally established from Kauai, with no differences in selective regime (See Supporting Methods and [25]). Multiple lines were included in each group to account for between-line variance and to enable detection of expression differences attributable to morph genotype. Females were homozygous diploid for the respective genotype while males were hemizygous (XX/XO). Dissections and Trizol RNA extractions were performed following [26].

Paired-end reads of all 36 samples were generated on an Illumina HiSeq 2000, and a *de novo* transcriptome was assembled from trimmed reads of all samples in Trinity using *in silico* normalisation [29]. Similar transcripts were clustered in CD-hit-est [30], and lowly expressed transcripts (those not expressed at >1 count per million in at least 3 samples) and transcripts without an open reading frame of

131 >100 amino acids were filtered from the transcriptome. Reads were aligned to the
132 transcriptome using Bowtie2 [31] with strand-specific settings, and quantified in
133 RSEM [32]. Differential expression (DE) analyses were performed in *edgeR* [33] at
134 the level of Trinity 'genes'; henceforth referred to as 'transcripts' in acknowledgement
135 that not all Trinity-identified genes passing filtering will represent genes in the
136 strictest sense. Because our analysis was at the gene level, isoform variants should
137 not contribute to the patterns of DE we observe. Clustering of similar genes by CD-
138 hit-est (see above) was used to further ensure isoform variants were not represented
139 as multiple genes, and we used the results of BUSCO analysis of conserved genes
140 [34] to verify that our transcriptome was not highly duplicated. Separate models were
141 constructed for somatic (neural, thoracic muscle) and gonad tissues, to examine
142 effects of sex and morph, with significance testing performed using likelihood ratio
143 tests. To restrict our analyses to transcripts showing strong evidence of DE, we
144 adopted a conservative significance threshold of FDR<0.01 to consider a transcript
145 significantly DE or sex-biased. We checked whether results qualitatively changed if
146 we used another common approach of imposing a fold-change threshold of >2 for a
147 transcript to be considered DE/sex-biased, with FDR <0.05 (e.g. [35]), and found
148 they did not (see Results).
149 Sequences of DE transcripts were entered as BLASTX queries against the
150 NCBI non-redundant protein database, with an e-value threshold of 10^{-3} and a
151 maximum of 20 hits. Mapping and annotation were performed in Blast2GO [36] with
152 default parameters. Functional enrichment of gene ontologies (GO) was assessed
153 using transcripts which passed filtering and showed homology with *Drosophila*

melanogaster proteins.

2.2 Gene expression feminisation, demasculinisation and tissue-specificity

We defined feminised and demasculinised expression, applied to males and females respectively, as up-regulation of female-biased transcripts (or down-regulation of male-biased transcripts) in males, and down-regulation of male-biased transcripts (up-regulation of female-biased transcripts) in females (Fig. 1). Thus, the terminology indicates the sex experiencing the effect. Identification of sex-biased genes was performed using differential expression analysis, averaging expression values across both morph genotypes in each sex; genes up-regulated at FDR<0.01 in males were considered male-biased, and genes up-regulated in females considered female-biased. To test for feminisation and demasculinisation, we took the subset of transcripts that were DE in both morph genotype and sex comparisons and compared the direction of change between the two for each tissue separately.

To understand whether changes in expression associated with morph genotype were correlated between sexes, we tested whether log-fold changes in expression for transcripts DE in one or both sexes were correlated between males and females. We also investigated the level of tissue-specificity of genotype-associated effects in each sex by comparing log-fold changes among all transcripts DE in either comparison [37]. To test whether sex-limited and tissue-specific transcripts were less likely to be DE between morph genotypes, which could support the involvement of pleiotropy affecting genes shared between sexes, we subset for each sex*tissue combination transcripts expressed at >1cpm in all 6 samples, and

177 transcripts expressed at <1cpm in all 6 samples, then compared identity across
178 tissues to define sets of sex-specific and tissue-specific transcripts.
179

180 **2.3 Reproductive tissue and condition measures**

181 We investigated whether sex-specific reproductive fitness measures differed between
182 separate, recently outcrossed (see Supporting Information) pure-breeding *NW* (N=4)
183 and *FW* (N=3) lines derived from the same base population. At 7 days post-adult
184 eclosion, gonad characteristics were measured in virgin male (N=140; 18 to 21 per
185 biological line) and female (N=145; 19 to 24 per biological line) crickets that had been
186 reared at standard stock densities. As proximate measures of reproductive output,
187 we obtained wet mass of dissected testes to the nearest mg, and for females
188 counted the number and measured the total wet mass in mg of eggs contained within
189 the ovaries.

190 Testes mass was analysed using a linear mixed model (LMM), while female
191 total egg mass was analysed using a generalised linear mixed model (GLMM) with a
192 negative binomial distribution. Total egg mass followed a negative binomial
193 distribution owing to the Poisson distribution of egg numbers. Both models included
194 predictor variables of morph genotype, log pronotum length, log somatic mass, and a
195 random effect of biological line. We calculated somatic (i.e. not including gonad
196 masses) scaled mass index (SMI) from pronotum length and somatic wet mass, often
197 used as a proximate measure for individual body condition [38]. Log-transformed SMI
198 was analysed using an LMM with predictor variables of morph genotype, sex, an
199 interaction between the two, and a random effect of biological line. Following the SMI
200 comparison, contributions of differences in pronotum length and somatic wet mass

were investigated using LMMs with the same predictors and random effect. Mixed models were run in the R package *lme4* [39], with *MASS* used to fit the negative binomial GLMM. Significance of predictor terms was tested using Wald's χ^2.

3. Results

3.1 Morph genotype has larger effects on gene expression in females

Female transcriptomes were more strongly impacted by carrying the *flatwing* genotype than were males'. The unfiltered *T. oceanicus* transcriptome contained complete sequences for 90.6% conserved insect BUSCO genes, with low duplication rates (1.8% of complete genes; see Supporting Information), and 42,496 transcripts (Trinity-identified 'genes') passed filtering. Differential expression results are summarised in Table 1. In all tissues the number of DE transcripts (FDR<0.01) associated with morph genotype was greater among females than males, and female thoracic muscle and ovaries were particularly strongly affected (neural tissue: χ^2_1=11.571, P<0.001; thoracic muscle: χ^2_1=310.77, P<0.001; gonads: χ^2_1=159.67, P<0.001) (Fig. 2a). This interpretation remained unchanged if a fold-change of >2 and FDR <0.05 was instead adopted (greater DE in females: all P<0.001).

Of 560 unique transcripts DE between genotypes in either sex, 296 (52.86%) had significant BLASTX hits. None of the annotated transcripts had obvious known functions or GO terms related to sexual dimorphism in insects. Overrepresented GO terms among transcripts up-regulated in each of the female genotypes are given in Table S1. Neither male morph showed significant overrepresentation for any GO categories.

3.2 Male trait loss is associated with demasculinised female gene expression

FW females showed demasculinised gene expression compared with *NW* females (Fig. 2b). Of the 119 sex-biased transcripts DE between female genotypes across all tissues, 87 (73.11%) showed expression patterns consistent with demasculinisation of *FW* females (either female-biased transcripts up-regulated in *FW* females or male-biased transcripts up-regulated in *NW* females), compared with only 32 transcripts (26.89%) showing the reverse pattern (χ^2_1=25.420, P<0.001). The pattern of demasculinisation in *FW* relative to *NW* samples was consistent across female thoracic muscle and ovaries tissues (thoracic muscle: χ^2_1=31.837, P<0.001; ovaries: χ^2_1=4.070, P=0.044), but numbers were too low for quantitative comparison in neural tissues. Interpretation of demasculinised expression remained unchanged under fold-change >2 and FDR <0.05 criteria (neural tissue: too few for comparison; thoracic muscle: χ^2_1=57.791, P<0.001; ovaries: χ^2_1=5.921, P=0.015).

3.3 Magnitude of DE associated with male trait loss across sexes and tissues

For transcripts DE between genotypes in one or both sexes, changes in gene expression were positively correlated between sexes in neural (Spearman's rank: r=0.920, N=26, P<0.001) and gonad (r=0.203, N=193, P=0.005) tissues, but not in thoracic muscle (r=0.046, N=378, P=0.372) (Fig. S1). Across the 19 transcripts showing concordant and significant DE in males and females, after relaxing the significance threshold to FDR<0.05 to increase sample size, there was no indication that females showed greater log-fold changes; male genotypes tended to exhibit

247 greater differences (male $\log_2$-fold change – female $\log_2$-fold change: average =
248 0.386, P=0.123). Changes in expression associated with the *FW* genotype were
249 concordant in pairwise comparisons across tissues within each of the sexes
250 (Spearman's rank: all r>0.465, P<0.01; Figs S1,2), suggesting a relatively high
251 degree of pleiotropy [37]. Interpretations above were unchanged under fold-change
252 >2 and FDR <0.05 criteria.
253 Transcripts showing sex-limited expression did not show substantial DE
254 between genotypes. In ovaries, the female tissue which showed the greatest degree
255 of sex-limited expression, sex-limited transcripts (expressed >1cpm in all ovaries
256 samples and <1cpm in all testes samples) tended to be underrepresented among
257 those DE between morph genotypes (11 of 185 DE transcripts sex-limited, vs 1,782
258 of the 17,254 transcripts >1cpm in all 6 samples; χ^2_1=3.350, P=0.067). No sex-limited
259 transcripts were DE between morph genotypes in testes, or neural and thoracic
260 muscle tissues of either sex.
261 Among transcripts showing tissue-specific expression within each sex (e.g.
262 expressed at >1cpm in all female neural samples but <1cpm in all female thoracic
263 muscle and ovaries samples) fewer than expected were DE between morph
264 genotypes in ovaries (7/178 DE transcripts showed tissue-specific expression, versus
265 1,576/17,254 of those expressed at >1cpm in all 6 samples; χ^2_1=5.161, P=0.023). No
266 tissue-specific transcripts were DE between genotypes in any of the other tissues;
267 including testes, despite the large number of tissue-specific transcripts (0/9 versus
268 6,658/20,998). In somatic tissues, tissue-specific transcripts were less likely to show
269 sex-bias than were non- tissue-specific transcripts also expressed at >1cpm in all 6
270 samples for the respective tissue (χ^2: P<0.001 in both tissues and sexes), but this

pattern was reversed in ovaries, where tissue-specific transcripts were more likely to show sex-bias (χ^2=26.763, P<0.001). There was no difference in testes samples (χ^2=0.300, P=0.584).

3.4 Sex and morph variation in reproductive tissues and condition

Adult *NW* males grew larger testes (LMM: χ^2_1=8.800, P=0.003; Fig. 3a), but there was no difference in the mass of eggs produced by females of either genotype (GLMM: χ^2_1=0.011, P=0.916; Fig. 3b) (Table S2). Nevertheless, *FW* females achieved better condition. Their SMI was greater than that of *NW* females, but a significant sex × morph interaction (LMM: χ^2_1=14.006, P<0.001) indicated there was no similar effect observed in males (Fig. 3c, Table S2). Thus, *FW* lines showed greater divergence in SMI between sexes, and this effect appeared largely related to changes in mass. (Table S2,3)

4. Discussion

Influential models of sexual selection and sexual conflict predict that sex differences in gene expression underlying sexually selected traits arise due to IASC [7]. However, such resolution of IASC is often expected to be incomplete, and costly elaboration of sexual traits should eventually be checked by natural selection [20-22]. Surprisingly, we found that the naturally-selected, genetic loss of a male sexual signal in crickets, via feminisation of male wing structures, affected gene expression more strongly in adult females than in males. There was no evidence of feminisation detectable in adult *flatwing* males, though this does not preclude such a role during

294 earlier stages of development (e.g. [40]), which is hinted at by their reduced testes
295 mass, and feminised CHCs [24]. In contrast, gene expression was demasculinised in
296 female carriers of the *flatwing* genotype, which also showed increased body
297 condition. These results support our predictions under a scenario of relaxed IASC
298 following male sexual trait loss (Fig. 1)

299 Sex-biased gene expression is likely to be associated with underlying IASC at
300 loci where selection pressures differ between males and females [4,6], and sexual
301 ornaments provide a clear example of a trait with contrasting fitness optima between
302 sexes [13]. The association between sexually selected traits and sexual conflict has
303 frequently been inferred by comparing laboratory lines reared under contrasting
304 selection regimes [19,27,41-43]. In *T. oceanicus*, our results raise the intriguing
305 possibility that relaxed IASC among females accompanied evolutionary loss of a
306 male sexual trait in the wild. Female release from IASC could occur more widely than
307 is generally considered, given repeated secondary losses of sexually-selected male
308 traits across taxonomic groups [28,44-46], and could even facilitate these losses
309 given the arms race-like dynamics with which IASC is frequently associated [47].

310 Recent evidence suggests increased sexually dimorphic gene expression is
311 associated with increased fitness [15]. We therefore expected males and females
312 from *flatwing* lines to show contrasting fitness effects of the mutant genotype, with
313 females benefitting from demasculinised gene expression and males showing no
314 variation. Flatwing males exhibited reduced testes mass, consistent with a previous
315 report [48], but females carrying the *flatwing* genotype did not differ in reproductive
316 output. Instead, they exhibited increased SMI, a proximate measure of body
317 condition, whereas *flatwing* males showed no such increase. While we are cautious

about making direct inference about fitness effects of SMI, evidence of IASC over body size in species as diverse as humans [49] and Indian meal moths *Plodia interpunctella* [50], illustrates that males and females are frequently subject to contrasting optima for mass and structural size. In *T. oceanicus*, structural body size is likely to have an important influence on male mating success through male-male competition and female choice, while females less subject to pressures of sexual selection may benefit from maximising energy reserves [51]. Phenotypic evidence suggests, therefore, that flatwing males are disadvantaged above and beyond their inability to signal, whereas female *flatwing* carriers are not strongly disadvantaged, and may actually benefit, potentially as a result of relaxed IASC.

While demasculinised gene expression and increased body condition in *flatwing*-carrying females support a hypothesis of relaxed IASC following male sexual trait loss, several caveats are worth considering. For example, demasculinised expression does not itself illustrate female benefit, though this interpretation is supported by the increased body condition observed, which may or may not be directly related to demasculinised gene expression, and by others' findings of an association between greater sex-biased gene expression and fitness-associated traits [15]. Additionally, while our focus was on sex-biased transcripts, genotype also affected many transcripts in both sexes which did not show sex-bias. It is difficult to make inferences about the importance of these changes, or relate them to phenotypic traits, however it would affect interpretation of female benefit from carrying the *FW* genotype if, for example, changes to non- sex-biased transcripts had contrasting fitness-associated effects [52]. Finally, we examined differences between pure-breeding lines derived from a single wild population, but interpretation of our

results would benefit from future work testing patterns of sex-specific selection across lines derived from wild populations with contrasting proportions of flatwing/normal-wing male phenotypes, to assess whether this influences IASC on a population level [18].

Comparing gene expression profiles across tissues within each sex revealed a strong pattern for transcripts differentially expressed between morphs in one tissue to show evidence of concordant differences in others. A lack of tissue specificity is often used as a proxy measure for pleiotropy (i.e. more pleiotropic loci are likely to be less tissue-specific) [37], and extensive pleiotropy is widely expected to constrain the rate of evolution due to the reduced likelihood of a net increase in fitness [53]. We found that very few transcripts showing tissue-specific or sex-limited expression differed in expression between genotypes. This supports the view that changes we observe to be associated with carrying *flatwing* are primarily among transcripts that have detectable levels of expression in both sexes, across tissues, and represent spillover effects of the *flatwing* locus in non-wing tissues. As well as showing *flatwing* has pervasive pleiotropic effects across multiple tissues, these results are consistent with the idea that the adaptive benefit of the flatwing phenotype in males outweighs costs associated with pleiotropic effects in non-focal tissues. Given the observed demasculinisation of female transcriptomes, and evidence for increased female body condition, our results also raise the intriguing prospect that positive pleiotropic effects of *flatwing* on females through relaxed IASC could actually have facilitated its rapid spread.

5. Conclusions

Our results are consistent with theoretical expectations for relaxed genomic conflict following reduction of sexual selection [10]. The relaxation of genomic conflict may be an underappreciated yet capacitating feature of the widely-observed loss of sexual ornaments, for which the genetic and evolutionary mechanisms are not well understood [28]. It is generally expected that the maintenance of sexually ornaments will be associated with IASC, and also acted against to varying degrees by natural selection. In *T. oceanicus*, the evolutionary loss of a male-specific sexual ornament may reduce IASC-associated constraints upon female gene expression, supporting the view that sex-biased gene expression only partially resolves underlying forces of intralocus sexual conflict even when phenotypes are sex-limited in their expression [11,13]. More generally, IASC may be an underappreciated driver during the evolutionary reduction or loss of secondary sexual traits.

Ethics

The species used in this study is not subject to ethical review.

Data Accessibility

Trimmed reads for each library are available at the European Nucleotide Archive under accession PRJEB27211, and phenotypic data are available from the Dryad digital repository (DOI:10.5061/dryad.5421j87).

Authors' contributions

JGR, SP & NWB designed experiments; JGR & SP performed experiments, JGR & NWB analysed data; JGR & NWB wrote the manuscript. All authors approved the final manuscript and agree to be held accountable for its content.

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References

1. Connallon, T. & Knowles, L.L. (2005). Intergenomic conflict revealed by patterns of sex-biased gene expression. *Trends Gen.* **21**(9), 495–499.

2. Ellegren, H. & Parsch, J. (2007). The evolution of sex-biased genes and sex-biased gene expression. *Nat. Rev. Genet.* **8**(9), 689–698.

3. Bachtrog, D., Mank, J.E., Peichel, C.L., Kirkpatrick, M., Otto, S.P., Ashman, T., Hahn, M.W., Kitano, J., Ming, R., Perrin, N. et al. (2014). Sex determination: why so many ways of doing it? *PLoS Biol.* **12**(7), 1–13.

4. Mank, J.E. (2017). The transcriptional architecture of phenotypic dimorphism. *Nature Ecol. Evol.* **1**(1), 0006.

5. Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**(2), 292–305.

6. Pizarri, T. & Snook, R.R. (2003). Perspective: sexual conflict and sexual selection: chasing away paradigm shifts. *Evolution* **57**(6), 1223–1236.

7. Bonduriansky, R. & Chenoweth, S.F. (2009). Intralocus sexual conflict. *Trends Ecol. Evol.* **24**(5), 280–288.

8. Harrison, P.W., Wright, A.E., Zimmer, F., Dean, R., Montgomery, S.H., Pointer, M.A. & Mank, J.E. (2015). Sexual selection drives evolution and rapid turnover of male gene expression. *PNAS* **112**(14), 4393–4398.

9. Rice, W.R. & Chippindale, A.K. (2001). Intersexual ontogenetic conflict. *J. Evol. Biol.* **14**, 685–693.

10. Cox, R.M. & Calsbeek, R. (2009). Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am. Nat.* **173**(2), 176–187.

11. Connallon, T., Cox, R.M. & Calsbeek, R. (2010). Fitness consequences of sex-specific selection. *Evolution* **64**(6), 1671–1682.

12. Berger, D., Berg, E.C., Widegren, W., Arnqvist, G. & Maklakov, A.A. (2014). Multivariate intralocus sexual conflict in seed beetles. *Evolution* **68**, 3457-3469.

13. Harano, T., Okada, K., Nakayama, S., Miyatake, T. & Hosken, D.J. (2010). Intralocus sexual conflict unresolved by sex-limited trait expression. *Curr. Biol.* **20**(22), 2036–2039.

14. Cheng, C. & Kirkpatrick, M. (2016). Sex-specific selection and sex-biased gene expression in humans and flies. *PLoS Genet.* **12**(9), e1006170.

15. Dean, R., Hammer, C., Higham, V. & Dowling, D.K. (2018). Masculinization of gene expression is associated with male quality in Drosophila melanogaster. *Evolution* **72**(12), 2736–2748.

16. Pointer, M.A., Harrison, P.W., Wright, A.E. & Mank, J.E. (2013). Masculinization of gene expression is associated with exaggeration of male sexual dimorphism. *PLoS Genet.* **9**(8): e1003697.

17. Stuglik, M.T., Babik, W., Prokop, Z. & Radwan, J. (2014). Alternative reproductive tactics and sex-biased gene expression: The study of the bulb mite transcriptome. *Ecol. Evol.* **4**(5), 623–632.

18. Joag, R., Stuglik, M., Konczal, M., Plesnar-Bielak, A. Skrzynecka, A., Babik, W., & Radwan, J. (2016). Transcriptomics of intralocus sexual conflict: Gene expression patterns in females change in response to selection on a male secondary sexual trait in the bulb mite. *Genome Biol. Evol.* **8**(8), 2351–2357.

19. Plesnar-Bielak, A., Skrzynecka, A.M., Miler, K. & Radwan, J. (2014). Selection for alternative male reproductive tactics alters intralocus sexual conflict. *Evolution*

455 **68**(7), 2137–2144.
456 20. Fisher, R.A. (1915). The evolution of sexual preference. *The Eugenics review*
457 **7**(3), 184–192.
458 21. Lande, R. (1981). Models of speciation by sexual selection on polygenic traits.
459 *PNAS* **78**, 3721–3725.
460 22. Kirkpatrick, M. (1982). Sexual selection and the evolution of female choice.
461 *Evolution* **36**, 1–12.
462 23. Zuk, M., Rotenberry, J.T. & Tinghitella, R.M. (2006). Silent night: adaptive
463 disappearance of a sexual signal in a parasitized population of field crickets. *Biol.*
464 *Lett.* **2**(4), 521–524.
465 24. Pascoal, S., Risse, J.E., Zhang, X., Blaxter, M., Cezard, T., Challis, R.J., Gharbi,
466 K., Hunt, J., Kumar, S., Langan, E., et al. (2018). Silent crickets reveal the
467 genomic footprint of recent adaptive trait loss. *bioRxiv*
468 https://doi.org/10.1101/489526.
469 25. Pascoal, S., Liu, X., Ly, T., Fang, Y., Rockliffe, N., Paterson, S., Shirran, S.L.,
470 Botting, C.H., & Bailey, N.W. (2016). Rapid evolution and gene expression: a
471 rapidly evolving Mendelian trait that silences field crickets has widespread
472 effects on mRNA and protein expression. *J. Evol. Biol.* **29**(6), 1234–1246.
473 26. Pascoal, S., Liu, X., Fang, Y., Paterson, S., Ritchie, M.G., Rockliffe, N., Zuk, M.,
474 & Bailey, N.W. (2018). Increased socially mediated plasticity in gene expression
475 accompanies rapid adaptive evolution. *Ecol. Lett.* **21**(4), 546–556.
476 27. Hollis, B., Houle, D., Yan, Z., Kawecki, T.J. & Keller, L. (2014). Evolution under
477 monogamy feminizes gene expression in Drosophila melanogaster. *Nat. Comm.*
478 **5**, 3482.

28. Wiens, J.J. (2001). Widespread loss of sexually selected traits: How the peacock lost its spots. *Trends Ecol. Evol.* **16**(9), 517–523.

29. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q. et al. (2011). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat. Biotechnol.* **29**(7), 644–652.

30. Li, W. & Godzik, A. (2006). Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**(13), 1658–1659.

31. Langmead, B. & Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**(4), 357–359.

32. Li, B. & Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**(1), 323.

33. Robinson, M.D., McCarthy, D.J. & Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**(1), 139–140.

34. Simao, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., & Zdobnov, E.M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**(19), 3210–3212.

35. Immonen, E., Sayadi, A., Bayram, H. & Arnqvist, G. (2017). mating changes sexually dimorphic gene expression in the seed beetle callosobruchus maculatus. *Genome Biol. Evol.* **9**(3), 677–699.

36. Conesa, A., Götz, S., Garcia-Gomez, J.M., Terol, J., Talón, M. & Robles, M. (2005). Blast2GO: A universal tool for annotation, visualization and analysis in

503 functional genomics research. *Bioinformatics* **21**(18), 3674–3676.
504 37. Dean, R. & Mank, J.E. (2016). Tissue specificity and sex-specific regulatory
505 variation permit the evolution of sex-biased gene expression. *Am. Nat.* **188**(3),
506 E74–E84.
507 38. Peig, J. & Green, A.J. (2009). New perspectives for estimating body condition
508 from mass/length data: the scaled mass index as an alternative method. *OIKOS*
509 **118**(12),1883–1891.
510 39. Bates, D., Martin, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects
511 models using lme4. *J. Stat. Softw.* **67**, 1–48.
512 40. Perry, J.C., Harrison, P.W. & Mank, J.E. (2014). The ontogeny and evolution of
513 sex-biased gene expression in drosophila melanogaster. *Mol. Biol. Evol.* **31**(5),
514 1206–1219.
515 41. Rice, W.R. (1996). Sexually antagonistic male adaptation triggered by
516 experimental arrest of female evolution. *Nature* **381**, 232–234.
517 42. Holland, B. & Rice, W.R. (1999). Experimental removal of sexual selection
518 reverses intersexual antagonistic coevolution and removes a reproductive load.
519 *PNAS* **96**(9), 5083-5088.
520 43. Crudgington, H.S., Beckerman, A.P., Brüstle, L., Green, K., Snook, R.R. (2005).
521 Experimental removal and elevation of sexual selection: does sexual selection
522 generate manipulative males and resistant females? *Am. Nat.* **165**: S72–S87.
523 44. Porter, M.L. & Crandall, K.A. (2003). Lost along the way: The significance of
524 evolution in reverse. *Trends Ecol. Evol.* **18**(10), 541-547.
525 45. Morris, M.R., Moretz, J.A., Farley, K. & Nicoletto, P. (2005). The role of sexual
526 selection in the loss of sexually selected traits in the swordtail fish Xiphophorus

527 continens. *Anim. Behav.* **69**(6), 1415-1424.
528 46. Ptacek, M.B., Childress, M.J., Petersen, J.A. & Tomasso, A.O. (2011).
529 Phylogenetic evidence for the gain and loss of a sexually selected trait in sailfin
530 mollies. *ISRN Zool.* 2011, 251925.
531 47. Pennell, T.M., de Haas, F.J., Morrow, E.H., van Doorn, G.S. (2016). Contrasting
532 effects of intralocus sexual conflict on sexually antagonistic coevolution. *PNAS*,
533 **113**(8), E978-86.
534 48. Bailey, N.W., Gray, B. & Zuk, M. (2010). Acoustic experience shapes alternative
535 mating tactics and reproductive investment in male field crickets. *Curr. Biol.* **20**,
536 845–849.
537 49. Stulp, G., Kuijper, B., Buunk, A.P., Pollet, T.V. & Verhulst, S. (2012). Intralocus
538 sexual conflict over human height. *Biol. Lett.* **8**(6), 976–978.
539 50. Lewis, Z., Wedell, N. & Hunt, J. (2011). Evidence for strong intralocus sexual
540 conflict in the indian meal moth, plodia interpunctella. *Evolution* **65**(7), 2085–
541 2097.
542 51. Whitman, D.W. (2008). The significance of body size in the Orthoptera: a review.
543 *J. Orthoptera Res.* **17**(2), 117–134.
544 52. Chevillon, C., Bourguet, D., Rousset, F., Pasteur, N. & Raymond, M. (1997).
545 Pleiotropy of adaptive changes in populations: comparisons among insecticide
546 resistance genes in Culex pipiens. *Genetics Research* **70**(3), 195–204.
547 53. Orr, H.A. (2000). Adaptation and the cost of complexity. *Evolution* **54**(1), 13–20.
548 54. Rayner, J.G., Pascoal, S., & Bailey, N.W. (2019). Data from: Release from
549 intralocus sexual conflict? Evolved loss of a male sexual trait demasculinises
550 female gene expression. Dryad Digital Repository (DOI:10.5061/dryad.5421j87).

Table 1. Numbers of DE genes for contrasts examining sex-biased expression (top section) and morph genotype in each tissue and sex (middle and bottom section).

Tissue	DE_Down[2]	DE_Up[2]	DE_Sum[2]
Sex (M)[1]			
Neural	379	152	631
Muscle	726	492	1218
Gonads	9030	11267	20297
Male genotype (*NW*)			
Neural	0	5	5
Muscle	9	10	19
Testes	5	4	9
Male total	**14**	**19**	**33**
Female genotype (*NW*)			
Neural	9	14	23
Muscle	160	204	364
Ovaries	50	135	185
Female total	**219**	**353**	**572**

[1] Reference group for each contrast is given in parentheses: M=males, NW=normal-wing
[2] All DE inferred using FDR<0.01

555 **Figure 1.** Hypothetical effects of male sexual trait loss on IASC at the level of gene
556 expression. The schematic shows expression levels (E) and fitness (W) for a
557 transcript assumed to be pleiotropically influenced by a sexual trait locus, thus
558 contributing to incompletely resolved IASC. Expression optima (E_θ) and observed
559 average expression values ($\bar{E}$) differ between the sexes, and shaded curves illustrate
560 frequency distributions for sex-specific expression. Within each sex, fitness is a
561 function of expression level, maximized at the optimum (top red and blue lines
562 indicating hypothetical stabilizing fitness functions for females and males,
563 respectively). Thus, ΔE describes displacement from the optimum level of expression
564 for each sex. The descriptors 'feminisation' and 'demasculinisation' refer to the
565 identity of the individual under consideration: females whose gene expression shifts
566 away from the male optimum are demasculinised, whereas males whose gene
567 expression shifts in the same direction are feminized.
568
569 **Figure 2.** The *flatwing* genotype's effect on gene expression. The top panel shows
570 tissues sampled. **a)** Numbers of transcripts up-regulated in *NW*-carrying crickets for
571 males (light blue) and females (light red), versus up-regulated in *FW*-carrying
572 individuals of either sex (dark blue/red). **b)** Sex-biased genes that differed between
573 female morph genotypes showed patterns of demasculinisation in *FW* females. (Too
574 few sex-biased genes were DE between male genotypes for statistical comparison.)
575 Numbers of sex-biased transcripts up-regulated in each morph genotype with respect
576 to the other are plotted, and colours show female-biased (red) vs. male-biased (blue)
577 expression. Significance (*** P<0.001, * P<0.05) is shown for differences between
578 genotypes in the number of transcripts showing masculinised/demasculinised

579 expression. Significance was not tested for neural tissue, in which just 5 sex-biased
580 transcripts were DE between genotypes.
581
582 **Figure 3.** Sex-specific differences in gonad phenotypes and body condition in *FW* vs.
583 *NW* genotypes. **a**) Male testes mass, and **b**) female total egg mass, at 7 days post-
584 eclosion. Black points illustrate means, and ** indicates a significant difference at
585 $P<0.01$ (see Table 2). **c**) *FW* females showed increased SMI compared to *NW*
586 females, but SMI did not differ between male genotypes. Points illustrate means,
587 error bars ± standard error.
588