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Original Article

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

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Abstract

The effects of within-generation plasticity vs. transgenerational plasticity on trait expression are poorly understood, but important for evaluating plasticity's evolutionary consequences. We tested how genetics, within-generation plasticity, and transgenerational plasticity jointly shape traits influencing rapid evolution in the field cricket *Teleogryllus oceanicus*. In Hawaiian populations attacked by acoustically orienting parasitoid flies, a protective, X-linked variant ("*flatwing*") eliminates male acoustic sexual signals. Silent males rapidly spread to fixation, dramatically changing the acoustic environment. First, we found evidence supporting *flatwing*-associated pleiotropy in juveniles: pure-breeding *flatwing* males and females exhibit greater locomotion than those with *normal*-wing genotypes. Second, within-generation plasticity caused homozygous-*flatwing* females developing in silence, which mimics all-flatwing populations, to attain lower adult body condition and reproductive investment than those experimentally exposed to song. Third, maternal song exposure caused transgenerational plasticity in offspring, affecting adult, but not juvenile, size, condition, and reproductive investment. This contrasted with behavioral traits, which were only influenced by within-generation plasticity. Fourth, we matched and mismatched maternal and offspring social environments and found that transgenerational plasticity sometimes interacted with within-generation plasticity and sometimes opposed it. Our findings stress the importance of evaluating plasticity of different traits and stages across generations when evaluating its fitness consequences and role in adaptation.

Keywords: adaptation, maternal effect, phenotypic plasticity, pleiotropy

Introduction

Dissecting how phenotypic plasticity operates within and across generations is necessary to evaluate 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 (Bailey, 2012; Bailey et al., 2018; Chevin et al., 2010; Ghalambor et al., 2007; Huey et al., 2003; Lande, 2009; Levis & Pfennig, 2018; Moczek et al., 2011; Pfennig, 2021; Robinson & Dukas, 1999; Snell-Rood et al., 2018; Sultan, 2017; West-Eberhard, 2003). Researchers have also explored plastic responses to parental or grandparental environmental conditions through transgenerational plasticity (TGP), a term which encompasses environmentally induced maternal and paternal effects (Dyer et al., 2010; LaMontagne & McCauley, 2001; Mousseau & Fox, 1998; Sheriff et al., 2010; Uller, 2008). It is clear from the latter work that TGP can similarly modify the dynamics of adaptive evolution. For example, both WGP and TGP might facilitate the spread and fixation of de novo adaptive variants under selection by mitigating fitness costs caused by negative pleiotropy affecting other traits (Bailey et al., 2021; Fisher, 1958; West-Eberhard, 2005). However, few empirical studies of any system have measured the effects on expression of relevant phenotypes for both TGP and WGP in the same individuals, leaving unanswered questions regarding the relationship between these two forms of plasticity as well as the trait types and life history stages that they affect. Such information would provide a fundamental baseline for evaluating more general hypotheses about WGP and TGP's fitness consequences and evolutionary roles.

It is generally expected that the strength of parental effects, including those which are environmentally induced, should be stronger in early life stages of offspring, while older offspring should rely on their own environmental cues through WGP (Moore et al., 2019; Mousseau & Dingle, 1991). Greater reliance on parental cues through TGP at very early ages is often necessary because many organisms do not develop sensory organs until a later life stage (Uller, 2008). However, once capable of assessing their own environment, an organism's own cues are expected to be more accurate than parental cues due to the time lag between parental environmental detection and offspring's selective environment (Auge et al., 2017). More practically, the mechanisms through which TGP acts (e.g., differential provisioning of nutrients, hormones, RNA) may constrain its effects to early stages of offspring development (Mousseau & Dingle, 1991). Consistent with these predictions, a recent meta-analysis found that maternal effects (both genetic and environmentally induced) more strongly impact juvenile offspring traits than adult offspring traits, though these effects still explained a non-trivial amount of variation in adults (Moore et al., 2019). Another outstanding

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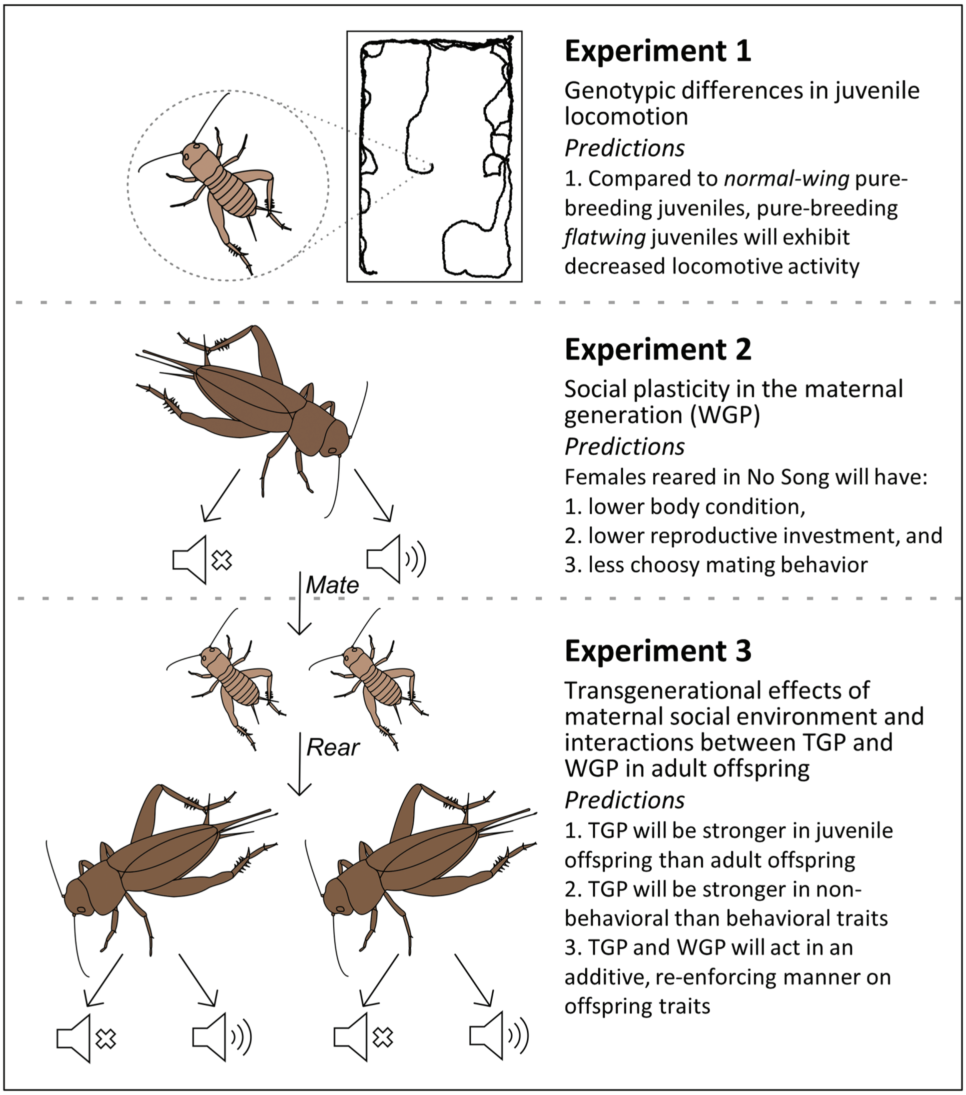
question is whether certain trait types, e.g., morphology and physiology vs. behavior, are predisposed to greater influence from TGP vs. WGP. Parental effects on offspring morphology and physiology (hereafter non-behavioral traits) are anticipated to be stronger than its effects on offspring behavior, because non-behavioral traits are more often fixed at an early stage when TGP is strongest, while behavioral traits remain flexible into adulthood, a pattern found in the freshwater snail *Physa acuta* (Beaty et al., 2016). However, there is also empirical evidence of TGP effects on offspring behavior in response to parental stressors including predator/parasite exposure (Cattelan et al., 2020; Giesing et al., 2011; Tschirren et al., 2007). Few studies which explore the simultaneous effects of both WGP and TGP have considered multiple trait types and different offspring life stages, limiting our ability to predict the relative contributions of these two forms of plasticity to phenotypic variation.

Although theoretical studies have explored the circumstances under which TGP vs. WGP is likely to evolve, it remains unclear how these two forms of plasticity interact when they occur simultaneously. Various outcomes could arise depending on the nature of their interaction. For example, a study of anti-predator defensive helmet formation in *Daphnia cucullata* found that WGP and TGP additively contribute to offspring phenotype in a reinforcing manner (Agrawal et al., 1999). Such a relationship could move a population toward 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) (Donelan & Trussell, 2015; Luquet & Tariel, 2016; Prasad et al., 2003; Stein et al., 2018; Zirbel et al., 2018). The relationship between TGP and WGP has been explored in the context of environmentally induced responses to predation risk (Agrawal et al., 1999; Donelan & Trussell, 2015; Luquet & Tariel, 2016; Stein et al., 2018), nutrition (Prasad et al., 2003; Zirbel et al., 2018), and temperature (Bernareggi et al., 2016) in a limited number of species, but the nature of this interaction in other important contexts, such as the social environment, remains largely unknown.

To experimentally dissect interactions between WGP and TGP, we tested phenotypic effects of maternal and offspring social environment in a rapidly evolving population of the field cricket, *Teleogryllus oceanicus*. This system enabled us to test the effects of WGP and TGP on multiple trait types and life stages in a controlled laboratory setting, with clear relevance to the dynamics of ongoing adaptation in the wild. In Hawaii, acoustically signaling (i.e., singing) males are attacked by an acoustically orienting parasitoid fly, *Ormia ochracea*. Recently, a single-locus, X-linked mutation, *flatwing* (*fw*), emerged and spread in fewer than 20 generations to affect ca. 90% of males in a population on the island of Kauai (Pascoal et al., 2020; Zuk et al., 2006). Males normally sing using their forewings, but *fw* disrupts normal male wing development, silencing the songs of “flatwing” males and shielding them from fly attack, while *fw*-carrying (either heterozygous or homozygous) females’ wing venation is left unchanged (Zuk et al., 2006). Its subsequent rapid spread to fixation (Rayner, Aldridge, et al., 2019; Tinghitella et al., 2018) dramatically changed the social environment by eliminating the conspicuous long-range male acoustic signal (song) that functions in mate attraction, courtship, and intrasexual aggression. Thus, although the mutation offers protection from parasitoid attack, it also has profound negative pleiotropic effects: flatwing males cannot use song to acoustically advertise for mates. Previous studies suggest that pre-existing WGP in response to male song, for both behavioral and non-behavioral traits, may have offset fitness costs associated with flatwing males’ inability to attract females, thus facilitating the rapid spread of *flatwing*. For example, both flatwing and normal-wing adult males show an overall increase in locomotor behavior when reared in the absence of male song (Balenger & Zuk, 2015). Balenger & Zuk (2015) argue that greater locomotive activity is an adaptive plastic response, as it likely increases a male’s chances of encountering a female in a flatwing-dominated, silent environment. Further supporting a role for WGP in this system, females raised in an environment lacking song increase responsiveness to calling males by moving more quickly toward them—enabling them to locate the few remaining singing males—and also become less discriminating among males to whom they respond (Bailey & Zuk, 2008, 2012). Regarding plasticity in non-behavioral traits, when reared without song, individuals of both sexes develop decreased reproductive tissue mass compared to their counterparts raised in the presence of song (Bailey et al., 2010; Heinen-Kay et al., 2019). Many organisms exhibit an adaptive shift from fecundity-supporting processes to survival-promoting processes in response to social signals of low mating opportunity or competition (Harshman & Zera, 2007). In the context of a flatwing-dominated social environment where mating encounters occur more often by chance than a typical singing cricket population, this plastic shift could be particularly adaptive by increasing an individual’s chances of surviving long enough to encounter a mate. Whether and how these traits also shift in response to socially induced TGP in this system remains unknown.

We performed three experiments to dissect the potential contributions of genetics, WGP, and TGP to the expression of a range of traits discussed above in a rapidly evolving population (see Figure 1 for a conceptual summary of experiments and predictions). In **Experiment 1**, we measured how juvenile (both male and female) locomotive behavior varied across *fw* and *normal-wing* (*nw*) genotypes using replicate lines fixed for each wing morph, before any maternal social manipulation. This allowed us to determine whether the rapid spread of flatwing males might have been associated with genetic changes in a trait relevant to the changing social environment. We expected that females homozygous for *fw* and males hemizygous for *fw* disperse less as juveniles, as remaining aggregated at higher densities upon reproductive maturity is expected to increase the chances of mating in a song-less environment.

In **Experiment 2**, we investigated WGP to the acoustic social environment. We focused on females that were homozygous for *fw* because the source population is now all-flatwing (Rayner, Aldridge, et al., 2019; Tinghitella et al., 2018) and previous work indicated that the *fw* variant is associated with increased socially induced WGP in gene expression (Pascoal et al., 2018). We predicted that, consistent with previous studies on females carrying *nw* genotypes (Bailey & Zuk, 2008, 2012; Bailey et al., 2010), *fw*-carrying females (which comprised the maternal generation for Experiment 3 below) raised in an environment without male song would exhibit less “choosy” or discriminative mating behavior and decreased reproductive investment.



Experiment 1

Genotypic differences in juvenile locomotion

Predictions

1. Compared to *normal-wing* pure-breeding juveniles, pure-breeding *flatwing* juveniles will exhibit decreased locomotive activity

Experiment 2

Social plasticity in the maternal generation (WGP)

Predictions

Females reared in No Song will have:

1. lower body condition,
2. lower reproductive investment, and
3. less choosy mating behavior

Experiment 3

Transgenerational effects of maternal social environment and interactions between TGP and WGP in adult offspring

Predictions

1. TGP will be stronger in juvenile offspring than adult offspring
2. TGP will be stronger in non-behavioral than behavioral traits
3. TGP and WGP will act in an additive, re-enforcing manner on offspring traits

Figure 1. Description of the study's three experiments and predictions for each. For Experiment 1, locomotion and pronotum length were measured. For Experiment 2, body condition, reproductive investment (ovary and egg weight), and mating behavior (i.e., likelihood of mounting by females) were measured in females homozygous for *flatwing*. For Experiment 3, locomotion and pronotum length were measured for juvenile offspring, and locomotion, pronotum length, somatic condition, and reproductive investment (egg and ovary or testes mass) were measured for adult offspring (both male and female) in a population pure-breeding for *flatwing*.

Finally, in **Experiment 3**, we tested transgenerational consequences of the maternal social environment by measuring size and locomotion in acoustically naïve juveniles, and final size, somatic condition, reproductive investment, and locomotion in adult offspring using individuals hemizygous or homozygous for *fw*. In this last experiment, adult offspring were exposed to either matched or mis-matched acoustic cues compared to their mothers. Using this comprehensive experimental design, we were able to directly test for interactions between WGP and TGP, informing several predictions about patterns of TGP in the context of a flatwing-dominated environment. First, we predicted that any TGP effects would be stronger in juvenile offspring compared to adult offspring. Crickets do not possess a fully developed auditory system until adulthood, though late juveniles may have limited auditory capabilities (Staudacher, 2009; Yack, 2004; Young & Ball, 1974), so young juveniles are not likely to be capable of accurately assessing their own social environment. Second, we predicted that the effects of TGP would be stronger in non-behavioral traits compared to behavioral traits. Finally, we predicted that TGP would affect offspring traits additively and in the same direction as WGP.

Methods

Experiment 1: Genotypic differences in juvenile locomotion

Cricket populations and rearing

We compared early juvenile behavior across the two cricket morph genotypes using 6 laboratory stock lines—3 pure-breeding for *fw* and 3 pure-breeding for *nw*. Lines were established in 2016 from a series of controlled crosses of Kauai-derived individuals to ensure homozygosity of allele(s) controlling wing morphology (Pascoal et al., 2016). As field crickets have XX/XO sex determination and *flatwing* is X-linked (Pascoal et al., 2014; Tinghitella, 2008), all males were hemizygous and females homozygous for the respective genotypes. Stock crickets were kept in 16-L plastic containers with cardboard egg cartons for shelter. Twice weekly, they were provided ad libitum food (Burgess Supa Rabbit Exel Junior pellets; blended for juveniles) and moistened cotton for water and oviposition. Crickets in isolated-rearing conditions were kept in 100 ml plastic deli cups (a cylindrical plastic container with a removable lid) with shelter, food, and water as above. All subjects were kept in the same growth chamber at 25 °C on a photo-reversed 12:12 hr light:dark cycle unless otherwise indicated. To obtain juveniles for this experiment, we collected eggs from each line twice weekly for four weeks. After approximately two weeks we monitored egg pads daily (16:00–18:00) and isolated new hatchlings.

Open field test

An open field test (OFT) was used to track individual crickets' movements in an unobstructed arena and measure their total distance traveled, a useful proxy for measuring behaviors related to dispersal, mate location, and foraging (Dingemanse et al., 2003; Fraser et al., 2001; Korsten et al., 2013). For this experiment, juveniles were isolated at hatching, and each was tested in an OFT at 15- and 45 days post-hatching. Juveniles of these ages do not have mature hearing structures (Young & Ball, 1974). All OFTs were performed under red light during the dark portion of the crickets' 12:12 light:dark cycle, between 23 and 25 °C. As in previous studies, red light was used because crickets cannot see at this wavelength (Bailey & Zuk, 2009; Hedrick & Kortet, 2012; Tinghitella et al., 2009). Subjects were placed in small glass vials within their deli cup to reduce handling disturbance before testing. The vial was gently turned over onto the center of an 11 × 17 cm clear plastic arena atop white poster paper and the cricket was allowed to acclimatize for 2 min. Upon lifting the vial, we began recording for 5 min at 30 frames/s using a camera (Nikon D3300) mounted ca. 40 cm above the arena. The arena was wiped down with 70% ethanol before each trial to minimize residual chemical cues. Two crickets were assayed at once in side-by-side arenas. It is unlikely that they were aware of one another due to their inability to see in red wavelengths of light. After the OFT, each cricket was photographed overtop a micrometer using a Leica DFC295 digital camera affixed to a Leica M60 dissecting microscope. ImageJ (v.1.8.0_112) was used to record pronotum length (a proxy for structural size, which refers to an inflexible measure of exoskeleton size which cannot change after the crickets' final molt except through physical damage) from the images. A total of 254 individuals were assayed at 15 days-post-hatching (130 from flatwing lines and 124 from normal-wing lines). Of those individuals, 228 survived to 45 days-post-hatching and were assayed again (115 from flatwing lines and 113 from normal-wing lines).

Locomotion measurements

We used DORIS v.0.0.17 (Friard, 2019) to extract coordinates of the test subject within each video frame, followed by coordinate path smoothing implemented in R (R Core Team, 2020) to increase measurement precision (see Supplementary Methods and Supplementary Figure S1). Using these coordinates, we measured total distance traveled ("*distance*") during trials. In this and later experiments involving OFTs, we also explored other movement parameters ("*proportion explored*" as a measure of exploratory activity; and "*origin time,*" "*middle time,*" and "*edge time*" as measures of space usage and thigmotaxis). However, variation in these parameters was largely accounted for by overall differences in distance moved, confirming that *distance* was the most salient locomotion trait in the experiment. For completeness, we discuss the measurement and analysis of all other movement traits in Supplementary Material.

Statistical analyses

All statistical tests were carried out using R version 4.0.2 (R Core Team, 2020). We compared *distance* between wing morph genotypes in 15- and 45-day old offspring using linear models (LMs) using the R package *lme4* (Bates et al., 2007). Individuals who jumped during their assay (n = 2 in 45-day assay) or whose video was inadvertently deleted before analysis (n = 2 in 45-day assay) were excluded. All data transformations are shown in Supplementary Table S1.

Morph and sex were modeled as categorical variables, with line nested within morph to account for inter-line variation within each morph. Pronotum length, temperature, and time of day were included as covariates. Thirty-nine individuals died before their sex could be identified, so to verify that sex did not qualitatively affect the findings, models including sex as a fixed effect were run on the subset of individuals for which sex could be identified. Sex did not approach significance in this model (all p > .2) and the qualitative outcome did not differ. Thus, the model retaining all individuals, and excluding sex as a fixed effect, was retained (Equation 1 of Supplementary Table S2). Finally, the model was run first with all individuals, then with only those who moved during the assay to confirm that genotypic variation in *distance* was not due to differences in the likelihood of initiating movement. Excluding crickets that failed to initiate movement did not affect interpretations of genotype differences (Supplementary Table S3) and a distance of 0 is still biologically relevant, as it could indicate a less exploratory or active behavioral tendency, so final models included stationary crickets. We also ran a generalized linear model (GLM) with a binomial distribution to determine if the likelihood of movement was impacted by morph.

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

Cricket populations and rearing

Given the focus on homozygous *fw* individuals and requirement for high replication in onward WGP and TGP experiments, the three pure-breeding *fw* lines used in Experiment 1 were reciprocally interbred to create an admixed pure-breeding *fw* stock population. Following previous work, we isolated juvenile females from this stock when sex became apparent to ensure virginity and more easily manipulate their acoustic environment (Bailey & Zuk, 2008; Pascoal et al., 2018). We also segregated a group of juvenile flatwing males into single-sex 16-L box to maintain their virginity. All group rearing conditions were identical to Experiment 1. Isolated females were placed in a separate, temperature-controlled 25 °C incubator on a 12:12 hr photo-reversed light cycle, with no male calling. Females were checked daily for adult eclosion, whereupon they were haphazardly assigned one of two acoustic social treatments: Song or No Song. Females do not achieve reproductive maturity until several days after adult eclosion, so our acoustic treatment targets the developmental period when mate assessment is possible but mating is not (Swanger & Zuk, 2015). We also recorded the number of days spent isolated prior to eclosion to account for any differences in growth rate that might be associated with time spent without song prior to adult acoustic treatment. We kept each female in their acoustic treatment for 15 days post-eclosion (i.e., post final molt into the adult stage) to maximize the opportunity for females to experience their own social environment.

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 12:12 hr 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 to the nearest 0.01 mm using digital calipers. Each female was placed in a 16 × 18 cm plastic container with cardboard, rabbit chow, and moistened cotton. We haphazardly selected an adult virgin male from the flatwing admixed stock population (and thus a male hemizygous for *flatwing*), weighed it, measured its pronotum width, and placed it in the container with the female. Trials were performed between 20 and 23 °C under red light between 16:00 and 18:00 hr. They lasted for 20 min, 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 hr, the male was removed to reduce potential paternal influences on offspring phenotype. After another 24–48 hr, the female was removed, and the egg pad was collected for use in Experiment 3. A total of 65 females were used for mating trials (33 No Song and 32 Song).

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 & Green, 2009) using the *smatr* package in R (Warton et al., 2012). SMI was measured in a total of 114 females (60 No Song and 54 Song), including the 65 females used for mating trials. A subset of these females drawn haphazardly from each treatment (total $n = 23$: 13 No Song and 10 Song) were dissected at 15 days post-eclosion rather than mated and thus contributed to data on SMI as well as reproductive investment. We recorded their pronotum width to the nearest 0.01 mm using digital calipers, weighed them, and then determined wet mass of their dissected ovaries (wet ovary mass included eggs and ovary tissue). Somatic mass was calculated by subtracting ovary mass from total weight.

Statistical analyses

First, we compared *SMI* across acoustic treatments using a LM (Equation 2 of Supplementary Table S2) with acoustic treatment as a categorical factor, days isolated before treatment as a covariate, and experimental replicate (block one or block two of the experiment). Replicate only had two factor levels so we included it as a fixed effect. Second, we compared mating behavior across acoustic treatments. We first ran a GLM with binomial error to examine presence vs. absence of *female mounting* during trials, including acoustic treatment, female SMI, and male SMI as predictors. Next, we ran a binomial GLM examining presence vs. absence of *spermatophore transfer*, with the same predictor variables. For this, we only included the 49 mating trials (out of 65 total) where mounting had occurred, because spermatophore transfer cannot occur without mounting. Equation 3 in Supplementary Table S2 gives the general form of these models.

Finally, we compared female reproductive investment (ovary mass) across acoustic treatments in the subset ($n = 23$) of females that had been dissected by running a LM on *ovary mass*, with acoustic treatment and days isolated as predictor and covariate, respectively. As in previous studies of reproductive investment, we controlled for body size by including log-transformed soma mass as an additional covariate (Bailey et al., 2010; Tomkins & Simmons, 2002) (Equation 4 in Supplementary Table S2).

Experiment 3: Transgenerational effects of maternal social environment and interactions between TGP and WGP in adult offspring

Cricket populations and rearing

Eggs produced by the maternal generation in Experiment 2 were first kept in a separate incubator under the same temperature and light conditions as the general incubator. As they began to hatch, the first U.K. national lockdown in response to the 2020 Covid-19 pandemic (March 23, 2020) required that all laboratory experiments be run under strict social distancing measures, which affected where and how some of our procedures were executed. A description of "socially-distanced" methods plus our design to statistically account for any variation it introduced is provided in Supplementary Methods.

We first tested TGP effects in juvenile offspring. Hatchlings were isolated as described previously and kept at 18–24 °C on a 12:12 hr photo-reversed light:dark cycle. We ran the experiment in two blocks (one during the United Kingdom's first Covid-19 lockdown and one after), with individuals in the second block kept at 25 °C in the lab incubator, as in Experiment 1. For the early juvenile offspring TGP experiment, we tested a total of 311 offspring (131 from 14 mothers treated with Song, 180 from 21 mothers treated with No Song) at 15 days post-hatching. 199 individuals were tested again at 45 days post-hatching (117 from 11 No Song mothers and 82 from 8 Song mothers).

For adult offspring experiments, hatchlings were kept in 10 replicate group-rearing boxes during development to match the demographic rearing conditions experienced by the previous generation. Once sex was apparent during development, individuals were isolated and assigned to either acoustic treatment using the same incubators and playback schedules as in Experiment 2. The distribution of adult offspring (n = 387) across the four maternal-offspring acoustic treatment combinations is shown in Supplementary Table S6. It is important to note that the adults measured in this experiment were not the same individuals as those used for the TGP juvenile trials described above; thus, the corresponding data sets are derived from different individuals of the same generation.

OFT

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

Non-behavioral trait measurements

In offspring, measures of size (e.g., structural size in juveniles and adults and somatic condition in adults) were recorded both because previous studies of TGP in other species have found that maternal crowding influences offspring size (Crocker & Hunter, 2018a; Dantzer et al., 2013; McCormick, 2006) and because size plays an important role in mate choice in field crickets (Simmons, 1988). Following the OFT, we photographed each juvenile overtop a micrometer using the same camera and dissecting scope as in Experiment 1 and measured pronotum length using ImageJ (v.1.8.0_112). We euthanized adults after OFTs at eight days post-eclosion, then weighed them and measured pronotum length to the nearest 0.01 mm using digital calipers. We then dissected, blotted excess fluid, and weighed their gonads (male testes and accessory glands, female eggs and ovaries). Here we used pronotum length and soma weight to calculate SMI, by subtracting gonad weight from total weight. In this experiment, SMI was thus a measure of somatic body condition, which allowed us to investigate whether differences in maternal or offspring acoustic environments influenced relative investment in somatic tissues while scaling to structural size. SMI was calculated separately for each sex. Gonad weight was later compared directly.

Statistical analyzes

First, we tested whether *juvenile offspring size* differed between maternal acoustic treatments by running a linear mixed model (LMM) using pronotum length as the response. Maternal treatment and experimental replicate were included as fixed effects, with maternal ID as a random effect. Experimental replicate was included to account for different rearing temperatures in trial 1 and trial 2 (see Supplementary Methods). Models took the general form of Equation 5 in Supplementary Table S2. We then examined the effect of maternal treatment on *juvenile offspring distance* using an LMM which included maternal treatment and experimental replicate as categorical factors; temperature, time of day, and pronotum length as covariates; and maternal ID as a random effect. The general form of the model is given by Equation 6 in Supplementary Table S2. In models of both *juvenile offspring size* and *juvenile offspring distance*, the treatment*replicate interaction ($p > .2$) was excluded from the final models. For each response variable, a similar model was run for 45-day old offspring, except experimental replicate was not included because we only had 45-day data for trial 1.

To investigate TGP, WGP, and their interaction, we tested the effects of maternal and offspring acoustic treatments on *adult pronotum length* and *somatic condition (SMI)*. Because there are large sex differences in physiology and the possibility of sex-specific maternal effects, we ran separate models for offspring of each sex. Each LMM included maternal and offspring treatments as factors plus their interaction. Non-significant ($p > .2$) interactions were removed. We analyzed the effect of acoustic treatments on *adult offspring reproductive investment* using sex-specific models with gonad weight as the response. First, we compared unscaled reproductive investment, then we added pronotum length as a covariate to examine whether variation in reproductive investment could be explained by structural size. Finally, we added log-transformed somatic mass to examine whether variation in reproductive investment might be explained by somatic weight. An additional random effect of "box replicate" was included to account for variation that could potentially have been introduced as a result of different cricket rearing locations necessitated by the 2020 Covid-19 lockdown (see Supplemental Methods for details about how this was experimentally controlled). These models took the general form shown in Equation 7 of Supplementary Table S2.

We then tested the impact of TGP and WGP on adult *distance* using separate LMMs for each sex. All models included maternal treatment, offspring treatment, temperature, time, and somatic SMI, plus box replicate as a random effect (Equation 8 in Supplementary Table S2). Non-significant ($p >$ .2) maternal*offspring treatment interactions were removed.

As a post hoc analysis of treatment and sex variation in attempted flight behavior, we modeled *flight attempts* using a generalized linear mixed model (GLMM) with a binomial error (1 if flight was attempted and 0 if not) with the predictors: maternal treatment, offspring treatment, sex, maternal treatment*sex, and somatic SMI. Box replicate was included as a random effect. Equation 9 of Supplementary Table S2 describes the final model after 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 ($F_{1,245} = 12.988$, $p < .001$) and in the subset of the same individuals who survived to 45 days post-hatching ($F_{1,210} = 16.554$, $p < .001$) (Figure 2, Supplementary Table S4). 15-day-old *fw* nymphs, which had a mean length of 3.81 mm, moved an average of 299.93 mm (ca. 79x their body length) further than *nw* nymphs. For 45-day-old nymphs, the average movement differential was 132.73 mm (ca. 12.9× their body length). Note that at both ages, the greater distance moved by *fw* nymphs were detected despite there being significant variation across replicate lines within each morph (Supplementary Table S4 and Supplementary Figure S2). At 15 days post-hatching, the morphs did not differ in their likelihood to initiate movement ($\chi^2_{1,245} = 0.835$, $p = .361$), but at 45 days, flatwing lines were more likely to initiate movement than normal-wing lines ($\chi^2_{1,214} = 4.408$, $p = .036$) (Supplementary Table S5).

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

The acoustic social environment affected physiology (Figure 3, Supplementary Table S7), but not mating behavior (Supplementary Table S8 and Supplementary Figure S4) in homozygous *fw* females. Those raised in Song attained higher condition (SMI) ($F_{1,110} = 6.038$, $p = .016$) (Figure 3A, Supplementary Table S7) and had heavier ovaries relative to somatic mass ($F_{1,19} = 5.015$, $p = .037$) (Figure 3B, Supplementary Table S7). Female mounting was not influenced by prior acoustic experience ($\chi^2_{1,61} = 0.340$, $p = .560$), though females of both acoustic treatments were more likely to mount higher condition males ($\chi^2_{1,61} = 6.789$, $p = .009$) (Supplementary Table S8 and Supplementary Figure S4A). Similarly, in trials where females did mount males ($n = 49$), there was no evidence that acoustic treatment affected spermatophore transfer ($\chi^2_{1,45} = 0.332$, $p = .565$), though males were more likely to transfer a spermatophore to higher condition females ($\chi^2_{1,45} = 5.361$, $p = .021$) (Supplementary Table S8 and Supplementary Figure S4B).

Experiment 3: Transgenerational effects of maternal social environment and interactions between TGP and WGP in adult offspring

Unexpectedly, TGP did not influence juvenile offspring traits. The total distance that juvenile offspring moved was similar regardless of their mothers' acoustic treatment ($F_{1,300} = 0.625$, $p = .429$ for 15-day-old juveniles; $F_{1,191} = 0.003$, $p = .955$ for 45-day-old juveniles) (Supplementary Table S9 and S10) as was their size (pronotum length: $\chi^2_{1,307} = 0.046$, $p = .829$ for 15-day-old juveniles; $\chi^2_{1,198} = 0.732$, $p = .392$ for 45-day-old juveniles) (Supplementary Table S11).

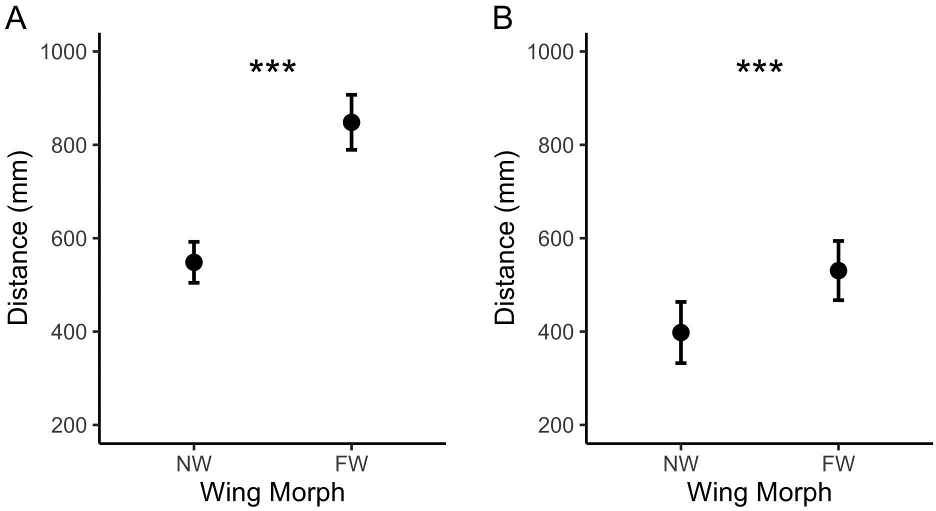


Figure 2. The effect of wing morph genotype—*normal-wing* (NW) versus *flatwing* (FW)—on total distance traveled by juveniles of both sexes combined in OFTs for (A) 15 day-old nymphs and (B) the same nymphs at 45 days. Plots illustrate pooled means across three replicate morph lines ± 1 standard error. ***$p < .001$.

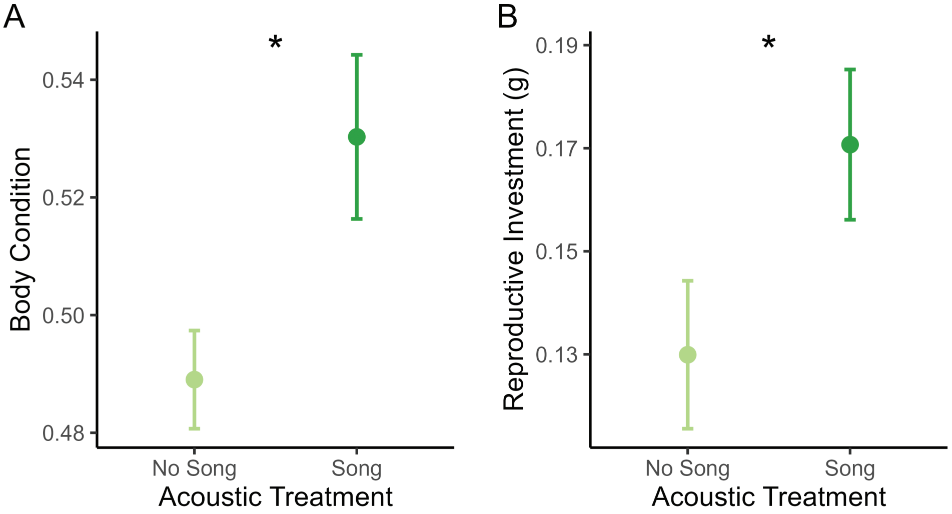


Figure 3. The effect of acoustic environment on (A) adult female body condition (scaled mass index: SMI) and (B) reproductive investment (ovary and egg mass) in females homozygous for *flatwing*. Plots show means ± 1 standard error. *$p < .05$.

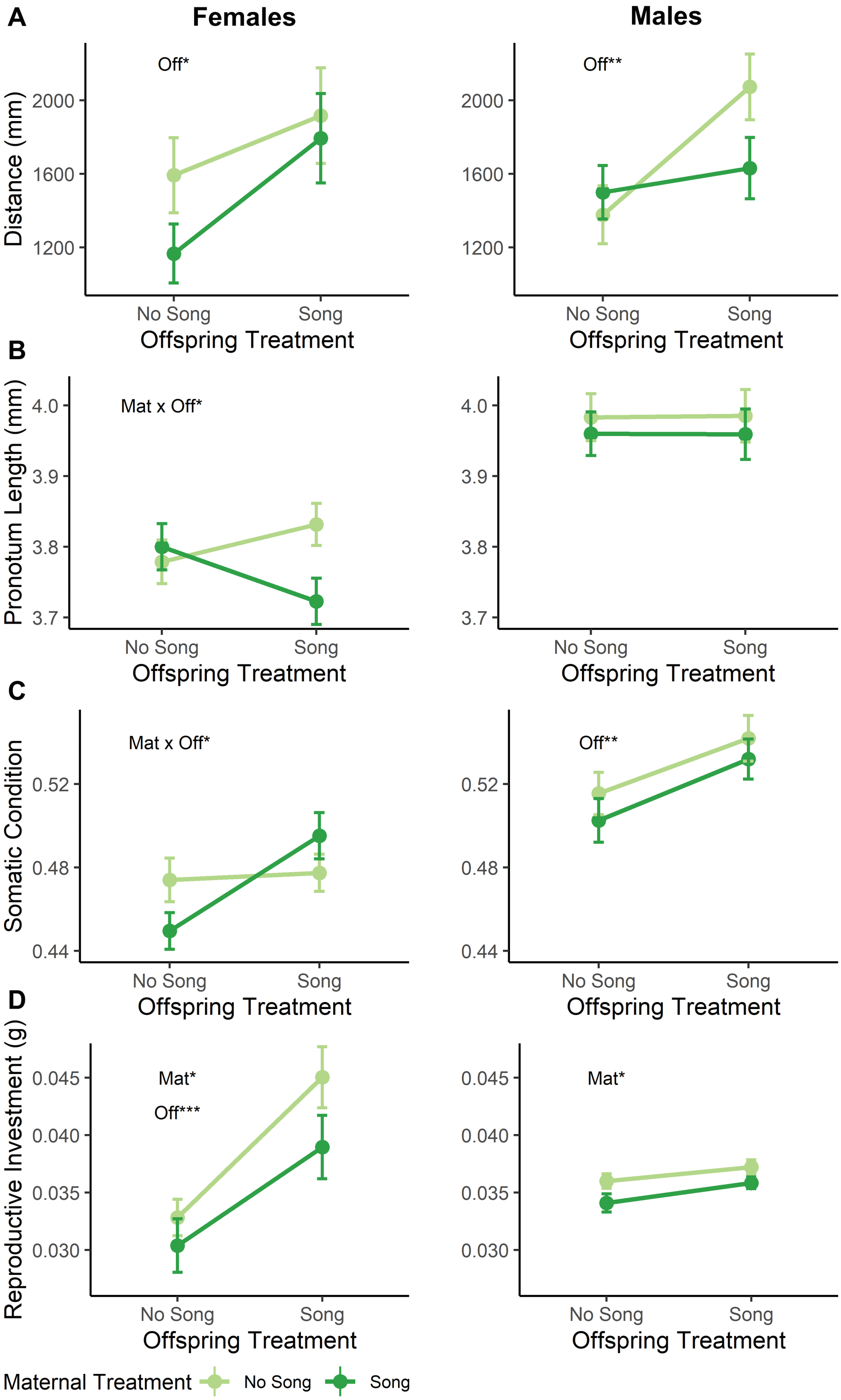


Figure 4. Effects of WGP and TGP in adult female (left) and adult male (right) offspring. (A) Distance traveled (B) pronotum length, (C) somatic condition (SMI), (D) reproductive investment, i.e., female ovaries and egg mass and male testis and accessory gland mass. Means are indicated by circles and bars indicate ± 1 standard error. Mat = effect of maternal treatment. Off = effect of offspring treatment. Mat × Off = effect of the interaction between maternal and offspring treatments. *$p < .05$, **$p < .01$, ***$p < .001$.

In adult offspring, acoustic effects on non-behavioral traits provided strong and consistent evidence for WGP, and TGP also affected several of these traits (Figure 4B–D, Supplementary Tables S16 and S17). We found no evidence that TGP affected these traits in the same direction as WGP; instead, for two traits (female pronotum length and female somatic condition), we observed significant interactions between TGP and WGP. Female offspring reared without song had similar pronotum lengths, but when they were reared with song, those whose mothers experienced No Song grew to be larger than those whose mothers experienced Song (*Maternal Treatment*Offspring Treatment* term $F_{1,186} = 4.741$, $p = .029$) (Figure 4B, left; Supplementary Table S16). Female somatic condition showed a similar crossing-over effect (*Maternal Treatment*Offspring Treatment* term $F_{1,187} = 4.670$, $p = .031$) (Figure 4C, left; Supplementary Table S16). Also, TGP and WGP affected female reproductive investment but in conflicting directions. Adult females raised in song developed heavier ovaries than those raised without song (*Offspring Treatment* term $F_{1,186} = 16.860$, $p < .001$), and female offspring from the No Song maternal treatment developed heavier ovaries than offspring from mothers who experienced Song (*Maternal Treatment* term $F_{1,186} = 3.910$, $p = .048$) (Figure 4D, left; Supplementary Table S17). Adult males raised in the presence of song developed higher somatic condition than those raised without song (*Offspring Treatment* term $F_{1,187} = 8.169$, $p = .004$), regardless of maternal treatment (Figure 4B, right; Supplementary Table S16). Consistent with females, adult male offspring reproductive investment was higher in individuals whose mothers had been reared in silence (*Maternal Treatment* term $F_{1,185} = 3.975$, $p = .046$) (Figure 4D, right; Supplementary Table S17), but in male offspring, offspring acoustic treatment did not significantly affect reproductive investment (*Offspring Treatment* term $F_{1,185} = 2.800$, $p = .094$) (Figure 4D, left; Supplementary Table S17).

In contrast to non-behavioral traits in adult offspring, adult offspring behavioral traits were largely influenced only by WGP, not TGP. Adult male and female offspring that experienced song themselves moved further (*Offspring Treatment* term $F_{1,182} = 6.985$, $p = .008$ for males; $F_{1,181} = 6.279$, $p = .012$ for females) and there was no significant WGP*TGP interaction for either sex (*Maternal Treatment*Offspring Treatment* term $F_{1,182} = 3.695$, $p = .055$ for males) (Figure 4A, Supplementary Table S14). Additionally, crickets raised without song attempted flight more than those raised with song (*Offspring Treatment* term $F_{1,371} = 8.788$, $p = .003$), with no effect of maternal treatment (*Maternal Treatment* term $F_{1,371} = 0.085$, $p = .770$) (Figure 5; Supplementary Table S18). Flight occurred more often in females (*Sex*: $F_{1,371} = 4.213$, $p = .040$) and individuals of higher somatic condition (*Somatic Condition* term $F_{1,371} = 4.161$, $p = .041$) (Supplementary Table S18; Figure 5; Supplementary Figure S5), though no interactions were significant.

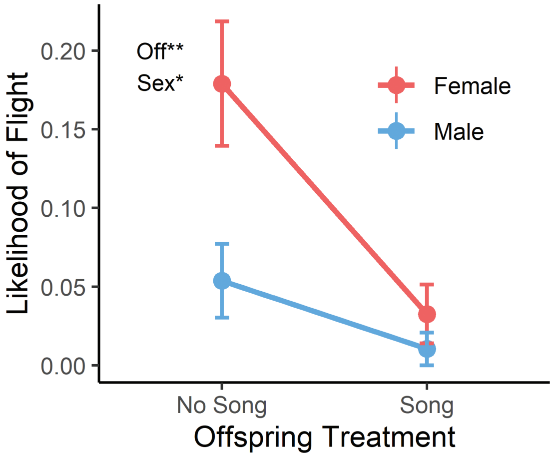


Figure 5. Likelihood of flight during open field trials. Means ± 1 standard error are represented by circles and bars, respectively. Significance of offspring ("Off") treatment and offspring sex are indicated with asterisks: *$p < .05$, **$p < .01$.

Discussion

Few empirical studies have been able to assess the relative effects of genetics and different forms of phenotypic plasticity such as WGP and TGP. Here we demonstrate how all three inputs contribute to variation in traits relevant to the rapid evolution of a parasitoid-avoidance adaptation in Hawaiian field crickets: the silencing of male song. Genotype had a surprisingly large effect on juvenile behavior, but in the opposite direction as predicted. At very early juvenile stages, individuals homozygous and hemizygous for *flatwing* moved nearly 80 body lengths further than *normal-wing* homozygotes and hemizygotes in a span of only 5 min. This genotypic difference could result from pleiotropic effects of the *fw* mutation or genomic hitchhiking and raises the possibility that the *fw* genotype may be exposed to selection at an earlier stage than previously considered, e.g., through associated effects on foraging efficiency or predation risk. Rather than facilitating local mating aggregations as we initially hypothesized, greater movement activity may instead permit individuals homozygous or hemizygous for *flatwing* to encounter one another more often. This result illustrates how genetic correlations manifesting during development might impact the trajectory of a mutant genotype which carries fitness benefits at adult stages. The idea that advantageous mutations can have pleiotropic effects during development has been explored extensively in the context of insecticide resistance and alternative reproductive morphs (Boivin et al., 2001; Giraldo-Deck et al., 2020). Against the background of this work, our findings suggest the combined phenotypic effects of adaptive mutations arising from positive pleiotropy, negative pleiotropy, and genomic hitchhiking may be non-intuitive, and phenotypic variation caused by such effects may alter the dynamics of adaptive evolution.

Consistent with our predictions and previous studies in this system and in other cricket species (Bailey et al., 2010; Conroy & Roff, 2018), mothers who experienced No Song invested less in reproductive tissue than mothers reared in the presence of male song. Such WGP is in line with adaptive predictions, as it could allow individuals to reallocate resources to non-reproductive tissue when competition and/or opportunity for mating is low (Harshman & Zera, 2007). This trade-off could be particularly adaptive in a flatwing-dominated social environment where no males can sing, because plastically shifting resources from reproduction to survival could increase the chances that a female survives long enough to find a mate.

WGP has been suggested to be more efficient than TGP, so once capable of assessing their environment, offspring are expected to rewrite parental cues with their own (Auge et al., 2017; Ezard et al., 2014; Moore et al., 2019). We predicted that TGP would be more likely to affect juvenile rather than adult offspring traits, but we found the opposite: the maternal social environment affected adult, but not juvenile, offspring phenotype. One reason for the delayed action of TGP might be that the maternal social environment influenced phenotype via mechanisms that would not cause observable differences until late in development. For example, many insects exhibit significant plasticity in the number of instars they undergo prior to sexual maturation, which can affect sexual size dimorphism at adulthood (Esperk et al., 2007; Stillwell et al., 2010). Another possibility is that the maternal social environment influenced offspring phenotype very early in development, such as size at hatching, but those effects dissipated prior to later phenotypic measurement, as was found in the salamander *Ambystoma talpoideum* (Moore et al., 2015). A third possibility is that TGP mediated by maternal social environments could be qualitatively different from TGP mediated by maternal physical environments. For example, maternal nutritional or thermal environments may have more direct impacts on juvenile offspring, whereas the social environment comprised of adult social cues is likely to be of greater relevance to offspring when they are adults themselves. The physiological mechanisms of TGP remain uncertain, but differential hormone provisioning of eggs appears a likely candidate in this system, as in another cricket, *Acheta domesticus*, low maternal densities increased ecdysteroid provisioning of eggs (Crocker & Hunter, 2018a, 2018b).

While non-behavioral traits were influenced by both TGP and WGP, we found that behavioral traits related to movement (locomotion and likelihood of flight) were only significantly influenced by WGP, with both sexes exhibiting more active walking behavior but lower propensity for flight when reared with song. This supports the prediction that traits whose expression remains flexible after development are more strongly affected by WGP. Song-exposed individuals may increase walking activity to locate conspecifics they perceive to be abundant nearby, even in the absence of an immediate acoustic cue. In contrast, crickets raised without song have no indication of nearby conspecifics and may decrease short-range mate-searching via walking to instead wait for an acoustic cue. This trade-off may be motivated by a high metabolic cost of mate-searching (Hack, 1998) and the resulting increase in predation risk (Bell, 1990). Our results contrast with previous studies in this species which found that adult males raised in song are less active than those raised in silence (Balenger & Zuk, 2015), that females exhibit limited flexibility in locomotion in response to acoustic environment (Heinen-Kay et al., 2018), and experience of silence increases boldness (Moschilla et al., 2022). One explanation for this apparent inconsistency is that the latter studies conducted movement trials in environments containing shelter or cover and, in the case of Moschilla et al. (2022), assessed movement toward acoustic stimuli. The availability of cover during trials could have reduced the perception of risk associated with walking, making increased undirected mate-searching in a song-less environment advantageous (Hedrick & Dill, 1993). In contrast, we used an OFT to mimic the experience of Hawaiian crickets within an all-flatwing population, in which locomotion in the absence of any immediately available acoustic signals is likely to have significant fitness consequences. Another explanation could be a trade-off between walking and flight; a song-less environment resulted in decreased walking but increased propensity to flight compared to offspring reared with song (Figure 5). Individuals reared without song may be more disposed to use flight as part of an undirected, long-range dispersal strategy akin to Lévy flight (Viswanathan et al., 1996) to increase their chances of reaching an area of greater conspecific resources. Trading off increased long-range dispersal via flight with decreased walking behavior in song-less, flatwing-dominated environments could have increased the speed at which *flatwing* alleles spread under pressure from parasitoid flies. More generally, the finding that behavior was more sensitive to WGP than TGP has important implications given the unique role behavior is purported to play in adaptive evolution, and future work should investigate the generality of this finding across systems (Bailey et al., 2018; Rayner et al., 2022; Zuk et al., 2014).

In contrast to our prediction that TGP would influence offspring traits additively and in the same direction as WGP, we found that these two forms of plasticity sometimes acted in opposition, sometimes interacted, and in some cases did not appear influential in shaping trait variation. Patterns of reproductive investment provide an illustrative example. As mentioned before, mothers who experienced No Song invested less in reproductive tissue, which is consistent with an adaptive WGP response in a song-less environment (Bailey et al., 2010). Reinforcing effects of TGP and WGP on offspring would facilitate this, but instead we found that TGP and offspring WGP acted in opposing directions on reproductive tissue in both sexes (Figure 4D). Though firm conclusions cannot be drawn about the fitness effects of this TGP without further study, if socially induced WGP in reproductive investment is adaptive in the flatwing system, the finding that TGP exhibits an opposing effect on this trait suggests that this TGP is not in line with adaptive predictions. Not all TGP is adaptive—it is possible that the observed effects are an incidentally transmitted physiological consequence of mothers responding to their social environment (selfish TGP [Marshall & Uller, 2007]) or cross-generation spillover of parental condition (parental condition-transfer effects [Bonduriansky & Crean, 2018]). It is important to note that plastic responses can exhibit high intra-specific variation (Scheepens et al., 2018), and future work would benefit from a more detailed quantitative genetic analysis of variation in plasticity than our analysis using three replicate lines per morph permitted, to establish the potential for gene-by-environment interactions for WGP and TGP.

Our results support the idea that the effects of TGP are contingent upon offspring environment. Put another way, sometimes TGP interacts with WGP, and sometimes it opposes WGP. The relative likelihood of these two outcomes may also vary according to trait type and stage of ontogenetic development. It is therefore necessary to consider the potentially conflicting effects of WGP and TGP when predicting phenotypic plasticity's fitness consequences or how it influences adaptive evolution. Theory predicts that, following rapid environmental change, populations may exhibit a transient increase in plasticity because genotypes which shift trait expression closer to a new optimum are favored (Lande, 2009). Additionally, genotypes coding for reaction norm slopes of other traits that offset negative effects of new variants spreading under selection may also be favored, as appears to be the case in *T. oceanicus* in Hawaii and other systems (Bailey et al., 2021). If increases in WGP during an adaptive evolutionary response result in a spillover of TGP (either adaptive or not) to the offspring generation, the role of plasticity in facilitating the establishment and spread of novel adaptations may be less straightforward than currently understood (Bell & Hellmann, 2019; Bonduriansky & Day, 2009; Lacey, 1998).

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpac036)

Data availability

All data underlying is available at Dryad (DOI: https://doi.org/10.5061/dryad.0rxwdbs4c).

Author contributions

S.L.S. and N.W.B. designed experiments; S.L.S. conducted experiments and analyzed the data; S.L.S. wrote the first draft of the manuscript; N.W.B. 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.

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Conflict of interest: We declare that we have no conflicts of interest.

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