Increased socially-mediated plasticity in gene expression accompanies rapid adaptive evolution

Sonia Pascoal[1*†], Xuan Liu[2†], Yongxiang Fang[2], Steve Paterson[2], Michael G. Ritchie[3], Nichola Rockliffe[2], Marlene Zuk[4] & Nathan W. Bailey[3*]

[1]Department of Zoology, University of Cambridge, CB2 3EJ, U.K.

[2]Centre for Genomic Research, University of Liverpool, Liverpool, L69 7ZB, U.K.

[3]Centre for Biological Diversity, University of St Andrews, St Andrews, KY16 9TH, U.K.

[4]Department of Ecology, Evolution and Behavior, University of Minnesota, St Paul, MN 55108, U.S.A.

Sonia Pascoal: scm77@cam.ac.uk; Xuan Liu: Xuan.Liu@liverpool.ac.uk; Yongxiang Fang: y.fang@liverpool.ac.uk; Steve Paterson: S.Paterson@liverpool.ac.uk; Michael G. Ritchie: mgr@st-andrews.ac.uk; Nichola Rockliffe: N.Rockliffe@liverpool.ac.uk; Marlene Zuk: mzuk@umn.edu; Nathan W. Bailey: nwb3@st-andrews.ac.uk

[*] Correspondence: scm77@cam.ac.uk (+44 (0)1223 334466) or nwb3@st-andrews.ac.uk (+44 (0)1334 463367)

[†] Equal contributors

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Conceived and designed the experiments: NWB, Sonia P, MGR, MZ. Performed the experiments: Sonia P, NR. Analyzed the data: NWB, XL, Sonia P, Steve P, YF. Contributed reagents/materials/analysis/tools: NWB, NR. Wrote the paper: NWB, Sonia P, MGR, MZ.

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Abstract

Recent theory predicts that increased phenotypic plasticity can facilitate adaptation as traits respond to selection. When genetic adaptation alters the social environment, socially-mediated plasticity could cause co-evolutionary feedback dynamics that increase adaptive potential. We tested this by asking whether neural gene expression in a recently arisen, adaptive morph of the field cricket *Teleogryllus oceanicus* is more responsive to the social environment than the ancestral morph. Silent males (flatwings) rapidly spread in a Hawaiian population subject to acoustically-orienting parasitoids, changing the population's acoustic environment. Experimental altering crickets' acoustic environments during rearing revealed broad, plastic changes in gene expression. However, flatwing genotypes showed increased socially-mediated plasticity, while normal-wing genotypes exhibited negligible expression plasticity. Increased plasticity in flatwing crickets suggests a coevolutionary process coupling socially flexible gene expression with the abrupt spread of flatwing. Our results support predictions that phenotypic plasticity should rapidly evolve to be more pronounced during early phases of adaptation.

Introduction

Adaptive mutations are likely to cause correlated phenotypic effects that extend beyond traits directly targeted by selection (Raymond et al. 2001). The fate of a new mutation during establishment and spread will therefore depend on the balance of costs and benefits of those associated effects, and phenotypic plasticity has been proposed as a mechanism that can mitigate the costs. Despite more than a century of debate focusing on how plasticity impacts rates of evolutionary change, the challenge of empirically testing the link between plasticity and the establishment of new mutations has defied resolution (Baldwin 1896; West-Eberhard 2005; Ghalambor *et al*. 2007; Scoville and Pfrender 2010; Stoks *et al*. 2015). An influential model of this process predicts that increased plasticity associated with traits directly affected by abrupt ("extraordinary") changes in selection should evolve over tens of generations, followed by a much longer period during which adaptive, previously plastic, phenotypes become genetically assimilated (Lande 2009). Increased plasticity can also increase the likelihood of adaptive evolutionary responses, even if some of the plasticity is initially counter-selected (Ghalambor *et al*. 2007; 2015).

An overlooked and unresolved question about the relationship between plasticity and rapid adaptive evolution concerns the extended phenotypic consequences of new mutations. Genomic invasion of mutations of large effect can indirectly cause major social changes that provoke plastic phenotypic responses, generating coevolutionary feedback (Bailey 2012). For example, adaptive mutations that affect social behaviour will alter the social environment as they spread, potentially altering the expression of other traits such as aggression or mating behaviour that are sensitive to the social environment (Schradin 2013). Pre-existing plasticity may enable persistence of new mutations with otherwise negative effects, but provided there is sufficient genetic variation for that plasticity, it could also

coevolve with adaptive mutations if they alter the environment that cues plastic responses (West-Eberhard 2005; Lande 2009). This scenario requires only a new genotype under selection that creates environmental feedback, plus genetic variation for plasticity, and it makes testable predictions about how plasticity modulates the rate of evolution.

We tested these predictions by capitalizing on the recent and rapid spread of a male-silencing wing morph in the Pacific field cricket (*Teleogryllus oceanicus*). Silence protects males in Hawaii from attack by an acoustically-orienting parasitoid fly, *Ormia ochracea*, and the phenotype, flatwing, segregates as a Mendelian trait on the X chromosome (Zuk *et al*. 2006; Tinghitella 2008; Pascoal *et al*. 2014). Males who carry flatwing mutation(s) develop wings that are incapable of normal sound production. These flatwing males appeared in 2003 and spread to near-fixation over approximately 20 generations, so dynamics of this system reflect the early stages of rapid adaptive evolution (Zuk *et al*. 2006). Flatwing males are protected from parasitoid attack, but they face difficulty in mate attraction because in this species, male calling song is the only known long-range mating signal and females cannot sing. Male song thus constitutes a dominant component of the social environment, and plasticity mediated by the acoustic environment appears to be advantageous in *T. oceanicus* populations that contain a large proportion of flatwing males. Females reared in environments lacking song are more responsive, which may enable them to compensate for the lack of signalling males by responding more quickly and with less discrimination to the few calling males that remain in the population (Bailey and Zuk 2008). Males reared in silence invest less in reproductive tissues but are more likely to adopt alternative reproductive tactics that increase the likelihood of encountering females (Bailey *et al*. 2010), present decreased immunity (Bailey *et al*. 2011) and show increased locomotion (Balenger and Zuk 2015).

Here, we asked whether enhanced socially-mediated plasticity is associated with the rapidly-evolving flatwing genotype, as theoretical arguments and models predict (West-Eberhard 2005; Lande 2009). We quantified transcriptome plasticity to the social environment in crickets that did or did not carry alleles for flatwing, and tested whether the genotypes respond to the social environment differently. We specifically evaluated the effects of prior social experience during development and maturation, rather than an instantaneous or short-term response as might be activated during mate choice and phonotaxis (Immonen and Ritchie 2012). We focused on longer-term effects of the acoustic environment because such exposure mimics variation that crickets would experience while developing in wild populations dominated by singing normal-wing males or silent flatwing males.

We examined socially-mediated gene expression using tissue derived from cricket heads, which comprised central and peripheral nervous tissues plus associated sensory structures contained within the head capsule, assayed during a relevant developmental interval of adulthood. In crickets, head capsule tissue contains the central brain structures, which themselves contain approximately 100 times more cells than any one of the ganglia distributed along the ventral nerve cord (Schildberger *et al*. 1989). We examined gene expression in tissue contained within head capsules (hereafter referred to as 'neural' or 'brain' tissue for convenience) because we were interested in genes and transcripts that might influence behavioural responses to the acoustic environment. Such responses need not rely exclusively on gene expression in the brain, but the tissue-specificity of our approach allowed us to exclude expression differences that might be associated with downstream effects of the obvious morphological variation between morphs (Zuk *et al*. 2006; Pascoal *et al*. 2014).

Examining neural expression allowed us to bypass difficulties that can arise from selecting and measuring plasticity of traits at the level of organismal phenotype. A growing literature focuses on how genomic approaches to the study of phenotypic plasticity can illuminate causal expression differences underlying plastic responses (Aubin-Horth and Renn 2009), or differential expression arising as a downstream consequence of earlier plastic changes (Aubin-Horth et al. 2005; Nyman et al. 2017). Others have characterized gene expression differences underlying environmentally-induced polyphenisms, as in morphs of the locust *Locusta migratoria* (Wang et al. 2014) or alternative male phenotypes in the bulb mite *Rhizoglyphus robini* (Stuglik et al. 2014). The present study had a different aim: our tests were focused on the prediction that rapid adaptation is facilitated by associated increases in phenotypic plasticity, and we focused on plasticity's relationship with a genetically-determined polymorphism evolving under selection. Thus, we tested whether flatwing and normal-wing genotypes show different neural transcriptome responses to the social environment in *T. oceanicus*, which would provide evidence that transcriptome plasticity to the social environment is coevolving with the segregating trait, flatwing, which directly alters that social environment. Our findings support the theoretical prediction that increased phenotypic plasticity characterizes early stages of rapid adaptation.

Material and methods

Crickets and acoustic environment manipulation

We used 3 replicate lines each of Kauai pure-breeding flatwing and normal-wing *T. oceanicus* to test whether neural gene expression in mutant and normal-wing crickets responds differently to changes in the acoustic environment. The lines were generated through a series of crosses to ensure homozygosity at the locus or loci causing the flatwing genotype

155 (the phenotype segregates as an X-linked, single locus trait), but the lines were not isogenic
156 (Zuk *et al*. 2006; Pascoal *et al*. 2014; Pascoal *et al*. 2016a). Stock crickets were reared in 16 L
157 plastic containers under common garden conditions in a temperature-controlled chamber at
158 25 ^{0}C with a 12:12h light:dark cycle. They were provided with moistened cotton and
159 cardboard egg cartons for shelter and fed Burgess Supa Rabbit Exel Junior and Dwarf rabbit
160 pellets *ad libitum*. When sex differences became apparent, males and females were isolated
161 in 118 mL plastic cups and thereafter reared individually and maintained twice weekly as for
162 the stock crickets. Isolated crickets were randomly assigned to one of four temperature-
163 controlled incubators under two treatments. We adapted previously-described methods
164 (Kasumovic et al. 2011; Thomas et al. 2011; Bailey and Zuk 2012; Bailey and Macleod 2014;
165 Pascoal *et al.* 2016b) to manipulate crickets' perceptions of their acoustic environment. Two
166 incubators were kept in silence ("no song" treatment mimicking a population with few or no
167 normal-wing males) and two incubators playing back two different average Kauai male
168 calling songs simultaneously ("song" treatment) mimicked a population with a high density
169 of singing males. Average calling song parameters were determined from laboratory
170 recordings made at 25 ± 2 °C of n = 24 normal-wing males from a Kauai stock population,
171 and the two average Kauai songs were artificially constructed by excising pulses representing
172 the correct length and carrier frequency from recordings, and manually arranging them into
173 the required pattern of pulse intervals (Table S1). Since *T. oceanicus* are mainly active at
174 night, we played back song only during the dark phase of the crickets' light:dark cycle. All
175 conditions other than the presence or absence of song were kept uniform in the two
176 treatments. Just after adult eclosion, the left wing scrapers were removed from all crickets
177 to prevent singing which would interfere with the silent treatment (flatwing males and

178 females cannot sing but were also clipped to control for confounding effects due to cutting).
179 One week later, cricket tissues were dissected and stored in RNALater at -20 ^{o}C.

180

181 **RNA extractions, library preparation and sequencing**

182 RNA extraction, library preparation and sequencing were performed as described in Pascoal
183 *et al*. (2016a). Briefly, we extracted total RNA from cricket heads (n=48; 3 biological
184 replicates for each sex, morph, social treatment and incubator, Table S2) using TRIzol plus
185 RNA purification kits (Life Technologies) and PureLink DNase treatment (Invitrogen),
186 followed by Qubit (Invitrogen) and Bioanalyser (Agilent) quantification and quality control.
187 We depleted total RNA with RiboZero following the manufacturer's protocol. Purified RNA
188 was checked for depletion and then libraries were constructed using the ScriptSeq protocol
189 (Epicentre). After fragmentation and conversion to cDNA, samples were purified with
190 Ampure XP beads, barcoded, PCR amplified for 14 cycles, and multiplexed. We checked
191 quantity and quality of final pools and performed qPCR using Illumina Library Quantification
192 Kits (Kapa) on a Roche Light Cycler LC480II. Denatured DNA was loaded at 9 pM with 1%
193 fragmented phage PhiX DNA spiked-in, then sequenced on an Illumina HiSeq 2000 (2×100 bp
194 paired end reads).

195

196 **RNA-seq data analysis**

197 Data analysis was conducted following the same pipeline as described in Pascoal *et al*.
198 (2016a). Briefly, CASAVA version 1.8.2 (Illumina), Cutadapt version 1.2.1 (Martin 2011) and
199 Sickle version 1.200 with a minimum window quality score of 20 were used for initial
200 processing and quality control of the data (Table S3). We used Trinity (Grabherr *et al*. 2011)

to create a combined transcriptome assembly using *in silico* normalisation of trimmed read data and a k-mer size of 25bp (Table S4). In common with other transcriptome assemblies, we recovered a large number of contigs and unitigs (Grabherr et al. 2011) (Table S4). These may relate to different isoforms or different exons deriving from the same gene, and differential expression of these transcripts between genes may therefore reflect differences in either transcription or splicing of genes, both of which may be biologically important. Quantification of transcript abundances was done with RSEM (Li and Dewey 2011): reads were mapped to the *de novo* transcriptome assembly using BOWTIE 2 (Langmead and Salzberg 2012), and expected raw read counts for downstream differential expression (DE) analysis were generated using the mapping BAM files. Prior to DE analysis, we applied a minimum expression level filter by only retaining transcripts that had non-zero counts in at least 6 samples, which is the number of samples in a group and thus the minimum number of non-zero samples likely to be biologically informative. It is possible to implement additional filtering by removing transcripts for which expression levels are lower than 1 count per million (cpm) in a specified number of groups; however, this must be balanced against the anti-conservative effect of increasing the false discovery rate when the number of DE transcripts recovered is reduced. We therefore present results based on data filtered as above, but performed additional filtering for the analysis presented in Figure 1 and verified that it does not qualitatively change the main patterns recovered (Fig. S6).

Read numbers mapping to each transcript were modelled with negative binomial error distributions using edgeR (Robinson *et al*. 2010). We implemented generalized linear models (GLMs) containing each of the three factors of interest (sex, morph and acoustic treatment) plus all two-way and three-way interactions. Normalisation factors were calculated to correct for differences in library size among samples, which might otherwise

cause bias in differential gene expression analysis. The "TMM" (Trimmed Mean M-values) method in edgeR (Robinson *et al*. 2010) was applied, with default parameters. Common, trended and tag-wise dispersion parameters were estimated. Tagwise dispersion was used for fold change estimating and significance testing. The estimated $\log_2$ fold change for each of the models and contrasts were tested in edgeR using a likelihood-ratios (LR) test (Wilks 1938). P-values associated with logFC (log2 fold change) were adjusted for multiple testing such that genes with a false discovery rate (FDR) adjusted P-value < 5% were defined as significantly differentially expressed (Benjamini and Hochberg 1995).

Pairwise comparisons of major interest (i.e. normal-wing male song vs. normal-wing male no song; flatwing male song vs. flatwing male no song; normal-wing female song vs. normal-wing female no song; flatwing female song vs. flatwing female no song; all females vs. flatwing males and all females vs. normal-wing males) were also tested. To visualise whether and how overall patterns of gene expression separated samples by sex, genotype and acoustic treatment, a multidimensional scaling (MDS) plot was drawn using the plotMDS function in edgeR applied to all transcripts. We used Trinotate (trinotate.sourceforge.net/) to annotate the transcriptome and DE sequences and Blast2GO (http://www.blast2go.com) (Conesa *et al*. 2005) to create gene ontology outputs.

Nanostring validation

To validate the RNA-seq data, we used Nanostring technology with a subset of 32 target probes that allowed us to analyse the same 48 samples used for the RNA-seq experiment. Nanostring technology directly obtains sample read count numbers without the need for cDNA synthesis and intermediate PCRs. Each selected probe represents an individual transcript or a group of transcript isoforms with the same gene expression pattern. For the

list of probes to test (nCounter CodeSet) we included: i) gene annotations of interest, ii) transcripts that were simultaneously DE in different contrasts (referred as overlap probes), iii) up- and down-regulated transcripts for each of the individual contrasts and iv) transcripts that were not DE in RNA-seq. 100 ng of total RNA, as quantified by Qubit assay, was used for each hybridization assay in a volume of 5 µl. Hybridisation buffer, reporter CodeSet and Capture probe set was added to each sample and incubated overnight (16-18H) at 65°C, according to manufacturer's instructions. Samples were handled in groups of 12. After hybridization, the samples were washed and loaded onto an nCounter cartridge. Each prepared cartridge was loaded into the counter with the associated CodeSet definition file allowing count generation for each transcript, including the negative and positive controls.

Data analysis was performed using the NanoString software nSolver Analysis Software Version 2.5.34. Background subtraction was done using all internal Nanostring negative controls, normalization was obtained using the internal Nanostring positive controls and 3 reference transcripts that were not DE in the RNA-seq experiment, and fold change ratios were estimated using data partitioning with NormalMaleSong treatment as baseline. Different normalization (just using the internal positive controls) and fold change methods (pairwise) were also tested but did not differ from the previous results. We chose to use the portioned method for fold change analysis because the same baseline was used in the RNA-seq global GLM analysis (dataset upon which the CodeSet selection was based). A direct fold change comparison for the different contrasts (sex, morph and acoustic treatment) between Nanostring and RNA-seq datasets was performed. Regression and paired t-test sample analyses were performed in SPSS Statistics 22.

Results

Neural gene expression

We assembled and characterised *de novo* transcriptomes for *T. oceanicus* (Tables S3-S5), generating a combined assembly to facilitate differential expression (DE) analysis. *T. oceanicus* lacks an annotated reference genome and is distantly-related to commonly employed model insects such as *Drosophila melanogaster*, so we performed expression analyses *de novo* at the level of isoforms. We recovered a characteristically large number of contigs and unitigs as a result, and we collectively refer to these as 'transcripts' for convenience. Our comparisons did not depend on the presence of annotation information, so we utilised the entire set of annotated and unannotated transcripts and followed this with homology-based identity and functional categorization where possible. Nanostring analysis performed on the same 48 samples used for RNA-seq yielded consistent results (see Figs. S1 and S2).

In a model that combined data from all treatments, sex differences accounted for the largest number of differentially-expressed neural transcripts (Fig. 1). Gene expression also differed between flatwing and normal-wing genotypes, and between acoustic treatments (Fig. 1). Gene Ontology (GO) terms associated with the latter group of socially-mediated plastic transcripts included sensory perception of sound, smell, touch; locomotion; and spermatogenesis, which correspond with known behavioural, physiological and morphological responses to the acoustic environment in this species, in particular the tendency of males to strategically allocate sperm resources depending on the perceived presence of rival males (Bailey *et al*. 2010; Gray and Simmons 2013).

Flatwing and normal-wing neural transcriptomes respond differently to the acoustic

environment

There were considerable differences in neural gene expression between flatwing and normal-wing genotypes, and annotations of interest included *rhomboid*, *hedgehog*, and *wingless*. Crucially, the morph genotypes showed different neural gene expression responses to the acoustic treatments. Interaction terms in the global model of gene expression illustrated the latter point: 7,927 transcripts showed different responses across acoustic treatments in males versus females (sex*acoustic treatment interaction), and 6,982 transcripts showed different responses across acoustic treatments in flatwing versus normal-wing crickets (morph*acoustic treatment interaction) (Fig. 1).

The large number of transcripts that showed different patterns of socially-mediated transcriptome plasticity in flatwing versus normal-wing genotypes (Fig. 1) supported the prediction that socially-mediated transcriptome plasticity is coevolving with the genetic mutation(s) that cause flatwing. Given our interest in the differential sensitivity of flatwing and normal-wing crickets to the social environment, we followed up our global analysis of transcriptome variation with individual pairwise contrasts testing differential expression between “song” and “no song” treatments in each of the four classes of cricket: normal-wing and flatwing males and females. This analysis was designed to investigate whether and how sexes and morphs differ in socially-mediated plastic gene expression, and it confirmed our main result: flatwing and normal-wing genotypes show strikingly different patterns of transcriptome plasticity (Fig. 2). Very few transcripts were differentially expressed between acoustic environments in normal-wing crickets, whereas flatwing crickets showed considerable transcriptomic responses to the social environment (Fig. 2, see also Fig. S3).

Thus the dominant pattern underlying transcripts recovered from the morph*acoustic interaction term in the main GLM is differential expression in flatwings

320 across social environments, but little to negligible socially-mediated plasticity in normal-wing
321 crickets. Gene expression also responded differently to the social environment in male
322 versus female neural tissue: there was no overlap of DE transcripts between the sexes. The
323 lack of overlap is in agreement with the finding above that a significant number of
324 transcripts show sexually dimorphic responses to the acoustic environment. While flatwing
325 genotypes showed greater plasticity than normal-wing genotypes (χ^2 = 767.30, df = 1, p <
326 0.001), flatwing males showed greater transcriptome sensitivity to the acoustic environment
327 than flatwing females (χ^2 = 206.32, df = 1, p < 0.001). The pattern of sex differences was
328 reversed in normal-wing crickets, although this is based on a very small number of DE
329 transcripts recovered in the normal-wing comparison (n = 15 in normal-wing females, versus
330 zero in normal-wing males) (χ^2 = 15.00, df = 1, p = 0.001).

331 In pairwise comparisons, only 15 transcripts showed socially-mediated plasticity in
332 normal-wing females. Nevertheless, GO analysis recovered annotations including response
333 to stimulus and locomotion among these, again consistent with prior findings about
334 flexibility in female mate choice and searching behaviours. Flatwing males showed 610
335 differentially expressed transcripts between acoustic treatments and 30% (n=179) had
336 annotations including GO terms such as localization, response to stimulus, signalling,
337 reproduction, reproductive process, and locomotion. Female flatwings had 201 DE
338 transcripts but only 6% (n=12) had associated annotations; this may reflect male-biased
339 availability in public datasets.

340 A final set of analyses tested how morph genotype, acoustic treatment effects and
341 their interaction impacted the transcriptomes of each sex separately. These broadly
342 supported our previous findings, and indicated that although both sexes show expression
343 variation depending on whether they carry flatwing vs. normal-wing alleles, the bulk of

plastic expression variation between morph genotypes appears to be driven by males. We interrogated patterns of socially-mediated plasticity between the morphs in greater detail by performing a clustering analysis of the 5,547 transcripts recovered in the morph*acoustic interaction term in the males-only analysis (Fig. 3). This analysis was only done for males owing to a paucity of differentially-expressed transcripts in females (see Table S6 and Fig. S4). The analysis produced 11 clusters describing differences in the way that gene expression was governed by the social environment in normal-wing versus flatwing males. Overall, expression differences appeared to be more extreme between social environments in flatwing males, although some transcripts showed reversed patterns of socially-mediated plasticity. For example, cluster 1 transcripts were downregulated in the “song” treatment compared to the “no song” treatment in flatwing males, whereas they were upregulated in the “song” treatment in normal-wing males. A similar reversal occurred in the opposite direction in cluster 3. Such patterns exemplify crossing reaction norms. In contrast, transcripts in cluster 7 and 11 appear to be downregulated in the “song” environment in flatwing males, but with little to no differential expression in normal-wing males. An assessment of functional annotations associated with transcripts in each cluster revealed several suggestive patterns related to behavioural phenotypes. For example, both clusters 7 and 11 contained transcripts with GO terms describing locomotor behaviour, and sensory perception of sound was annotated in clusters 7, 9, 10, and 11. Additional behavioural annotations included flight from cluster 6, inter-male aggression from cluster 7, and male courtship from cluster 11.

Nearly half (45%) of the 5,547 transcripts implicated in the male morph*acoustic interaction had an associated annotation. Metabolic and cellular processes were highly represented, and biologically relevant recovered GO terms include response to stimulus,

368 developmental process, reproduction, locomotion, reproductive process, behaviour,
369 immune system process and growth (Fig. S5). These enriched GO terms are suggestive of
370 differences in the mechanisms by which flatwing and normal-wing genotypes respond to
371 acoustic cues in their rearing environment. Previous experiments have provided evidence
372 that each of these processes are affected by exposure to the acoustic environment during
373 development and rearing, providing corroboration for gene expression data, and potential
374 candidates for future study of the functional genomics of socially-mediated plasticity.

375

376 **Transcriptome feminisation and sex differences in plasticity**

377 The nearly 7,000 transcripts identified as significant in the overall sex*morph interaction
378 (Fig. 1) suggested that brain transcriptomes showed different levels of sex-biased expression
379 in the two morphs. A comparison of differential expression between flatwing males versus
380 all females, and between normal-wing males versus all females, revealed that there were
381 fewer sex differences in flatwing male brain transcriptomes compared to normal-wing male
382 brain transcriptomes (Fig. 4a) ($\chi^2 = 2011.79$, df = 1, $p < 0.001$). Flatwing males thus had more
383 female-like patterns of neural gene expression. We used multidimensional scaling (MDS) to
384 plot similarities among samples in expression measured across all transcripts (Fig. 4b). The
385 first and second dimensions separated the sexes and morph genotypes, respectively. As with
386 the previous analysis, flatwing male brain transcriptomes appeared more female-like than
387 those of normal-wing males, but this feminisation was most prominent in flatwing males
388 that had been reared in silence (Fig. 4b). Thus, flatwing males not only showed the greatest
389 degree of transcriptome plasticity in response to acoustic signals in their environment, but
390 their exposure to song appeared to mitigate female-like patterns of gene expression in the
391 brain. Despite the fact that expression of the flatwing phenotype is sex-limited, female

carriers of the flatwing mutation(s) also showed altered neural gene expression compared to normal-wing females. On average, expression patterns differed the most between normal-wing males and flatwing females, although neural expression differences between genotypes were more pronounced in males than in females (Fig. 4b).

The pattern of transcriptome feminisation in flatwing males is consistent with the well-documented female-like venation patterns on their forewings (Zuk *et al*. 2006), and it is notable that both *doublesex* and *fruitless* were identified as differentially expressed between the sexes. However, female-like expression patterns of flatwing brains are not consistent with the idea that the causative mutation(s) underlying flatwing exert effects that are strictly compartmentalised to wing venation. Instead, flatwing and normal genotypes appear to constitutively differ in the expression of brain transcripts, suggesting widespread genomic effects associated with the mutation(s) arising either through pleiotropy, linkage disequilibrium, or coevolution (Pascoal *et al*. 2016a).

Discussion

There is much debate and controversy concerning the role of phenotypic plasticity in evolutionary change, and both adaptive and non-adaptive plasticity have been proposed to increase the likelihood of adaptive evolution (West-Eberhard 2005; Ghalambor *et al*. 2015). Plasticity can create opportunities for divergent selection to act, accelerate responses to selection, pre-adapt populations to respond to novel selective pressures, increase the likelihood of diversification, or deflect the effects of selection (West-Eberhard 1989; West-Eberhard 2003; DeWitt and Scheiner 2004; West-Eberhard 2005; Wund 2012; Zuk *et al*. 2014). These predictions have received mixed empirical support. Comparative work has

415 linked diversity with patterns of ancestral plasticity in spadefoot toad species (Gomez-
416 Mestre and Buchholz 2006), and patterns of plasticity have been found to recapitulate
417 macroevolutionary patterns of trait divergence in *Polypterus*, the ray-finned fishes (Standen
418 *et al*. 2014). Despite the intense interest and focus this topic has received, however,
419 plasticity is often treated as a static property, rather than an evolvable quantity. For
420 example, the idea that pre-existing phenotypic plasticity acts as a pre-adaptation is
421 appealing, and has received support in the cricket system we used here (Bailey *et al*. 2008;
422 Tinghitella *et al*. 2009; Zuk *et al*. 2014), yet we still do not understand how plasticity interacts
423 with traits under selection throughout the ongoing process of adaptive evolution. Our
424 findings in *Teleogryllus oceanicus* reveal a genetic association between a rapidly evolving
425 genotype and plasticity in neural gene expression supporting the view that plasticity itself is
426 subject to evolutionary forces, and, in particular, can increase during the early stages of
427 adaptive evolution in line with theoretical predictions (West-Eberhard 2005; Garland and
428 Kelly 2006; Lande 2009). Box 1 and Fig. 5 provide a graphical description and explanation of
429 this process.

430 Prior work has revealed acoustically-mediated plasticity in a broad spectrum of traits
431 related to mating and reproduction in *T. oceanicus* from the island of Kauai, where alleles
432 causing the erasure of sound-producing structures on male wings have rapidly spread,
433 almost always in a manner that would be expected to increase fitness in a silent
434 environment dominated by silent flatwing males (Zuk *et al*. 2006; Pascoal *et al*. 2014; Zuk *et*
435 *al.* 2014). The constitutive difference in acoustically-mediated plastic gene expression in *T.*
436 *oceanicus* crickets carrying flatwing versus normal-wing genotypes is consistent with the
437 rapid evolution of increased plasticity in neural gene expression in flatwing genotypes –
438 increased plasticity to the acoustic environment accompanied the rapid spread of flatwing.

In contrast, we recovered very few socially-mediated plastic transcripts in crickets carrying normal-wing genotypes; in individual comparisons for normal-wing males, there were none. Flatwings of either sex, however, showed hundreds of transcripts DE between social environments. While it is possible that a single, or very few, transcripts could control responses to the social environment at the phenotypic level in female crickets carrying normal-wing genotypes, for example if some genes within regulatory networks exert greater control over such plasticity than others, they nevertheless exhibited a different pattern of neural transcriptome plasticity than females carrying the recently-derived flatwing genotype. Both the order of magnitude difference in the number of socially-cued DE transcripts between morph genotypes in pairwise comparisons and the existence of nearly a dozen distinct expression clusters in the morph*acoustic environment interaction for males, indicated that numerous genetic modules are implicated in responses to acoustic social cues.

It is unclear whether the socially-mediated plasticity in gene expression we have documented is causally linked to adaptive phenotypic responses. For example, enhanced adaptive plasticity is expected following episodes of rapid adaptation to extreme environmental pressures (Lande 2009), although this may be accompanied by the release of cryptic genetic variation for both adaptive and non-adaptive plasticity (Fischer et al. 2016). In situations where non-adaptive plastic responses to environmental change enhance responses to directional selection by exposing cryptic variation, those plastic responses that persist in newly-adapted populations may be of lower magnitude, but are likely to lie along adaptive phenotypic trajectories (Ghalambor *et al*. 2015, though see Crispo et al. 2010). We note that exposure to song in the acoustic environment of *T. oceanicus* appeared not to change neural transcriptomes in the same direction as morph-associated changes, but instead predominately shifted transcriptome profiles along a sex-biased gene expression axis

463 (x-axis on MDS plot in Fig. 4b) in a male-biased direction.

464 Evidence from other systems suggests that stress responses may represent a
465 frequent underlying mechanism for acoustically-induced expression changes. Acoustically-
466 mediated plasticity has been suggested to facilitate adaptive responses to the presence of
467 signalling rivals in other cricket species (*T. commodus*; Kasumovic et al. 2011) and to
468 anthropogenic noise pollution in birds (the nightingale *Luscinia megarhynchos*; Brumm
469 2004). In *Drosophila melanogaster*, courtship song signals activate stress-related gene
470 expression pathways (Immonen and Ritchie 2012), and in the zebrafish *Danio rerio*, gene
471 expression changes in the inner ear have been linked to recovery from trauma caused by
472 over-exposure to extremely loud (179 dB) stimuli (Schuck et al. 2011). A future objective in *T.*
473 *oceanicus* is therefore to determine whether enhanced brain transcriptome plasticity
474 associated with flatwing genotypes is causally linked to adaptive phenotypic responses,
475 either as a mechanistic driver of those responses or as a consequence of them (Mateus *et al.*
476 2014; Aubin-Horth et al. 2005).

477 We would not have expected a difference in plastic responses of flatwing and
478 normal-wing genotypes if the average genotype in the population had been subjected to
479 similar selection favouring the rapid evolution of socially-mediated plasticity. It appears that
480 the initial spread of flatwing was facilitated by pre-existing plasticity, followed by further
481 differential selection on plasticity in flatwing versus normal-wing genotypes. It is important
482 to note that pre-existing genotypic variation in plasticity is necessary for plasticity to
483 subsequently evolve: the existence of reaction norm variation prior to dramatic
484 environmental change favouring increased plasticity is a key assumption of the Lande (2009)
485 model. There is evidence for such reaction norm variation in *T. oceanicus* (Bailey and Zuk
486 2012), and it seems likely that the different morphs experience distinct selective pressures

because of the differences in both parasitoid attack rates and mating tactics employed by either type of male (Zuk *et al*. 2006). Because of the short timeframe in which the evolution and spread of flatwing has taken place, the difference in plasticity between flatwing versus normal-wing genotypes strongly suggests a pleiotropic effect of flatwing allele(s) or loci maintained in linkage disequilibrium. Rapid evolution of *de novo* physical linkage is an unlikely scenario. Two intriguing possibilities are that both morphs may demonstrate plasticity at the level of observable reproductive or physiological phenotypes, yet be subject to different environmental triggers or neurogenomic mechanisms of socially-mediated plasticity, or that selection has favoured canalized responses to the social environment in normal-wing genotypes, with correspondingly different consequences for plastic changes in the brain transcriptome (Cardoso *et al.* 2015).

The constitutive differences in how flatwing and normal-wing transcriptomes respond to cues in the social environment support key theoretical predictions about the coevolution of plasticity with novel adaptations. Lande (2009) and others (West-Eberhard 2005; Garland and Kelly 2006) predict a rapid evolutionary increase in plasticity at the onset of dramatic environmental changes. In Hawaiian *T. oceanicus*, the acoustic environment underwent an abrupt and profound change because of the rapid spread of silent males: in the span of several dozen generations, the population on Kauai shifted from one in which long-range acoustic signals were the dominant mode of social communication, to a population effectively depauperate in song (Zuk *et al*. 2006). Feedback between the rapid change from a song-rich to a silent environment, and plasticity in response to the acoustic environment, appears to have created a situation favourable for the rapid coevolution of socially-cued plasticity and alleles that cause the silent flatwing phenotype. Over time, genetic assimilation is predicted to more permanently link these traits, but it is likely to

511 occur on the order of hundreds to thousands of generations, not dozens (Box 1) (Lande
512 2009). Similar feedback effects are pervasive in evolving systems (Crespi 2004), and the
513 relationship between flatwing and transcriptome plasticity in *T. oceanicus* demonstrates
514 how the general impact of phenotypic plasticity on evolutionary change in other systems is
515 likely to be inextricably linked to its own coevolution with traits under selection.

516

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525

526 References

527 Aubin-Horth N, Renn SCP. 2009. Genomic reaction norms: using integrative biology to understand
528 molecular mechanisms of phenotypic plasticity. Mol Ecol. 18: 3763-3780.

529 Aubin-Horth N, Landry CR, Letcher BH, Hofmann HA. 2005. Alternative life histories shape brain gene
530 expression profiles in males of the same population. Proc Roy Soc Lond B. 272: 1655–1662.

531 Baldwin JM. A new factor in evolution. 1896. Am Nat. 30: 441-451, 536-553.

532 Bailey NW. 2012. Evolutionary models of extended phenotypes. Trends Ecol Evol. 27: 561-569.

533 Bailey NW, Macleod E. 2014. Socially flexible female choice and premating isolation in field crickets
534 (*Teleogryllus* spp.) J Evol Biol. 27:170-180

535 Bailey NW, Zuk M. 2012. Socially flexible female choice differs among populations of the Pacific field
536 cricket: geographic variation in the interaction coefficient psi (Ψ). Proc Roy Soc Lond B. 279: 3589-
537 3596.

538 Bailey NW, Gray B, Zuk M. 2011. Exposure to sexual signals during rearing increases immune defence
539 in adult field crickets. Biol Lett. 7: 217-220.

540 Bailey NW, Gray B, Zuk M. 2010. Acoustic experience shapes alternative mating tactics and
541 reproductive investment in male field crickets. Curr Biol. 20: 845-849.

542 Bailey NW, McNabb JR, Zuk M. 2008. Preexisting behavior facilitated the loss of a sexual signal in the
543 field cricket *Teleogryllus oceanicus.* Behav Ecol. 19: 202-207.

544 Bailey NW, Zuk M. 2008. Acoustic experience shapes female mate choice in field crickets. Proc Roy
545 Soc Lond B. 275: 2645-2650.

546 Balenger SL, Zuk M. 2015. Roaming Romeos: male crickets evolving in silence show increased
547 locomotor behaviours. Anim Behav. 101: 213-219.

548 Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful
549 approach to multiple testing. J Roy Stat Soc B. 57: 289-300.

550 Brumm H. 2004. The impact of environmental noise on song amplitude in a territorial bird. J Anim
551 Ecol. 73:434-440.

552 Cade W. 1975. Acoustically orienting parasitoids: fly phonotaxis to cricket song. Science. 190: 1312-
553 1313.

554 Cardoso SD, Teles MC, Oliveira RF (2015) 2015. Neurogenomic mechanisms of social plasticity. J Exp
555 Biol. 218: 140-149.

556 Crespi BJ. 2004. Vicious circles: positive feedback in major evolutionary and ecological transitions.
557 Trends Ecol Evol. 19: 627-633.

558 Crispo E, DiBattista JD, Correa C, Thibert-Plante X, McKellar AE, Schwartz AK, Berner D, De Léon LF,
559 Hendry AP. 2010. The evolution of phenotypic plasticity in response to anthropogenic disturbance.
560 Evol Ecol Res. 12:47-66.

561 Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: a universal tool for
562 annotation, visualization and analysis in functional genomics research. Bioinform. 21: 3674–3676.

563 DeWitt TJ, Scheiner SM. 2004.Phenotypic plasticity: functional and conceptual approaches. Oxford
564 University Press, Oxford.

565 Fischer EK, Ghalambour CK, Hoke KL. 2016. Can a network approach resolve how adaptive vs
566 nonadaptive plasticity impacts evolutionary trajectories? Int Comp Biol 56:877-888.

567 Garland Jr T, Kelly SA. 2006. Phenotypic plasticity and experimental evolution. J Exp Biol. 209: 2344-
568 2361.

569 Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015. Non-adaptive plasticity
570 potentiates rapid evolution of gene expression in nature. Nature. 525: 372-375.

571 Ghalambor CK, McKay JK, Carroll S, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic
572 plasticity and the potential for contemporary adaptation in new environments. Funct Ecol. 21:
573 394-407.

574 Gibson G, Dworkin I. 2004. Uncovering cryptic genetic variation. Nat Rev Genet. 5: 681-690.

575 Gomez-Mestre I, Buchholz DR. 2006. Developmental plasticity mirrors differences among taxa in
576 spadefoot toads linking plasticity and diversity. Proc Natl Acad Sci USA. 103: 19021-19026.

577 Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. 2011. Full-length
578 transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotech. 29: 644-
579 652.

580 Gray B, Simmons LW. 2013. Acoustic cues alter perceived sperm competition risk in the field cricket
581 *Teleogryllus oceanicus*. Behav Ecol. 24: 982-986.

582 Immonen E, Ritchie MG. 2012. The genomic response to courtship song stimulation in female
583 *Drosophila melanogaster*. Proc R Soc Lond B. 279:1359-1365.

584 Kasumovic MM, Hall MD, Try H, Brooks RC. 2011. The importance of listening: juvenile allocation
585 shifts in response to acoustic cues of the social environment. J Evol Biol. 24:1325-1334.

586 Lande R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and
587 genetic assimilation. *J Evol Biol.* 22: 1435-1445.

588 Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nature Method. 9: 357-
589 359.

590 Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a
591 reference genome. BMC Bioinform. 12: 323.

592 Logue DM, Abiola IO, Rains D, Bailey NW, Zuk M, Cade WH. 2010. Does signalling mitigate the costs
593 of agonistic interactions? A test in a cricket that has lost its song. Proc Roy Soc Lond B. 277: 2571-
594 2572.

595 Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.
596 EMBnet.journal. 17: 10-12.

597 Mateus ARA, Marques-Pita M, Oostra V, Lafuente E, Brakefield PM, Zwaan BJ, Beldade P. 2014.
598 Adaptive developmental plasticity: Compartmentalized responses to environmental cues and to
599 corresponding internal signals provide phenotypic flexibility. BMC Biol. 12: 97.

600 Nyman C, Fischer S, Aubin-Horth N, Taborsky B. 2017. Effect of the early social environment on
601 behavioural and genomic responses to a social challenge in a cooperatively breeding vertebrate.
602 Mol Ecol. 26: 3186-3203.

603 Pascoal S, Liu X, Ly T, Fang Y, Rockliffe N, Paterson S, et al. 2016a. Rapid evolution and gene
604 expression: a rapidly-evolving Mendelian trait that silences field crickets has widespread effects on
605 mRNA and protein expression. J Evol Biol. 29: 1234-1246.

606 Pascoal S, Mendrok M, Mitchell C, Wilson AJ, Hunt J, Bailey NW. 2016b. Sexual selection and
607 population divergence I. The influence of socially flexible cuticular hydrocarbon expression in male
608 field crickets (*Teleogryllus oceanicus*). Evolution. 70: 82-97.

609 Pascoal S, Cezard T, Eik-Nes A, Gharbi K, Majewska J, Payne E, et al. 2014. Rapid convergent evolution
610 in wild crickets. *Curr Biol.* 24: 1369-1374.

611 Raymond M, Berticat C, Weill M, Pasteur N, Chevillon C. 2001. Insecticide resistance in the mosquito
612 *Culex pipiens*: what have we learned about adaptation? Genetica. 112-113: 287-296.

613 Robinson M, McCarthy D, Smyth G. 2010. edgeR: a Bioconductor package for differential expression
614 analysis of digital gene expression data. Bioinform. 26: 139-140.

615 Rotenberry JT, Swanger E, Zuk M. 2015. Alternative reproductive tactics arising from a continuous
616 behavioral trait: Callers versus satellites in field crickets. Am Nat. 185: 469-490.

617 Rotenberry JT, Zuk M, Simmons LW, Hayes C. 1996. Phonotactic parasitoids and cricket song
618 structure: an evaluation of alternative hypotheses. Evol Ecol. 10: 233-243.

619 Schildberger K, Huber F, Wohlers DW. 1989. Central auditory pathway: neuronal correlates of
620 phonotactic behavior. In: Cricket Behavior and Neurobiology (Eds. Huber F, Moore TE, Loher W.)
621 Cornell University Press, Ithaca, NY.

622 Schradin C. 2013. Intraspecific variation in social organization by genetic variation, developmental
623 plasticity, social flexibility or entirely extrinsic factors. Phil Trans Roy Soc Lond B. 368: 20120346.

624 Schuck JB, Sun H, Penberthy WT, Cooper NGF, Li X, Smith ME. 2011. Transcriptome analysis of the
625 zebrafish inner ear points to growth hormone mediated regeneration following acoustic trauma.
626 BMC Neurosci. 12:88.

627 Scoville AG, Pfrender ME. 2010. Phenotypic plasticity facilitates recurrent rapid adaptation to
628 introduced predators. Proc Natl Acad Sci USA. 107: 4260-4263.

629 Standen EM, Du TY, Larsson HC. 2014. Developmental plasticity and the origin of the tetrapods.
630 Nature. 513: 54-58.

631 Stoks R, Govaert L, Pauwels K, Jansen B, De Meester L. 2015. Resurrecting complexity: the interplay
632 of plasticity and rapid evolution in the multiple trait response to strong changes in predation
633 pressure in the water flea *Daphnia magna*. Ecol Lett. 19: 180-190.

634 Stuglik MT, Babik W, Prokop Z, Radwan J. 2014. Alternative reproductive tactics and sex-biased gene
635 expression: the study of the bulb mite transcriptome. Ecol Evol. 4: 623-632.

636 Thomas ML, Gray B, Simmons LW. 2011. Male crickets alter the relative expression of cuticular
637 hydrocarbons when exposed to differend acoustic environments. Anim Behav. 82:49-53.

638 Tinghitella RM, Wang JM, Zuk M. 2009. Preexisting behavior renders a mutation adaptive: flexibility
639 in male phonotaxis behavior and the loss of singing ability in the cricket *Teleogryllus oceanicus*.
640 Behav Ecol. 20: 722-728.

641 Tinghitella RM. 2008. Rapid evolutionary change in a sexual signal: genetic control of the mutation
642 'flatwing' that renders male field crickets (*Teleogryllus oceanicus*) mute. Heredity. 100: 261-267.

643 Wang X, Fang X, Yang P, Jiang X, Jiang F, Zhao D, et al. 2014. The locust genome provides insight into
644 swarm formation and long-distance flight. Nat Comm. 5: 2957.

645 West-Eberhard MJ. 2005. Developmental plasticity and the origin of species differences. Proc Natl
646 Acad Sci USA. 102: 6543-6549.

647 West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Syst. 20:
648 249-278.

649 West-Eberhard MJ. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

650 Wilks S. 1938. The large-sample distribution of the likelihood ratio for testing composite hypotheses.
651 Annal Mathemat Stat. 9: 60-62.

652 Wund MA. 2012. Assessing the impacts of phenotypic plasticity on evolution. Integ Comp Biol. 52: 5-
653 15.

654 Zuk M, Bastiaans E, Langkilde T, Swanger E. 2014. The role of behaviour in the establishment of novel
655 traits. Anim Behav. 92: 333-344.

656 Zuk M, Rotenberry JT, Tinghitella RM. 2006. Silent night: adaptive disappearance of a sexual signal in
657 a parasitized population of field crickets. Biol Lett. 2: 521-524.

658 Zuk M, Rotenberry JT, Simmons LW. 1998. Calling songs of field crickets (*Teleogryllus oceanicus*) with
659 and without phonotactic parasitoid infection. Evolution. 52: 166-171.

660 Zuk M, Simmons LW, Cupp, L. 1993. Calling characteristics of parasitized and unparasitized
661 populations of the field cricket *Teleogryllus oceanicus*. Behav Ecol Sociobiol. 33: 339-343.

Figures

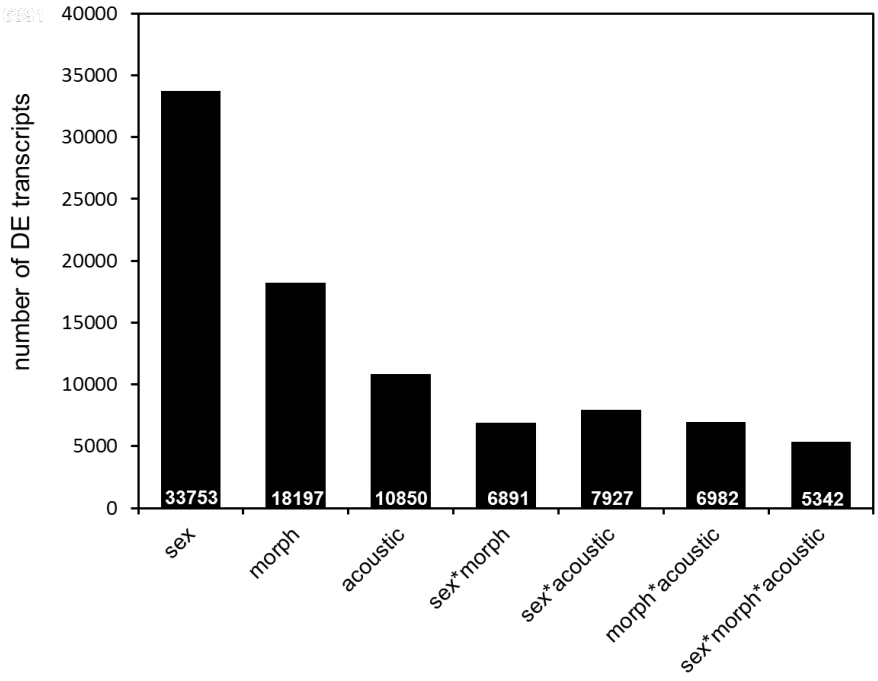
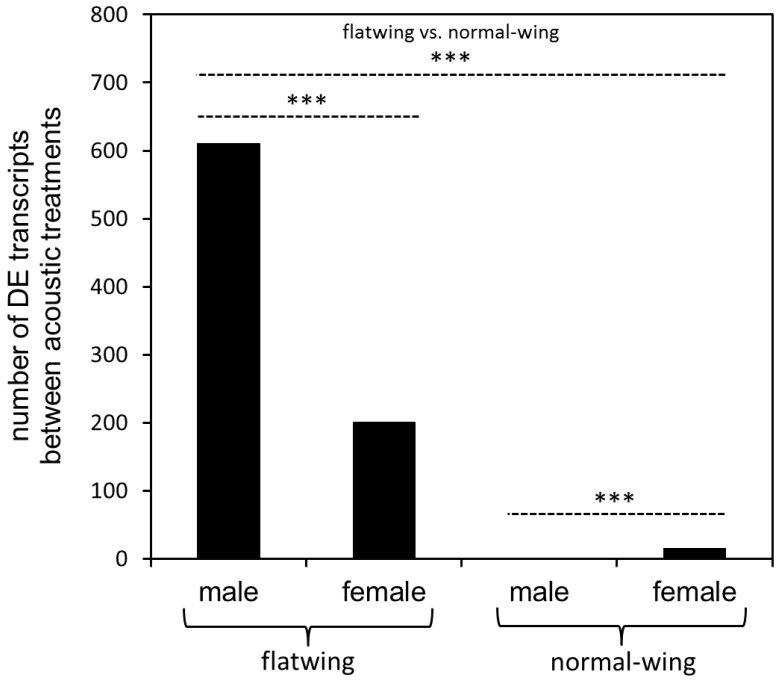


Figure 1 Differential transcript expression in cricket neural tissue. Expression differences were inferred using generalized linear models (GLMs). The bars show numbers (given in white text) of transcripts that were DE between sexes, between wing morphs, and between acoustic treatments. Interaction terms indicate transcripts whose differential expression was not heterogeneous, i.e. not in the same direction or magnitude in different groups.



670

671 **Figure 2 Socially-mediated plasticity in gene expression is constitutively different between**
672 **morph genotypes.** The number of differentially-expressed transcripts in the brains of adult
673 crickets that had been reared in song vs. silence is indicated for each morph and sex.
674 Differential expression was separately assessed for each of the four types of crickets using
675 pairwise comparisons between the “song” and “no song” acoustic treatment groups.
676 Asterisks highlight significant differences in the proportion of differentially expressed
677 transcripts for the comparisons indicated (Chi-square tests using a total of n = 1,545,564
678 observations for all groups. All $p < 0.001$ after Bonferroni correction at $\alpha = 0.0003$

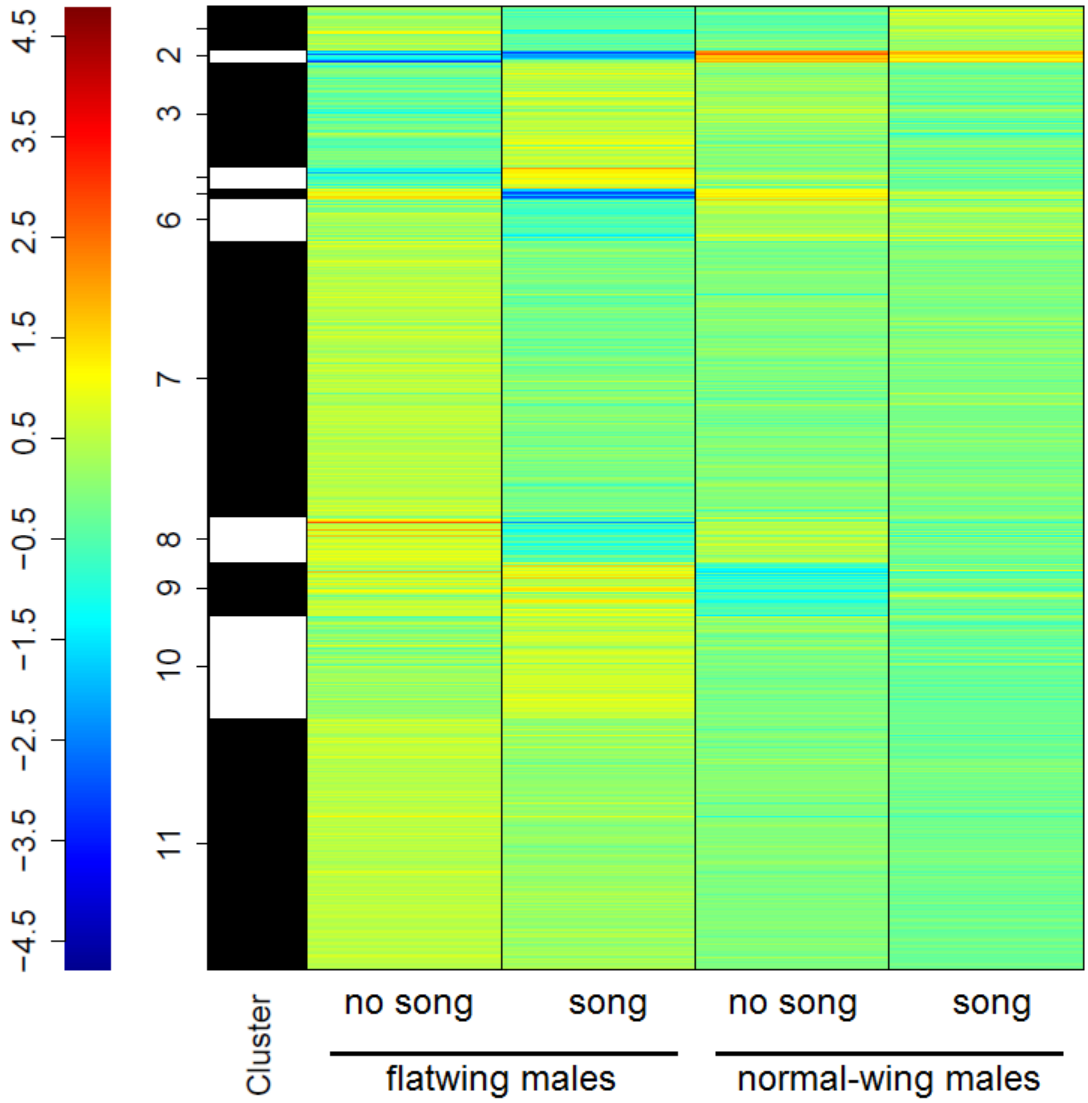


Figure 3 Comparison of socially-mediated gene expression in flatwing vs. normal-wing males. Transcripts whose expression was significant in the morph*acoustic interaction of the male-specific expression analysis are depicted. The significance of the interaction term indicated that the two morph genotypes regulate expression of that transcript differently in response to the acoustic environment. Transcripts are grouped into 11 clusters describing similar patterns of socially-mediated plasticity. The color gradient represents the difference in log2 fold change compared to the across-treatment average, with larger values (red) indicating up-regulation, and smaller values (blue) indicating down regulation. For each gene, data from all samples are zero-centred to facilitate visual interpretation

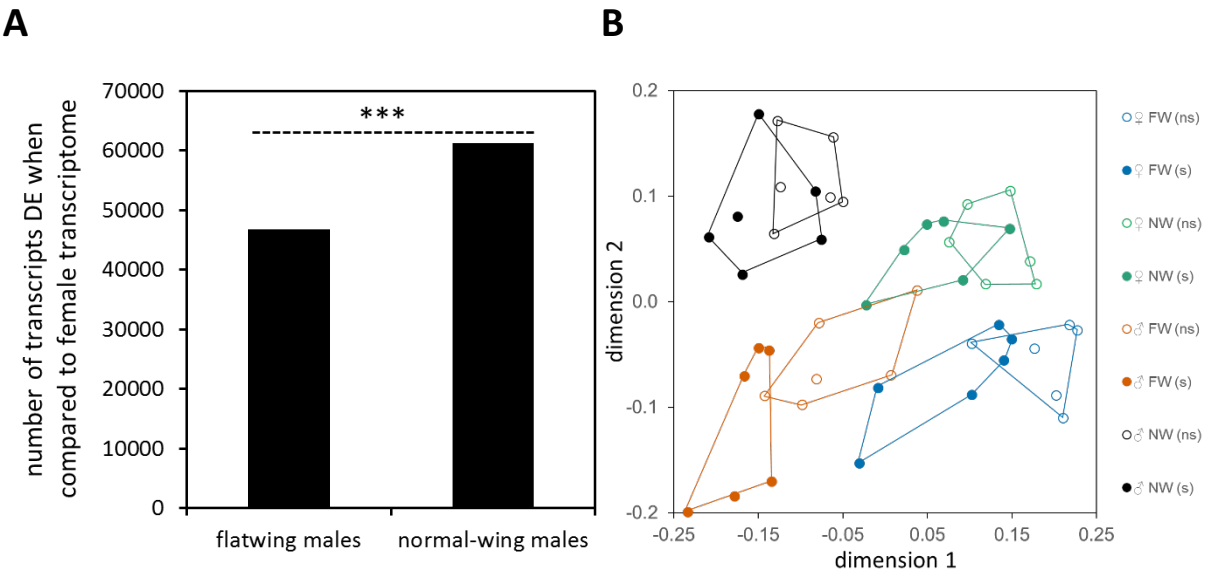
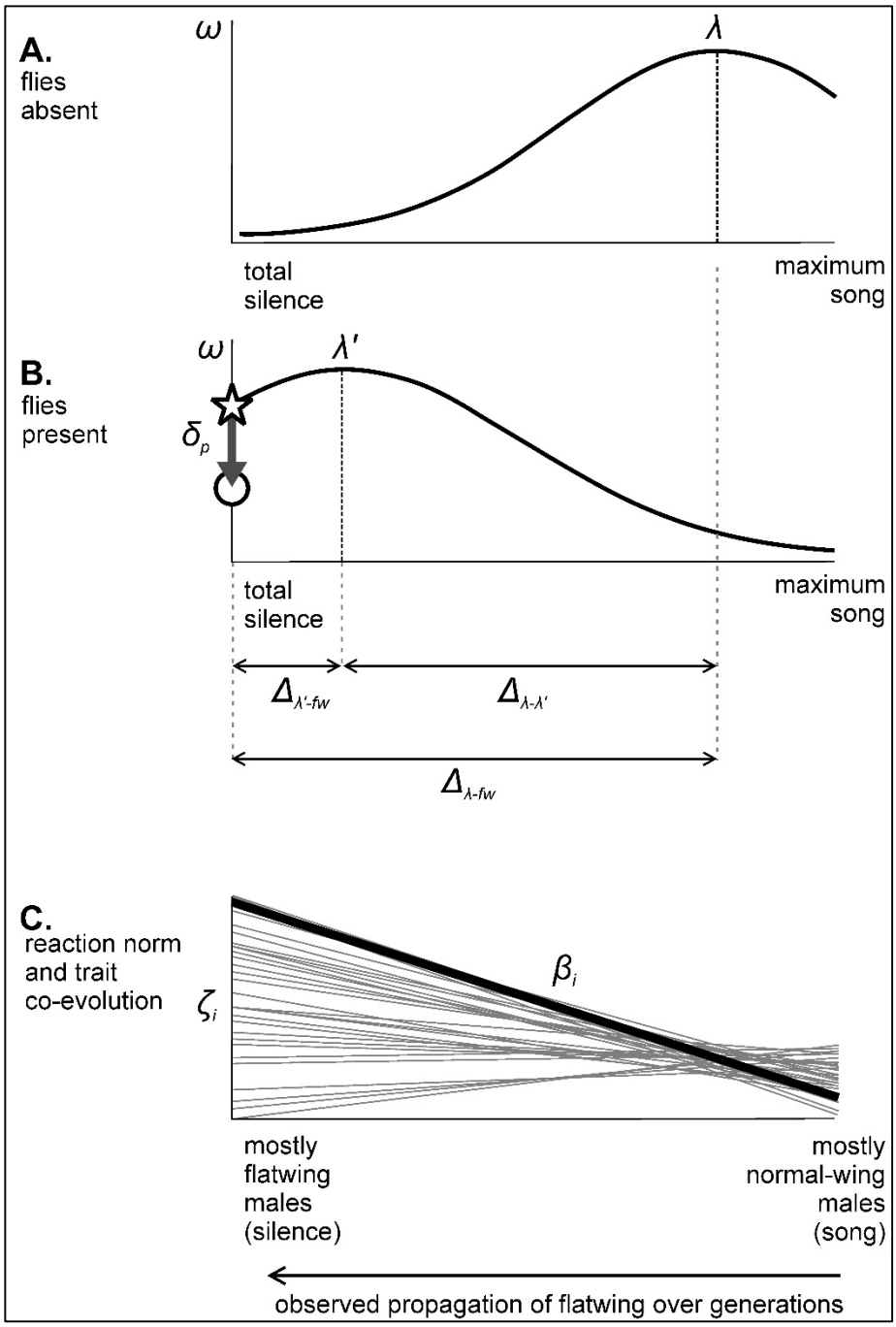


Figure 4 Neural transcriptomes are feminised in flatwing males. (a) Number of transcripts differentially expressed between flatwing males versus all females and between normal-wing males versus all females. Greater similarity between flatwing males and females than between normal-wing males and females indicates transcriptional feminisation of flatwing male neural tissue; asterisks indicate a significant difference ($\chi^2 = 2011.79$, df = 1, $p < 0.001$). **(b)** Multidimensional scaling (MDS) plot showing overall patterns of neural gene expression in each of the 48 samples, for all mapped transcripts. Open symbols represent crickets reared in silence and solid symbols represent those reared with song. Polygons have been drawn to enclose all the replicates of each type of cricket. The factors “sex”, “morph”, and “acoustic treatment” explain 8%, 4%, and 3% of the total variation (Bray distance) in transcriptome profiles, respectively.



704

705 **Figure 5 Schematic illustration depicting coevolution between phenotypic plasticity and a**

706 **novel adaptive phenotype, as described in Box 1.** Panels (a)-(c) illustrate a scenario of rapid

707 evolution of male silence in *Teleogryllus oceanicus*, and a hypothetical role for plasticity

708 based on Lande 2009.

709

Box 1. Rapid coevolution of socially-mediated plasticity and a trait under selection. The evolutionary loss of male song in *Teleogryllus oceanicus* is used as an example (Figure 5).

[A] Hypothetical Gaussian fitness function for male singing tendency in an ancestral environment. The y-axis represents relative male fitness (ω), which depends on how much males sing (x-axis). Song is advantageous owing to its role in mate attraction, courtship and aggression, but energetic and mechanical constraints reduce male fitness beyond an optimal level of song production, λ (Fig. 5A).

[B] Shift of the optimal male singing tendency when acoustically-orienting parasitoids are present. The y-axis still represents relative male fitness (ω) and the x-axis how much males sing. Song still functions in mate acquisition and thus carries a sexually selected benefit. However, optimal levels of male song production are now lower (λ') because of countervailing natural selection exerted by fatal parasitoids that use it to locate hosts. The shift in optimum male phenotype along the x-axis is indicated by $\Delta_{\lambda-\lambda'}$, and can be conceptualised as selection on quantitative variation underlying the tendency to sing, by forcing a shift in the distribution of singers vs. non-singers in the population or alternatively through a change in average behaviour across males. Early field studies found support for the latter (Cade 1975; Zuk *et al*. 1993; Rotenberry *et al.* 1996; Zuk *et al*. 1998). Despite the benefits of song reduction, complete cessation of singing still carries costs, for example because of the need to acquire mates via other means (Bailey *et al.* 2010, Rotenberry *et al.* 2015) and poorer performance in agonistic encounters (Logue *et al*. 2010).

The star indicates the phenotype of obligately silent flatwing males. The invasion of flatwing allele(s) into the population marks the emergence of a new, discrete phenotype favoured because it places males closer to the optimal phenotype when flies are present. If there were no flies, the flatwing male phenotype would carry a severe cost owing to its distance from the population optimum, $\Delta_{\lambda-fw}$, yet when flies are present it clearly confers an advantage despite having "overshot" the optimal phenotype, $\Delta_{\lambda'-fw}$. Flatwing is also known to cause a range negative pleiotropic effects in males that express it: they cannot advertise for or court females, and they experience dysfunction in agonistic encounters (Zuk *et al*. 2006; Bailey *et al*. 2008; Logue *et al*. 2010). Flatwing males also have reduced investment in reproductive tissues (Bailey *et al*. 2010) and partially-feminised cuticular hydrocarbon profiles (unpublished data). The fitness decrement due to negative pleiotropy in flatwing males, δ_p, is indicated by the solid grey arrow, which shows how the potential maximum fitness benefits of flatwing (star) exceed the realised fitness benefits (circle). Plasticity to the changed signalling environment caused by the spread of silent flatwing males is known to enable males to mitigate consequences of obligate silence, reducing the fitness decrement δ_p associated with flatwing (Fig. 5B).

[C] Evolution of phenotypic plasticity during "extraordinary" environmental change caused by proliferation of silent flatwing males. Here, the y-axis represents a generic trait ζ_i that mitigates negative pleiotropic effects of flatwing by responding to the acoustic social environment—for example, the tendency of males to adopt satellite mating tactics. The x-axis now represents the proportion of flatwing males present in the population, which determines the amount of song present within the environment. Here, we consider the shift towards a silent social environment an "extraordinary" environmental change, cf. Lande (2009). An optimal reaction norm with slope β_i is indicated by the thick line, and selection

will favour individuals expressing phenotypes close to this line. If there is genetic variation for plasticity, for example as a result of past environmental stochasticity caused by demographic fluctuations or environmental signal interference (indicated by "silence" and "song" in parentheses on the x-axis), then reaction norms for individual genotypes are predicted to be distributed as indicated by the light grey lines, with little genetic variance available to selection under ordinary environmental circumstances that characterise populations rich in singing, normal-wing males, but with increasing exposure of cryptic genetic variation as the social environment shifts due to the proliferation of flatwing males (Gibson and Dworkin 2004). As the environment changes (following the lower arrow from right to left along the x-axis), phenotypes that mitigate negative effects of flatwing (i.e. reducing δ_p) will be positively selected, favouring reaction norms with increasingly large slopes β. Short-term reaction norm evolution over a timescale of tens to hundreds of generations is expected to be rapid, whereas a longer period of genetic assimilation is predicted to occur subsequently over many thousands of generations (Lande 2009). The evolution of flatwing crickets in Hawaii is very recent as they appear to have arisen approximately 15 years ago, thus the rapid spread of flatwings represents the earliest phase of this process (Zuk *et al.* 2006) (Fig. 5C). Figure based on Lande (2009) (Fig. 1).