Within-generation and transgenerational social plasticity interact during rapid adaptive evolution

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Conflicts of Interest

We declare that we have no conflicts of interest.

Author Contributions

SLS and NWB designed experiments; SLS conducted experiments and analyzed the data; SLS wrote the first draft of the manuscript; NWB provided feedback and made conceptual contributions to the following drafts. All authors approved the final manuscript and agreed to be held accountable for its content.

Data Availability Statement

All data underlying the manuscript will be made publicly available in an appropriate repository (e.g., Dryad) upon acceptance for publication.

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Abstract

1. The role of within-generation phenotypic plasticity (WGP) versus transgenerational plasticity (TGP) during evolutionary adaptation are not well understood, particularly for socially-cued TGP.

2. We tested how genetics, WGP, and TGP jointly influence expression of fitness traits facilitating adaptive evolution in the field cricket *Teleogryllus oceanicus*. A male-silencing mutation ("*flatwing*") spread to fixation in ca. 50 generations in a Hawaiian cricket population attacked by acoustically-orienting parasitoids. This rapid loss of song caused the social environment to dramatically change.

3. Juveniles carrying the *flatwing* (*fw*) genotype exhibited greater locomotive activity than those carrying the *normal-wing* (*nw*) allele, consistent with genetic coupling of increased locomotion with *fw*.

4. Consistent with adaptive WGP, homozygous *fw* females developing in the absence of song showed reduced body condition and reproductive investment at adulthood.

5. Adult but not juvenile offspring exhibited TGP in response to maternal social environment for structural size, somatic condition, and reproductive investment, whereas adult locomotion and flight was only influenced by WGP. WGP and TGP interacted to shape multiple traits at adulthood, though effect sizes were modest.

6. Interactions between genetic effects and social plasticity within and across generations are likely to have influenced the evolutionary spread of flatwing crickets. However, interactions among these effects can be complex, and it is notable that TGP manifested most strongly later in development. Our findings stress the importance of evaluating trait plasticity at different developmental stages and across generations when studying phenotypic plasticity's role in evolution.

Key words: adaptive evolution, maternal effects, open field test, phenotypic plasticity, pleiotropy, social environment, transgenerational plasticity

Introduction

Dissecting how phenotypic plasticity affects trait expression within and across generations is necessary to fully understand its role in adaptive evolution. Within-generation plasticity (WGP), where an individual's phenotype shifts as a response to its own environmental conditions, has long been argued to influence evolutionary processes (Robinson and Dukas 1999; Huey et al. 2003; West-Eberhard 2003; Ghalambor et al. 2007; Lande 2009; Chevin et al. 2010). More recently, researchers have explored plastic responses to parental or grandparental environmental conditions through transgenerational plasticity (TGP) (LaMontagne and McCauley 2001; Dyer et al. 2010; Sheriff et al. 2010). It is clear from this work that TGP can also modify a species' evolutionary trajectory. In particular, both WGP and TGP may mitigate indirect fitness costs caused by new genetic variants under selection (negative pleiotropy), thus facilitating spread and fixation of *de novo* adaptive variants. However, few empirical studies of rapid adaptation have considered the consequences of both these forms of plasticity acting simultaneously.

Depending on the nature of the interaction between WGP and TGP, various evolutionary outcomes could occur. For example, a study of anti-predator defensive helmet formation in *Daphnia cucullate* found that WGP and TGP additively contribute to offspring phenotype (Agrawal et al. 1999). Such a relationship could move a population towards a new trait optimum faster than if just one form of plasticity were acting (Auge et al. 2017). Several other studies have detected a more complicated pattern, where parental environment interacts with the effects of offspring environment (i.e., TGP alters the extent and/or direction of offspring WGP response or reaction norm) (Prasad et al. 2003; Donelan and Trussell 2015; Luquet and Tariel 2016; Stein et al. 2018; Zirbel et al. 2018). This interaction could be adaptive, for example, by allowing parents to produce pre-adapted offspring that do not need to themselves express costly plasticity (Luquet and Tariel 2016). Alternatively in cases where TGP is non-adaptive for offspring, WGP acting on offspring traits in the opposite direction as TGP could allow offspring a means of 'escaping' negative fitness effects carried over from parental environment (Auge et al. 2017). The relationship between TGP and WGP has been somewhat explored in response to the environmental effects of predation (Agrawal et al. 1999; Donelan and Trussell 2015; Luquet and Tariel 2016; Stein et al. 2018), nutrition (Prasad et al. 2003; Zirbel et al. 2018), and temperature (Bernareggi et al. 2016), but the nature of this

57 interaction in other important contexts, such as the social environment, remains largely
58 unknown.

59   To experimentally dissect this interaction, we tested phenotypic and fitness effects of
60 maternal and offspring social environment in a rapidly-evolving population of the Oceanic
61 field cricket, *Teleogryllus oceanicus*. In Hawaii, acoustically signaling males are attacked by
62 an acoustically orienting parasitoid fly, *Ormia ochracea*. Recently, a single-locus, X-linked
63 mutation, *flatwing* (*fw*), arose and spread in fewer than 20 generations to affect ca. 90% of
64 males in a population on the island of Kauai (Zuk et al. 2006). *Fw* segregates as a single-locus
65 mutation and disrupts normal wing development, thus silencing 'flatwing' males and
66 shielding them from fly attack (Zuk et al. 2006; Pascoal et al. 2020). Its rapid spread to
67 fixation dramatically changed the social environment by eliminating the conspicuous long-
68 range male acoustic signal that functions in mate attraction, courtship, and intrasexual
69 aggression. Thus, the mutation has obvious negative indirect (pleiotropic) effects: flatwing
70 males cannot acoustically advertise for mates. They also show partial, apparently
71 maladaptive feminization of phenotypes unrelated to wings (Bailey et al. 2010; Pascoal et al.
72 2016; Pascoal et al. 2018; Rayner et al. 2019b; Pascoal et al. 2020). Pre-existing WGP to
73 acoustic cues in the environment that offsets some of these fitness costs may have facilitated
74 the rapid spread of *flatwing*. When raised in an environment lacking song, females relax
75 mate preferences and increase responsiveness to calling males, likely enabling them to locate
76 the few remaining singing males, or satellite flatwing males near them, in flatwing-
77 dominated populations (Bailey and Zuk 2008; 2012). Similarly, males raised in silence are
78 more likely to express satellite mating tactics (Bailey et al. 2010). Males also show an overall
79 increase in locomotive behavior when reared in silence (Balenger and Zuk 2015). Finally,
80 when exposed to song during rearing, individuals of both sexes develop increased
81 reproductive tissue mass and have enhanced immune responses compared to their
82 counterparts raised in the absence of song (Bailey et al. 2010, 2011; Lierheimer and
83 Tinghitella 2017; Heinen-Kay et al. 2019).

84   Several features of this field cricket system suggest that socially-induced TGP could
85 also contribute to the rapid spread of flatwing males. Like many insect species, *T. oceanicus*
86 suffers high juvenile mortality, favoring fitness-increasing alterations to early juvenile
87 phenotype via TGP. Recent findings suggest that nongenetic inheritance can affect the
88 interplay between predation risk vs. movement towards singing males, though this has only

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89 been examined for adults (Moschilla et al. 2021). Second, the fragmented distribution of *T.*

90 *oceanicus* habitat in Hawaii, the inability of juveniles to fly, and overlapping generations

91 should result in high autocorrelation between parental and offspring social environments,

92 which is predicted to favor adaptive TGP (Leimar and McNamara 2015). Third, crickets do

93 not possess a fully-developed auditory system until adulthood, though late juveniles may

94 have limited auditory capabilities (Young and Ball 1974; Yack 2004; Staudacher 2009).

95 Young juveniles are therefore not likely capable of accurately assessing their own social

96 environment acoustically, which theory predicts will favor the evolution of TGP (Leimar and

97 McNamara 2015).

98   We performed three experiments to dissect the potential contributions and

99 interactions of genetic evolutionary responses, WGP, and TGP to rapid adaptation observed

100 in Hawaiian *T. oceanicus*. Supplementary figure S1 provides an experimental overview.

101 Across these experiments, we focused on four traits relevant to mate competition and the loss

102 of acoustic sexual signaling during the evolutionary spread of flatwing crickets: structural

103 size (in juveniles and adults), body condition (in adults), investment in reproductive tissues

104 (in adults), and locomotive activity (in juveniles and adults). Locomotion was a key trait

105 because there is evidence from other insect species that individuals exposed to crowded

106 conditions increase dispersal themselves (WGP) or produce offspring with increased dispersal

107 tendencies (TGP) (Denno and Roderick 1992; Allen et al. 2008; Yu et al. 2019). Locomotive

108 activity is also expected to have especially important fitness consequences in the cricket

109 system given the challenges of locating conspecifics in a song-less, flatwing-dominated

110 population.

111   In **Experiment 1**, we measured how juvenile locomotive behavior varied across *fw*

112 and *normal-wing* (*nw*) genotypes, before any maternal social manipulation. This allowed us

113 to determine whether the rapid spread of flatwing males might have been associated with

114 genetic changes in juvenile expression of a trait relevant to the changing social environment.

115 We expected that *fw-carriers* benefit from dispersing less as juveniles and therefore

116 aggregating at higher densities upon reproductive maturity, increasing the chances of mating

117 in a song-less environment. In **Experiment 2**, we investigated WGP to the acoustic social

118 environment. We focused on *fw*-carrying individuals because the source population is now

119 all-flatwing (Tinghitella et al. 2018; Rayner et al. 2019a) and previous work indicated that

120 the mutation is associated with increased socially-induced WGP (Pascoal et al. 2018). We

121   tested whether, consistent with previous studies on females carrying *nw* genotypes, *fw*-
122   carrying females (the maternal generation) raised in different acoustic environments
123   increase reproductive investment and alter mating behaviors in a way that increases
124   probability of mating with a silent male. Finally, in **Experiment 3**, we tested
125   transgenerational consequences of the maternal social environment by measuring size and
126   locomotive activity in acoustically-naïve juveniles, and final size, somatic condition,
127   reproductive investment, and locomotive activity in adult offspring. In this last experiment,
128   adults were exposed to either matched or mis-matched acoustic cues compared to their
129   mothers, permitting a direct test of the interaction between WGP and TGP.

Methods

Experiment 1: Genotypic differences in juvenile locomotion

Cricket populations and rearing

133   We compared early juvenile behavior across the two cricket morph genotypes using 6
134   laboratory stock lines – 3 pure-breeding for *fw* and 3 pure-breeding for *nw*. Lines were
135   established in 2016 from a series of controlled crosses of Kauai-derived individuals to ensure
136   homozygosity (Pascoal et al. 2016). Stock crickets were kept in 16 litre plastic containers with
137   cardboard egg cartons for shelter. Twice weekly, they were provided *ad libitum* food (Burgess
138   Supa Rabbit Exel Junior pellets; blended for juveniles) and moistened cotton for water and
139   oviposition. Crickets in isolated-rearing conditions were kept in 100 mL plastic deli pots with
140   shelter, food, and water as above. All subjects were kept in the same growth chamber at 25°C
141   on a photo-reversed 12:12 hour light:dark cycle unless otherwise indicated. To obtain
142   juveniles for this experiment, we collected eggs from each line twice weekly for 4 weeks. After
143   approximately two weeks we monitored egg pads daily (16:00 - 18:00) and isolated new
144   hatchlings.

Open-field test

146   An open field test (OFT) was used to track individual crickets' movements in an unobstructed
147   arena and measure their total distance travelled, a useful proxy for measuring behaviors
148   related to dispersal, mate location and foraging (Fraser et al. 2001; Dingemanse et al. 2003;
149   Korsten et al. 2013). For this experiment, juveniles were isolated at hatching, and each was

150 tested in an OFT at 15-days and 45 days post-hatching. Juveniles of these ages do not have

151 mature hearing structures (Young and Ball 1974). All OFTs were performed under red light

152 during the dark portion of the crickets' 12:12 light:dark cycle, between 23-25°C. Subjects were

153 placed in small glass vials within their deli pot to reduce handling disturbance before testing.

154 The vial was gently turned over onto the center of an 11x17 cm clear plastic arena atop white

155 poster paper and the cricket was allowed to acclimatize for two minutes. Upon lifting the vial,

156 we began recording for 5 minutes at 30 frames/second using a camera (Nikon D3300)

157 mounted ca. 40 cm above the arena. The arena was wiped down with 70% ethanol before each

158 trial to minimize residual chemical cues. Two crickets were assayed at once in side-by-side

159 arenas. It is unlikely that they were aware of one another due to their inability to see in red

160 wavelengths of light. After the OFT, each cricket was photographed overtop a micrometer

161 using a Leica DFC295 digital camera affixed to a Leica M60 dissecting microscope. ImageJ

162 (v.1.8.0_112) was used to record pronotum length (a proxy for structural size) from the

163 images.

164 *Locomotion measurements*

165 We used DORIS v.0.0.17 (Friard 2019) to extract coordinates of the test subject within each

166 video frame, followed by coordinate path smoothing implemented in R (R Core Team 2020)

167 to increase measurement precision (see Supplemental Methods and Figure S2). Using these

168 coordinates, we measured total distance traveled ("*distance*") during trials. In this and later

169 experiments involving open field tests, we also explored other movement parameters

170 ("*proportion explored*" as a measure of exploratory activity; and "*origin time*", "*middle time*",

171 and "*edge time*" as measures of space usage and thigmotaxis). However, variation in these

172 parameters was largely accounted for by overall differences in distance moved, confirming

173 that *distance* was the most salient locomotion trait in the experiment. For completeness, we

174 discuss the measurement and analysis of all other movement traits in the Online

175 Supplementary Information.

176 *Statistical analyzes*

177 All statistical tests were carried out using R version 4.0.2 (R Core Team 2020). We compared

178 *distance* between wing morph genotypes in 15-day old and 45-day old offspring using a linear

179 model. Individuals who jumped during their assay (n = 2 in 45-day assay) or whose video was

180 inadvertently deleted before analysis (n = 2 in 45-day assay) were excluded. All data
181 transformations are shown in Table S1.

182   Morph and sex were modelled as categorical variables, with line nested within morph
183 to account for inter-line variation. Pronotum length, temperature, and time of day were
184 included as covariates. Thirty-nine individuals died before their sex could be identified, so to
185 verify that sex did not qualitatively affect the findings, models including sex as a fixed effect
186 were run on the subset of individuals for which sex could be identified. Sex did not approach
187 significance in this model (all $p > 0.2$) and the qualitative outcome did not differ. Thus, the
188 model retaining all individuals, and excluding sex as a fixed effect, was retained (Equation 1
189 of Supplementary Table S2). Finally, the model was run first with all individuals, then with
190 only those who moved during the assay to confirm that genotypic variation in *distance* was
191 not due to differences in the likelihood of initiating movement. Excluding crickets that failed
192 to initiate movement did not affect interpretations of genotype differences, so final models
193 included stationary crickets (Supplementary Table S3).

Experiment 2: Social plasticity in the maternal generation (WGP)

Cricket populations and rearing

196 The three pure-breeding *fw* lines used in Experiment 1 were reciprocally interbred to create
197 an admixed pure-breeding *fw* stock population. Following previous work, we isolated juvenile
198 females from this stock when sex became apparent to ensure virginity and more easily
199 manipulate their acoustic environment (Bailey and Zuk 2008; Pascoal et al. 2018). We also
200 segregated a group of juvenile males into single-sex 16-L box to maintain their virginity. All
201 group rearing conditions were identical to Experiment 1. Isolated females were placed in a
202 separate, temperature-controlled 25°C incubator on a 12h:12h photo-reversed light cycle,
203 with no male calling. Females were checked daily for adult eclosion, whereupon they were
204 haphazardly assigned one of two acoustic social treatments: Song or No Song. Females do not
205 achieve reproductive maturity until several days after adult eclosion, so our acoustic
206 treatment targets the developmental period when mate assessment is possible but mating is
207 not (Swanger and Zuk 2015). We also recorded the number of days spent isolated prior to
208 eclosion to account for any differences in growth rate that might be associated with time
209 spent without song prior to adult acoustic treatment. We kept each female in their acoustic
210 treatment for 15 days post-eclosion.

Acoustic treatments

In the Song treatment, Kauai male calls reflecting population averages for key song parameters were played at 80-85 dB (measured at the lid of the deli cup which has an acoustic impedance of ca. 10 dB) during the night portion of the crickets' light:dark cycle to best match calling dynamics in the wild (Zuk et al. 1993). Playbacks used in the Song treatment have been previously described (Pascoal et al. 2018) (see Supplementary Methods). Acoustic treatments were run in two separate LMS Series 4 (Model 600) controlled temperature incubators at 25°C on the same 12h:12h photo-reversed light:dark cycle as the general incubator. Calls were broadcast from computer speakers (Logitech Z120 2.0) and the calling schedule programmed using the Task Scheduler application on a desktop computer. Twice a week, we switched which incubator housed each acoustic treatment to prevent any incubator-related experimental confounds.

Mating trials of acoustically treated females

At 15 days post-eclosion, isolated adult females were weighed and their pronotum width was measured using digital calipers. Each female was placed in a 16 x 18 cm plastic container with cardboard, rabbit chow, and moistened cotton. We haphazardly selected an adult virgin male from the flatwing stock population, weighed it, measured its pronotum width, and placed it in the container with the female. Trials were performed between 20-23°C under red light between 16:00h and 18:00h. They lasted for 20 minutes, and we noted whether the female mounted the male and whether the male transferred a spermatophore. Afterwards, pairs were placed in a separate incubator without male song at the same temperature and light:dark cycle as in Experiment 1. After 24 hours, the male was removed to reduce potential paternal influences on offspring phenotype. After another 24-48 hours, the female was removed, and the egg pad was collected for use in Experiment 3.

Body condition, size, and reproductive tissue measurements

To compare female body condition, we used pronotum width and total body weight to calculate the scaled mass index (SMI) of each individual (Peig and Green 2009). A subset of females drawn haphazardly from each treatment (total n = 23) were dissected at 15 days post-eclosion rather than mated. We recorded their pronotum width using digital calipers,

240   weighed them, and then determined wet mass of their dissected ovaries. Somatic mass was

241   calculated by subtracting ovary mass from total weight.

Statistical analyzes

243   First, we compared *SMI* across acoustic treatments using a linear model (Equation 2 of Table

244   S2) with acoustic treatment as a categorical factor, days isolated before treatment as a

245   covariate, and experimental replicate (block one or block two of the experiment). Replicate

246   only had two factor levels so we included it as a fixed effect. Second, we compared mating

247   behavior across acoustic treatments. We first ran a generalized linear model (GLM) with

248   binomial error to examine presence vs. absence of *female mounting* during trials, including

249   acoustic treatment, female SMI, and male SMI as predictors. Next, we ran a binomial GLM

250   examining presence vs. absence of *spermatophore transfer*. For this, we only included the 49

251   mating trials (out of 65 total) where mounting had occurred, because spermatophore transfer

252   cannot occur without mounting. Acoustic treatment was included as a categorical factor and

253   female SMI and male SMI were included as covariates. Equation 3 in Table S2 gives the

254   general form of these models.

255   Finally, we compared female reproductive investment (ovary mass) across acoustic

256   treatments in the subset (n = 23) of females that had been dissected by running a linear

257   model (LM) on *ovary mass*, with acoustic treatment and days isolated as predictor and

258   covariate, respectively. As in previous studies of reproductive investment, we controlled for

259   body size by including log-transformed soma mass as an additional covariate (Tomkins and

260   Simmons 2002; Bailey et al. 2010) (Equation 4 in Table S2).

261   **Experiment 3: Transgenerational effects of maternal social environment and**

262   **interactions between TGP and WGP in adult offspring**

Cricket populations and rearing

264   Eggs produced by the maternal generation in Experiment 2 were first kept in a separate

265   incubator under the same temperature and light conditions as the general incubator. As they

266   began to hatch, the first UK national lockdown in response to the 2020 Covid-19 pandemic

267   (23 March 2020) required that all laboratory experiments be run under strict social

268   distancing measures, which affected where and how some of our procedures were executed.

269 A description of 'socially-distanced' methods plus our design to statistically account for any
270 variation it introduced is provided in the Supplementary Methods.

271 We first tested TGP effects in juvenile offspring. Hatchlings were isolated as described
272 previously and kept at 18-24°C on a 12h:12h photo-reversed light:dark cycle. We ran the
273 experiment in two blocks, with individuals in the second block kept at 25°C in the lab
274 incubator, as in Experiment 1. For the early juvenile offspring TGP experiment, we tested
275 311 offspring (131 from 14 mothers treated with Song, 180 from 21 mothers treated with No
276 Song) at 15 days post-hatching. 199 individuals were tested again at 45 days post-hatching
277 (117 from 11 No Song mothers and 82 from 8 Song mothers).

278 For adult offspring experiments, hatchlings were kept in 10 replicate group-rearing
279 boxes during development to match the demographic rearing conditions experienced by the
280 previous generation. Once sex was apparent during development, individuals were isolated
281 and assigned to either acoustic treatment using the same incubators and playback schedules
282 as in Experiment 2. The distribution of adult offspring (n = 387) across the 4 maternal-
283 offspring acoustic treatment combinations is shown in Supplementary Table S4. It is
284 important to note that the adults measured in this experiment were not the same individuals
285 as those used for the TGP juvenile trials described above; thus, the corresponding data sets
286 are derived from different individuals of the same generation.

Open field test

288 OFT procedures were identical to Experiment 1. For juvenile offspring, OFTs were performed
289 15 days post-hatching and 45 days post-hatching. Adult offspring OFTs were performed 8-
290 days post-adult eclosion. All recordings were performed at 23-28°C between 12:00 and 17:00
291 under dim red lighting. Adult OFTs were identical to those of juveniles, except a larger plastic
292 arena was used (41 cm wide, 37 cm long, and 28 cm high). In the course of the experiment,
293 we noticed that some adults attempted to fly out of the arena during the assay. When that
294 happened, we stopped the recording and placed the subject into an incubator without song
295 for 10 minutes. After re-acclimation, we started the trial again. The number of flight attempts
296 was recorded for each individual. We collected movement coordinates and calculated *distance*
297 using DORIS (v.0.0.17) as in Experiment 1.

Morphological measurements

299   Following the OFT, we photographed each juvenile overtop a micrometer using the same
300   camera and dissecting scope as in Experiment 1 and measured pronotum length using
301   ImageJ (v.1.8.0_112). We euthanized adults after OFTs at 8 days post-eclosion, then weighed
302   them and measured pronotum length to the nearest 0.01 mm using digital calipers. We then
303   dissected, blotted excess fluid, and weighed their gonads (male testes and accessory glands,
304   female ovaries). Here we used pronotum length and soma weight to calculate SMI, by
305   subtracting gonad weight from total weight. In this experiment, SMI was thus a measure of
306   somatic body condition, which allowed us to investigate whether differences in maternal or
307   offspring acoustic environments influenced relative investment in somatic tissues while
308   scaling to structural size. SMI was calculated separately for each sex. Gonad weight was later
309   compared directly.

310   *Statistical analyzes*

311   First, we tested whether *juvenile offspring size* differed between maternal acoustic
312   treatments by running a linear mixed model (LMM) using pronotum length as the response.
313   Maternal treatment and experimental replicate were included as fixed effects, with maternal
314   ID as a random effect. Experimental replicate was included to account for different rearing
315   temperatures in trial 1 and trial 2 (see Supplementary Methods). The treatment*replicate
316   interaction ($p > 0.2$) was excluded from the final model. Pronotum length of 45-day old
317   offspring was modelled similarly except experimental replicate was not included because we
318   only had 45-day nymph data for trial 1. Models took the general form of Equation 5 in
319   Supplementary Table S2.

320   We then examined the effect of maternal treatment on juvenile offspring *distance*
321   using an LLM which included maternal treatment and experimental replicate as categorical
322   factors; temperature, time of day, and pronotum length as covariates; and maternal ID as a
323   random effect. The treatment*replicate interaction ($p > 0.2$) was excluded from the final
324   model. A similar model was run for 45-day old offspring, except experimental replicate was
325   not included because we only had 45-day data for trial 1. The general form of the model is
326   given by Equation 6 in Supplementary Table S2.

327   To investigate TGP and WGP and their interaction, we tested the effect of maternal
328   and offspring acoustic treatments on *adult pronotum length* and *somatic condition (SMI)*.
329   Because there are large sex differences in physiology and the possibility of sex-specific

330   maternal effects, we ran separate models for each sex. Each LMM included maternal and

331   offspring treatments as factors plus their interaction. Non-significant ($p > 0.2$) interactions

332   were removed. We analyzed the effect of acoustic treatments on *adult offspring reproductive*

333   *investment* using sex-specific models with gonad weight as the response. First we compared

334   unscaled reproductive investment, then we added pronotum length as a covariate to examine

335   whether variation in reproductive investment could be explained by structural size. Finally,

336   we added log-transformed somatic mass to examine whether variation in reproductive

337   investment might be explained by somatic weight. Replicate was included as a random effect.

338   These models took the general form shown in Equation 7 of Supplementary Table S2.

339   We then tested the impact of TGP and WGP on adult *distance* using separate LMMs

340   for each behavior and sex. All models included maternal treatment, offspring treatment,

341   temperature, time, and somatic SMI, plus replicate as a random effect (Equation 8 in Table

342   S2). Non-significant (p > 0.2) maternal*offspring treatment interactions were removed.

343   As a *post hoc* analysis of treatment and sex variation in attempted flight behavior, we

344   modelled *flight attempts* using a generalized linear mixed model (GLMM) with a binomial

345   error (1 if flight was attempted and 0 if not) with the predictors: maternal treatment,

346   offspring treatment, sex, maternal treatment*sex, and somatic SMI. Replicate was included

347   as a random effect. Equation 9 of Supplementary Table S2 describes the final model after

348   removing non-significant interaction terms.

Results

Experiment 1: Genotypic differences in juvenile locomotion

Fw-carrying nymphs moved further than *nw* nymphs, both at 15 days and 45 days post-hatching (Table 1, Figure 1). The effect size of distance differences was considerable. 15-day-old *fw* nymphs, which had a mean length of 3.81 mm, moved an average of ca. 300 mm further than *nw* nymphs. This difference in distance moved is ca. 79x their body length in a relatively short period of 5 minutes. For 45 day-old nymphs, the average movement differential was 132.73 mm (ca. 12.9x the mean body length of a 45 day-old nymph).

Table 1. Linear models examining the effects of genotype on total distance travelled in open field tests by 15 day-old (top) and 45 day-old (bottom) juveniles

		df^*	F	$P^†$
15 days old	Morph	1, 245	12.988	**<0.001**
	Morph:Line	4, 245	2.806	**0.026**
	Pronotum	1, 245	0.360	0.549
	Temperature	1, 245	0.680	0.410
	Assay Time	1, 245	0.311	0.578
45 days old	Morph	1, 210	16.554	**<0.001**
	Morph:Line	4, 210	12.696	**<0.001**
	Pronotum	1, 210	12.122	**<0.001**
	Temperature	1, 210	0.561	0.455
	Assay Time	1, 210	33.055	**<0.001**

* numerator, denominator
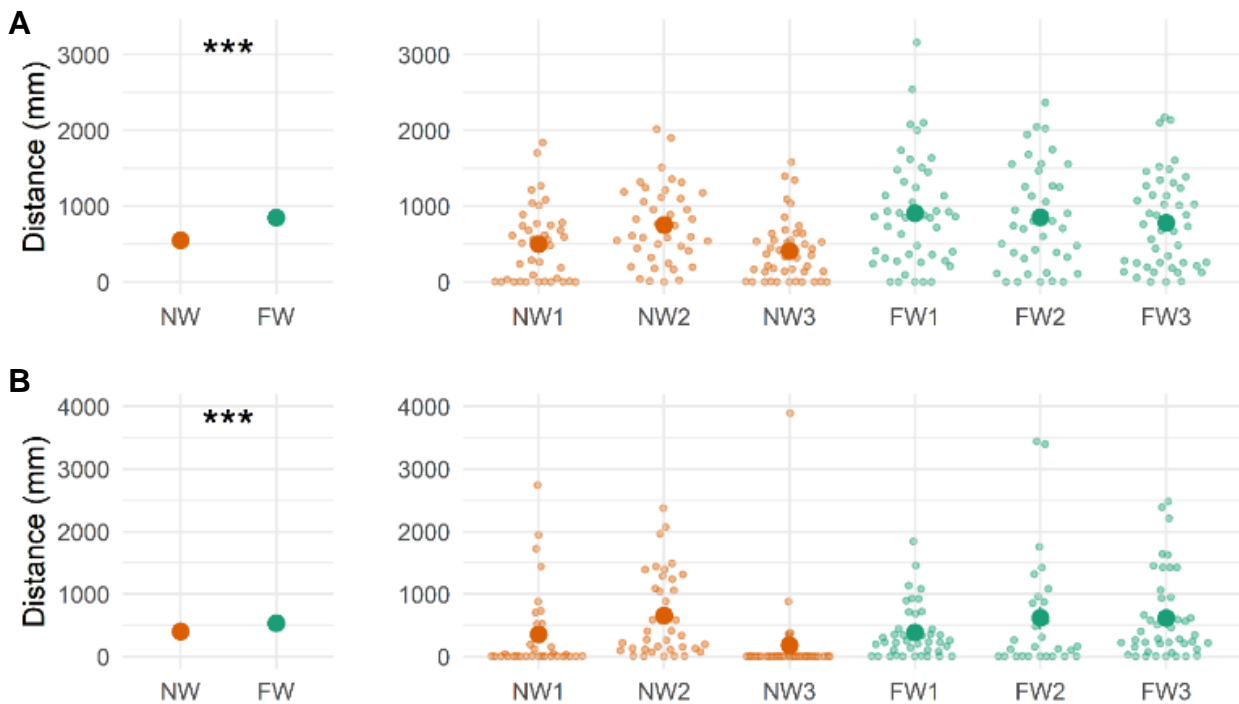† p < 0.05 indicated in bold

Figure 1. The effect of wing morph genotype on total distance traveled by juveniles in open field tests for (**A**) 15 day-old nymphs, (**B**) 45 day-old nymphs. Plots on the left illustrate pooled means across three replicate morph lines. Bars indicating ± 1 standard error are not shown as these are too small to indicate graphically without being obstructed by the symbols for means. Violin plots on the right show data for each replicate morph line, with dark circles indicating means, small light circles showing each data point, and bars indicating ± 1 standard error (when visible). *** indicates a morph difference with p < 0.001.

Experiment 2: Social plasticity in the maternal generation (WGP)

The acoustic social environment affected physiology (Table 2, Figure 2), but not mating behavior (Supplementary Table S5; Supplementary Figure S4), in homozygous *fw* females. Those raised in Song attained higher condition (SMI) (Table 2, Figure 2A) and had heavier ovaries relative to somatic mass (Table 2, Figure 2B). Female mounting was not influenced by prior acoustic experience, though females were more likely to mount higher condition males (Supplementary Table S5 and Supplementary Figure S4A). Similarly, in trials where females did mount males (n = 49), there was no evidence that acoustic treatment affected spermatophore transfer, though males were more likely to transfer a spermatophore to higher condition females (Supplementary Table S5 and Supplementary Figure S4B).

Table 2. Linear models examining WGP arising from the acoustic environment on female condition (scaled mass index) and reproductive weight (g)

	Condition			Reproductive Weight		
	df [*]	*F*	*P*[†]	*df*	*F*	*P*
Acoustic treatment	1,110	6.038	**0.016**	1,19	5.015	**0.037**
Days isolated	1,110	0.129	0.720	1,19	1.037	0.321
Experimental replicate	1,110	0.948	0.332	-	-	-
Somatic mass	-	-	-	1,19	0.211	0.652

[*] numerator, denominator
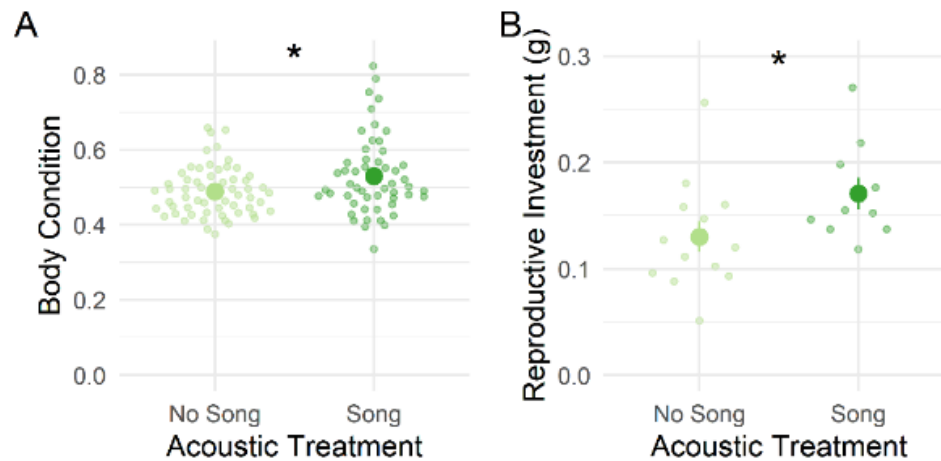[†] p < 0.05 indicated in bold

Figure 2. The effect of acoustic environment on (**A**) adult female body condition (scaled mass index) and (**B**) reproductive investment (ovaries mass). Plots show means ± 1 standard error, except for (A), where standard error bars are too small to indicate graphically without being obstructed by the symbols for means. Asterisks indicate $p < 0.05$.

366 **Experiment 3: Transgenerational effects of maternal social environment and**

367 **interactions between TGP and WGP in adult offspring**

368 Unexpectedly, TGP affected adult, but not juvenile, traits (Figure 3). For example, the

369 acoustic treatment of mothers was not associated with locomotion and morphology of their

370 15-day-old and 45-day old juvenile offspring (Table 4, Supplementary Tables S6 and S7).

371 However, adult offspring that experienced song themselves during rearing moved further

372 (WGP) (Table 5; Figure 3A), and in the case of males, this WGP was considerably exaggerated

373 if their mothers had been raised without song (TGP) (Figure 3A, right). It must be noted, this

374 WGP*TGP interaction was only marginally significant ($p = 0.055$; Table 5), though the effect

375 size appears non-trivial (mean movement differential of ca. 400 mm for adult song-reared

376 males; Figure 3A, right).

Table 4. Linear models examining the effects of TGP arising from the maternal acoustic environment on total distance travelled by 15 day-old (top) and 45 day-old (bottom) juveniles

		df^*	F	$P^\dagger$
15 days old	Maternal treatment	1, 300	0.625	0.429
	Experimental replicate	1, 300	45.092	**<0.001**
	Pronotum	1, 300	0.086	0.769
	Temperature	1, 300	0.530	0.467
	Assay time	1, 300	1.073	0.300

45 days old	Maternal treatment	1, 191	0.003	0.955
	Pronotum length	1, 191	5.714	**0.017**
	Temperature	1, 191	2.278	0.131
	Assay time	1, 191	0.338	0.561

* numerator, denominator
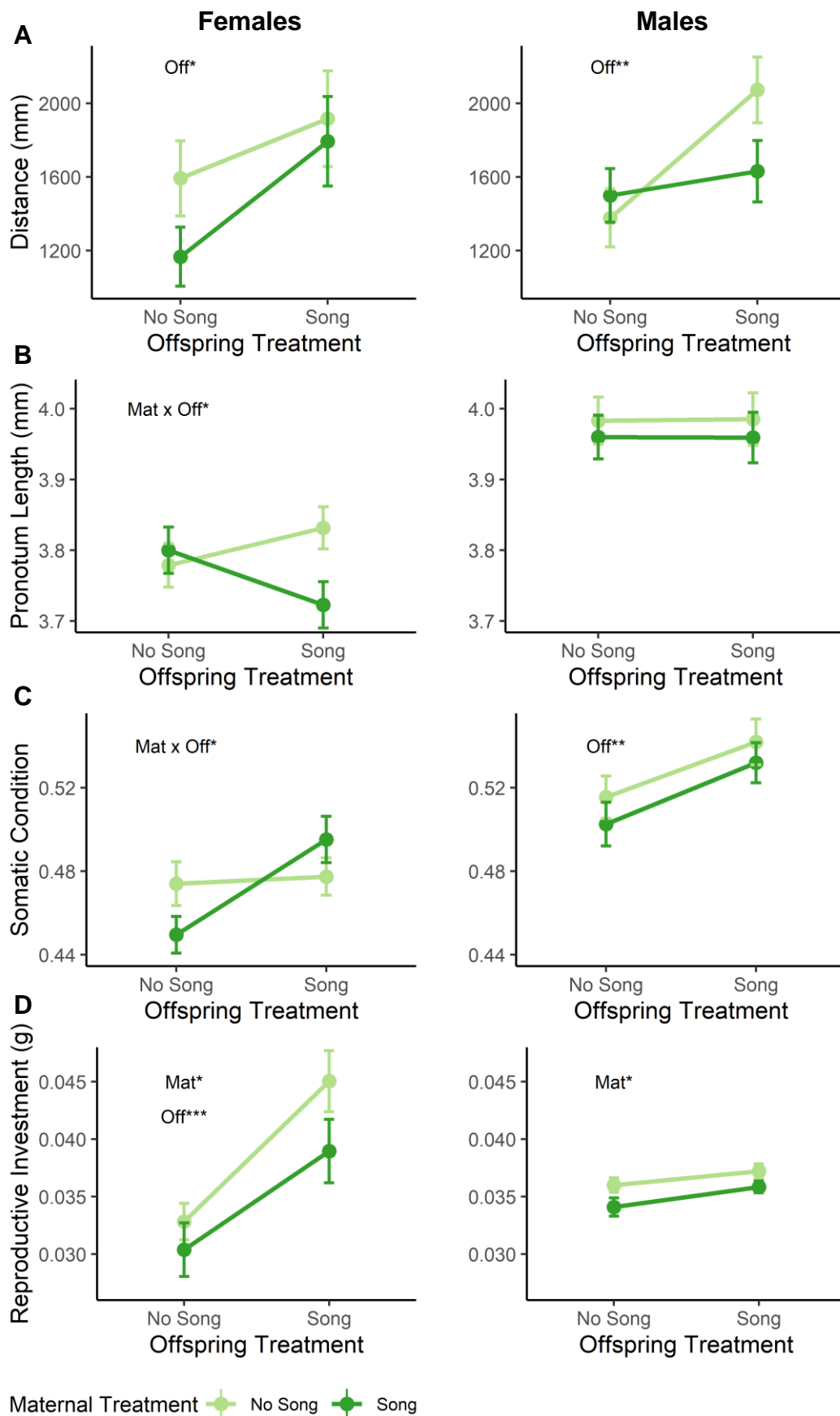† p < 0.05 indicated in bold

Figure 3. Effects of TGP and WGP in adult female (left) and adult male (right) offspring. (**A**) Distance travelled (**B**) pronotum length, (**C**) somatic condition, (**D**) reproductive investment, i.e., female ovaries mass and male testis mass. Means are indicated by circles and bars indicate ± 1 standard error. Significance of maternal ("Mat") treatment, offspring ("Off") treatment, and their interaction ("Mat x Off") are indicated with asterisks:
*p <0.05
**p <0.01
***p <0.001

Table 5. Linear models examining TGP and WGP on total distance moved by adult females (top) and adult males (bottom)

		df^*	F	$P^†$
Females	Maternal treatment	1, 181	2.055	0.152
	Offspring treatment	1, 181	6.279	**0.012**
	Temperature	1, 181	2.535	0.111
	Assay time	1, 181	1.923	0.166
	Somatic condition	1, 181	1.211	0.271
Males	Maternal treatment	1, 182	0.789	0.375
	Offspring treatment	1, 182	6.985	**0.008**
	Temperature	1, 182	6.620	**0.010**
	Time	1, 182	0.724	0.395
	Somatic condition	1, 182	3.567	0.059
	Maternal treatment* Offspring treatment	1, 182	3.695	0.055

* numerator, denominator

† p < 0.05 indicated in bold

377   By contrast, acoustic effects on adult offspring morphology provided strong and
378   consistent evidence for WGP, and TGP also affected several aspects of adult offspring
379   morphology (Tables 6 and 7; Figure 3B,C,D). TGP often did not affect traits in the same
380   direction as WGP; interactions between TGP and WGP combined to shape adult traits in a
381   way that suggests TGP activates WGP, or put another way, that the manifestation of TGP is
382   contingent on current environmental conditions. For example, female offspring reared
383   without song had similar pronotum lengths, but when they were reared with song, those
384   whose mothers experienced No Song grew to be larger than those whose mothers experienced
385   Song (Table 6; Figure 3B, left). Female somatic condition showed a similar crossing-over
386   effect (Table 6; Figure 3C, left). Also, TGP and WGP affected female investment in ovaries,
387   but in conflicting directions. Those raised in song developed heavier ovaries than those raised
388   without song, and offspring from the No Song maternal treatment developed heavier ovaries
389   than offspring from mothers who experienced Song (Table 7; Figure 3D, left). Adult males
390   raised in the presence of song developed higher somatic condition than those raised without
391   song, regardless of maternal treatment (Table 6; Figure 3B, right). We also found that
392   crickets raised without song attempted flight more than those raised with song, particularly
393   for females and lower condition individuals (Table 8; Figure 6; Supplementary Figure S5).

Table 6. Linear models examining TGP and WGP on adult morphology for females (top) and males (bottom)   394

		Pronotum Length			Somatic Condition		
		df^{*}	χ^2	$P^{\dagger}$	df	χ^2	P
Females	Maternal Treatment	1, 186	0.621	0.431	1, 187	3.060	0.080
	Offspring Treatment	1, 186	1.452	0.228	1, 187	0.079	0.779
	Maternal Treatment* Offspring Treatment	1, 186	4.741	**0.029**	1, 187	4.670	**0.031**
Males	Maternal Treatment	1, 187	0.199	0.655	1, 187	1.265	0.261
	Offspring Treatment	1, 187	0.001	0.983	1, 187	8.169	**0.004**

* numerator, denominator
† p < 0.05 indicated in bold

Table 7. Linear models examining TGP and WGP on reproductive investment for adult females (top) and males (bottom)

		df	χ^2	P
Females	Maternal treatment	1, 186	3.910	**0.048**
	Offspring treatment	1, 186	16.860	**<0.001**
	Pronotum length	1, 186	0.894	0.345
	Somatic weight	1, 186	15.601	**<0.001**
Males	Maternal treatment	1, 185	3.975	**0.046**
	Offspring treatment	1, 185	2.800	0.094
	Pronotum length	1, 185	1.698	0.193
	Somatic weight	1, 185	8.675	**0.003**

* numerator, denominator
† p < 0.05 indicated in bold

Table 8. GLMM examining the effects of WGP and TGP on flight attempts

	df	χ^2	P
Maternal treatment	1, 371	0.085	0.770
Offspring treatment	1, 371	8.788	**0.003**
Sex	1, 371	4.213	**0.040**
Somatic condition	1, 371	4.161	**0.041**
Maternal treatment*Sex	1, 371	2.140	0.144

* numerator, denominator
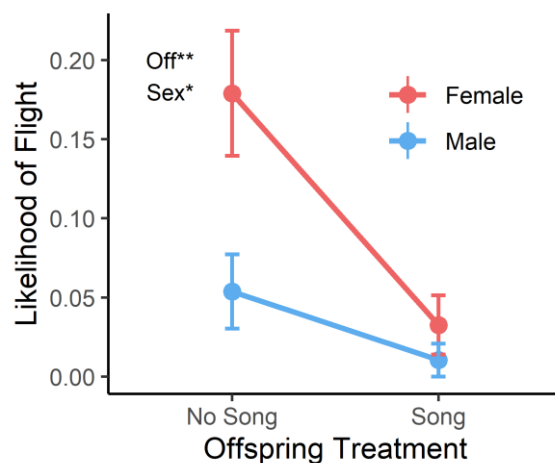† p < 0.05 indicated in bold

Figure 4. Likelihood of flight during open field trials. Means and ± 1 standard error are represented by circles and bars, respectively. Significance of offspring ("Off") treatment and sex are indicated with asterisks: *p <0.05, **p <0.01.

Discussion

Phenotypic plasticity's role in evolution stimulates vigorous debate, but one barrier to a general resolution may be that plasticity is not a monolithic phenomenon. Influential verbal models have suggested that "buffering" effects of plasticity can permit novel adaptations to escape loss at low frequencies and subsequently spread under selection, but few empirical studies have been able to assess the contributions and interactions of genetics and different forms of phenotypic plasticity such as within-generation and transgenerational plasticity. Here we demonstrate how all three inputs – genetics, WGP and TGP – interact to affect traits that potentially facilitate the rapid evolution of a parasitoid-avoidance adaptation in Hawaiian field crickets, male silence.

Genotype had a surprisingly large effect on juvenile behavior, but in the opposite direction predicted. At very early juvenile stages, *flatwing* carriers moved nearly 80 body lengths further than *normal-wing* carriers in a span of only 5 minutes. This genotypic difference could result from pleiotropic effects of the *fw* mutation or genomic hitchhiking. Genotypic differences in juvenile locomotion are consistent with phenotypic differences that have been detected between *fs* and *nw* carriers in other sexually dimorphic adult traits

411   (Pascoal et al. 2016; Rayner et al. 2019b; Pascoal et al. 2020), which raises the possibility
412   that the *fw* genotype may be exposed to selection at an earlier stage than previously
413   considered, for example through associated effects on foraging efficiency or predation risk.
414   Further, it is possible that increased locomotion of *fw* juveniles might have accelerated the
415   speed at which the mutation initially spread in the wild. Rather than facilitating local mating
416   aggregations as we initially hypothesized, greater movement activity may instead permit
417   silent crickets and females carrying male-silencing variants to encounter one another. The
418   ultimate fitness consequences of these differences remain to be tested, but more broadly, this
419   result illustrates how genetic correlations manifesting during development might impact the
420   trajectory of a mutant genotype which carries fitness benefits at adult stages. The idea that
421   advantageous mutations can have pleiotropic effects during development has been explored
422   extensively in the context of insecticide resistance and alternative reproductive morphs
423   (Boivin et al. 2001; Giraldo-Deck et al. 2020). Against the background of this work, our
424   findings suggest the combined phenotypic and fitness effects of adaptive mutations arising
425   from positive pleiotropy, negative pleiotropy, and genomic hitchhiking may be non-intuitive,
426   and phenotypic variation caused by such effects can alter the dynamics of adaptive evolution
427   as well as the manner in which plasticity affects that evolution.

428   In the crickets, transgenerational plasticity and within-generation plasticity
429   sometimes acted in concert, sometimes in opposition, and in some cases did not appear
430   influential in shaping trait variation. If TGP is adaptive for offspring (and therefore also for
431   mothers), a match between parental and offspring environment should result in higher
432   offspring fitness when compared to mis-matched offspring (Marshall and Uller 2007; Uller et
433   al. 2013). However, we found no evidence supporting this expectation for offspring
434   performance traits. Patterns of reproductive investment provide an illustrative example.
435   Consistent with previous studies in this system and in other cricket species (Bailey et al.
436   2010; Conroy and Roff 2018), mothers who experienced No Song invested less in reproductive
437   tissue. Such WGP is in line with adaptive predictions, as it allows individuals to reallocate
438   resources to non-reproductive tissue when competition and/or opportunity for mating is low
439   (Harshman and Zera 2007). This trade-off could be particularly adaptive in a flatwing-
440   dominated social environment where no males can sing, because plasticity shifting resources
441   from reproduction to survival could increase the chances that a female survives long enough
442   to find a mate. Consistent effects of TGP and WGP on offspring would facilitate this, but

443 instead we found that TGP and offspring WGP acted in opposing directions (Figure 3D). It is
444 therefore unlikely that this TGP in reproductive investment is an adaptive, anticipatory
445 effect to increase offspring fitness, but instead may be an incidentally-transmitted
446 physiological consequence of mothers responding to their social environment ("selfish TGP"
447 cf. Marshall and Uller 2007) or cross-generation spillover of parental condition ("condition-
448 transfer effects" cf. Bonduriansky and Crean 2018). Lack of support for adaptive TGP is
449 consistent with a recent meta-analysis which recovered weak evidence for it across taxa
450 (Uller et al. 2013).

451 WGP has been suggested to be more efficient than TGP, so once capable of assessing
452 their environment, offspring are expected to rewrite parental cues with their own (Ezard et
453 al. 2014; Auge et al. 2017; Moore et al. 2019). Nevertheless, we found that the maternal social
454 environment affected adult, but not juvenile, offspring phenotype in *T. oceanicus*. One reason
455 for the delayed action of TGP might be that the maternal social environment influenced
456 phenotype via mechanisms that would not cause observable differences until late in
457 development. For example, many insects exhibit significant plasticity in the number of
458 instars they undergo prior to sexual maturation, which can affect sexual size dimorphism at
459 adulthood (Esperk et al. 2007; Stillwell et al. 2010). Another possibility is that the maternal
460 social environment influenced offspring phenotype very early in development, such as size at
461 hatching, but those effects dissipated prior to later phenotypic measurement, as was found
462 in the salamander *Ambystoma talpoideum* (Moore et al. 2015). A third possibility is that TGP
463 mediated by maternal social environments could be qualitatively different from TGP
464 mediated by maternal physical environments. For example, nutritional or thermal
465 environments that mothers experience may have more direct impacts on juvenile offspring,
466 whereas the social environment comprised of adult social cues is likely to be of greater
467 relevance to offspring when they are adults themselves.

468 In contrast to morphological traits, we found that behavioral traits related to
469 movement (locomotive activity and likelihood of flight) were more influenced by WGP than
470 TGP. This supports the prediction that traits whose expression remains flexible after
471 development are more strongly affected by WGP (Beaty et al. 2016). Specifically, offspring of
472 both sexes were more active when reared in song. They may increase walking activity to
473 locate conspecifics they perceive to be abundant nearby, even in the absence of an immediate
474 acoustic cue. In contrast, crickets raised without song have no indication of nearby

475 conspecifics and may decrease short-range mate-searching via walking to instead wait for an
476 acoustic cue. This trade-off is likely motivated by a high metabolic cost of mate-searching
477 (Hack 1998) and the resulting increase in predation risk (Bell 1990). Our results contrast
478 with previous studies in this species which found that adult males raised in song are less
479 active than those raised in silence (Balenger and Zuk 2015) and that females exhibit limited
480 flexibility in locomotive behavior in response to acoustic environment (Heinen-Kay et al.
481 2018), but are consistent with other findings that suggest increases in exploratory behavior
482 under predation risk (Moschilla et al. 2021).

483   One explanation for this apparent inconsistency is that the latter studies conducted
484 movement trials in environments that contained shelter or cover and in some cases assessed
485 movement towards acoustic stimuli, whereas we used an open field test to mimic the
486 experience of Hawaiian crickets within an all-flatwing population, in which locomotion in the
487 absence of any immediately available acoustic signals is likely to have significant fitness
488 consequences. Cover during movement trials could have reduced the perception of risk
489 associated with walking, making increased undirected mate-searching in a song-less
490 environment advantageous (Hedrick and Dill 1993). Additionally, two of the previous studies
491 relied on indirect measures of activity (e.g., time spent walking, gridlines crossed, farthest
492 grid reached), whereas we directly measured distance traveled using automated and
493 validated coordinate collection, giving us greater resolution to resolve variation in activity.
494 Finally, we made an incidental discovery during the course of the experiment which provides
495 another intriguing explanation: offspring reared in No Song were more likely to attempt
496 flight during the open field test, a pattern that was particularly strong in females who were
497 ca. 4 times more likely to attempt fly if they had experienced no acoustic signals during
498 development (Figure 4). Individuals reared without song may be more disposed to use flight
499 as part of an un-directed, long-range dispersal strategy akin to Lévy flight (Viswanathan et
500 al. 1996) to increase their chances of reaching an area of greater conspecific resources.
501 Trading off increased long-range dispersal via flight with decreased walking behavior in
502 song-less, flatwing-dominated environments could have increased the speed at which
503 *flatwing* alleles spread under pressure from parasitoid flies.

504   Our results support the idea that the effects of TGP can be contingent upon offspring
505 environment. Put another way, sometimes TGP potentiates WGP, and sometimes it
506 suppresses WGP. It is therefore necessary to consider the potentially conflicting effects of

507 WGP and TGP when predicting how phenotypic plasticity influences adaptive evolution.

508 Theory predicts that, following rapid environmental change, populations may exhibit a

509 transient increase in plasticity because genotypes which shift trait expression closer to a new

510 optimum are favored (Lande 2009). Additionally, genotypes coding for reaction norm slopes

511 of other traits that offset negative effects of new variants spreading under selection may also

512 be favored, as appears to be the case in *T. oceanicus* in Hawaii and other systems (Bailey et

513 al. 2021). If increases in WGP during an adaptive evolutionary response result in a spillover

514 of non-adaptive TGP to the offspring generation, the role of plasticity in facilitating the

515 establishment and spread of novel adaptations may be less straightforward than currently

516 understood (Lacey 1998; Bonduriansky and Day 2009; Bell and Hellmann 2019). Previous

517 work in the Hawaiian flatwing cricket system supports predictions of increased WGP which

518 mitigates negative pleiotropy of male silence, in this case facilitating mate location and

519 reproduction in a song-less social environment (Bailey and Zuk 2008; Bailey et al. 2018;

520 Tinghitella et al. 2009; Bailey 2011; Bailey and Zuk 2012; Balenger and Zuk 2015; Pascoal et

521 al. 2018), but see (Rayner et al. 2020). However, we did not find a prominent signature of

522 either adaptive TGP or of an adaptive interaction between TGP and WGP. Instead, our

523 results suggest that plasticity transmitted across generations may have more complex effects

524 and, in some cases, counterbalance facilitating effects of WGP.

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