SOCIAL PLASTICITY WITHIN AND ACROSS GENERATIONS : TESTING THE ROLE OF PLASTICITY IN RAPID EVOLUTION IN FIELD CRICKET *TELEOGRYLLUS OCEANICUS*

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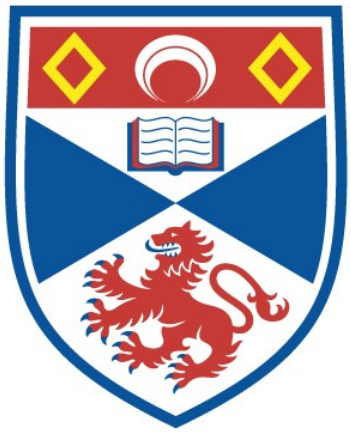

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Declarations

1. **Candidate's declarations:**

I, Sam Sturiale, hereby certify that this thesis, which is approximately …21,412… words in length, has been written by me, and that it is the record of work carried out by me, or principally by myself in collaboration with others as acknowledged, and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student at the University of St Andrews as a candidate for the degree of MSc(Res) in [August, 2019]

Date …5/Apr/2021… signature of candidate ……

2. **Supervisor's declaration:**

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of MSc(Res) in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date: 5/Apr/2021 …… signature of supervisor ……

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Abstract

The field of evolutionary biology lacks a full understanding of how phenotypic plasticity influences adaptive evolution, despite over a century of research effort. One under-studied question within this topic is whether plastic responses occurring within the lifetime of an individual (within-generational plasticity or WGP) versus across generations (transgenerational plasticity or TGP) produce different evolutionary outcomes. To understand how these two forms of plasticity interact, I investigated to what extent an individual's phenotype is shaped by its own social environment and the social environment experienced by its mother using a field cricket model, *Teleogryllus oceanicus*. Crickets in the Hawaiian Islands are targeted by acoustically orienting parasitoid flies, and a mutant form of silent male ('flatwing') recently invaded and rapidly spread under this pressure. Some populations have undergone a full shift from all normal, singing males to all flatwing, silent males, dramatically changing the social environment, and providing the opportunity to explore the hypothesis that both WGP and TGP play a role in adaptive evolution following abrupt environmental change. First, I explored what variation exists in the juvenile morphology and behavior of individuals carrying normal-wing vs. flatwing genotypes, prior to maternal social manipulation. At multiple juvenile stages, flatwing-carrying individuals exhibit greater locomotive activity than normal-wing individuals, suggesting a co-evolved genetic response or the prior genetic coupling of locomotor activity with *flatwing* variants. Second, I tested consequences of social plasticity for the fitness traits of reproductive investment and mating behavior in adult females carrying the *flatwing* genotype, which might permit them to adjust to the absence of song in their environment. This demonstrated that females homozygous for *flatwing* raised in silence exhibit reduced body condition and reproductive investment compared to those raised in song, as predicted. Mating behavior, in contrast, was not sensitive to social environment in these females. Third, I tested whether maternal acoustic environment affects juvenile offspring behavior and morphology, whether effects of maternal acoustic environment persist through offspring development into adulthood, and how the adult social environment of offspring interacts with transgenerational plasticity induced by their mothers' social environment. Unexpectedly, adult, but not juvenile, offspring of mothers raised in different social environments exhibit differences in several ecologically relevant characteristics such as pronotum length, somatic condition, and reproductive investment. Taken together, these results illustrate that genetic and plastic responses may jointly influence the dynamics of rapid adaptive evolution. Further, when exploring the effects of plasticity in adaptive evolution, it is important to consider how WGP and TGP may act simultaneously, and in a non-additive nature, to influence an individual's phenotype.

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Chapter I

INTRODUCTION

A) The role of plasticity in rapid adaptation

Adaptation to environmental pressures can occur one of two ways. The first is an evolutionary response to selection through changes in allele frequencies across generations. The second is through phenotypic plasticity, or the ability of a single genotype to express different phenotypes depending on the environmental conditions experienced. Because such plasticity can invoke environmentally-sensitive responses (including both irreversible developmental changes and more dynamic behavioral flexibility or learning) within an individual's lifetime, it has been argued to produce a more rapid adaptive response to environmental pressure than evolutionary responses (Chevin et al. 2010; Snell-Rood et al. 2018). In this way, when faced with rapid environmental change, plasticity may "buy time" for populations to adapt via slower evolutionary responses (Robinson and Dukas 1999; Lande 2009). Others argue that, rather than buying time for evolutionary responses to occur, plasticity shields genetic variation from selection, effectively retarding adaptation on a longer timescale (Huey et al. 2003; Ghalambor et al. 2007). For example, thermoregulatory behavioral plasticity is expected to allow many lizard species to track climate change in the short-term, but will ultimately prevent the evolution of the increased thermal tolerance that is necessary for long-term persistence in an increasingly warming climate (Buckley et al. 2015). Another hypothesis, often referred to as plasticity-first-evolution (PFE), suggests that rather than shielding genetic variation from selection, plasticity promotes the storage and release of "cryptic genetic variation" (Levis and Pfennig 2018). In this way, when an environmental change occurs, pre-existing plasticity can facilitate an evolutionary response to create a new adaptive trait (Levis et al. 2018).

Resolving the relative contributions and interactions of evolutionary responses and phenotypic plasticity to rapid adaptation requires us to disentangle whether observed phenotypic shifts in natural populations are primarily driven by genetic or plastic responses. Further, it is necessary to better understand the relative incidence of adaptive versus non-adaptive plasticity to build a general framework for the role of plasticity in evolution (Ghalambor et al. 2007). It is crucial that we characterize the nature of phenotypic shifts – adaptive or maladaptive, plastic or genetic, rapid or much too slow – to better predict the ultimate outcome for species experiencing abrupt environmental changes. I performed a series of experimental studies that illustrate the influences of intrinsic (genotype and sex) and extrinsic (social environment, maternal social environment) factors on several ecologically relevant characteristics in a species which has recently undergone rapid adaptation to a novel predator via loss of an important sexual signal. Focusing on the important form of plasticity which is responsive to the social environment (hereafter 'social plasticity'), I further dissect the contribution of phenotypic plasticity to adaptive evolution by investigating whether social plasticity expressed across generations (transgenerational plasticity) mirrors the effects of that within a generation (within-generational plasticity).

B) Social plasticity

An organism's social environment is defined by the quantity and/or characteristics (sex, age, condition, etc.) of conspecifics present in its vicinity. The social environment can have dramatic consequences for individual fitness in many animal species, including those that rely on social groups to defend against predators, gather resources and raise offspring (*Reviewed in* Silk 2007; Stanton and Mann 2012). For example, females of the long-lived social rodent, *Marmota marmota*, show increased lifetime reproductive success when born into groups with many helpers (Berger et al. 2015). However, even in species that do not exhibit strict group dynamics and are not considered eusocial, social environment can influence individual fitness by modulating levels of conspecific competition for resources and mates (Relyea 2002). Because of these clear impacts on fitness, selection is expected to optimize a population's average phenotype to best fit its social environment. However, while changes in abiotic environment often occur gradually over many generations, changes in social environment could arise rapidly within the lifetime of an individual, precluding genetic adaptation. Many researchers have therefore investigated the role of phenotypic plasticity in species' adaptation to heterogeneous social environments. Much of this work has examined the contributions of within-generation plasticity (WGP), where an individual shifts its developmental or phenotypic trajectory in response to environmental cues that it detects during its lifetime (Stockley and Seal 2001; Kasumovic et al. 2011; Kasumovic et al. 2012; Kotrschal et al. 2012; Rodríguez et al. 2013). These shifts can take the form of morphological changes affecting characteristics such as developmental rate, metabolic rate, and investment in reproductive tissue (Stockley and Seal 2001; Walling et al. 2007; Stoltz et al. 2012). Additionally, social plasticity, or plasticity occurring in response to variation in the social environment, can result in differences in behavioral strategies such as male aggression, dispersal patterns, mate choice, and mating strategies (Carroll and Corneli 1995; Simpson et al. 2001; Kasumovic et al. 2012). Variation in the social environment has even been shown to trigger phase shifts, where several distinct phenotypes of an individual change in concert (Simpson et al. 2001).

Social plasticity begins with an individual's ability to assess its social environment. The specific component of social environment that an individual detects may vary depending on what is most relevant to its fitness. For example, in organisms with complex social structure, many group qualities such as number of helpers and social bonds can influence individual fitness (Silk 2007; Stanton and Mann 2012; Berger et al. 2015). However, other species that lack group structure may respond to parameters such as sex ratio, mate quality, or density (Carroll and Corneli 1995; Stockley and Seal 2001; Immler et al. 2010; Kasumovic et al. 2011; Kasumovic et al. 2012; Stoltz et al. 2012). Within a species, the same social information could also be interpreted differently by individuals of different groups (e.g., sexes, ages, reproductive morphs), and thus elicit varying plastic responses (Kotrschal et al. 2012). For example, the presence of male-calling may be interpreted by both sexes as a cue of general high conspecific density.

Alternatively, females could interpret this information as a cue of high mating potential, while males view it as a cue of high competition.

Species also vary considerably in the modality used to detect these components of social environment. Often, assessments of social environment can be made based on direct contact with conspecifics, either through tactile, olfactory, or visual interpretation of this interaction. Green swordtails (*Xiphophorus helleri*), for instance, assess and respond plastically to rival quality through visual inspection of male ornamentation (Walling et al. 2007). Alternatively, assessment of the social environment might rely on tactile cues, as demonstrated by the shift from 'solitarious' to 'gregarious' phases in the desert locust (*Schistocerca gregaria*) at high population density. After exposing various body parts of these locusts to mechanical stimulation, researchers found that this transition was more likely to occur following stimulation of hind femurs (Simpson et al. 2001). Some species, however, either do not have the sensory apparatus necessary to detect these cues, or do not interact frequently enough with conspecifics to achieve an accurate assessment of social environment. Instead, in organisms capable of producing song, individuals may use acoustic cues as a measure of conspecific density and quality (Bailey and Zuk 2008; Leary et al. 2008; Bailey et al. 2010; Balenger and Zuk 2015).

Regardless of what modality is used, the information obtained must then be analyzed and translated into phenotypic change. Many suspect this processing of social information to be endocrine-mediated. Hormones have long been known to orchestrate concerted changes in phenotype as an individual progresses through developmental stages (Seeman and Mcewen 1996; DeVries 2002). For example, shifts in androgen levels regulate the timing of sexual development in many vertebrates. Similarly, in insects, juvenile hormone (JH) and ecdysteroids (ESH) together time metamorphosis and molting, while in adulthood, they help regulate sexual maturation and behaviors involving mating, foraging, and flight (Robinson 1987; Zera 2006; Riddiford 2008; Ganter et al. 2011). In addition to internal influences – such as sex, genotype, or age – external factors, such as variation in the social environment, can also give rise to observable shifts in hormone levels. Work in both vertebrate and invertebrate systems, for example, has meticulously characterized patterns in hormone levels resulting from male-male fighting, parental care, bond-forming, isolation, and crowding (Dingle and Winchell 1997; Stout et al. 1998; Bloch et al. 2000; Oliveira et al. 2001; Oliveira et al. 2002; Hirschenhauser et al. 2003; Scott and Panaitof 2004; Ruscio et al. 2007). Their ability to influence phenotype, combined with their sensitivity to environmental cues, has led many researchers to suspect hormones as playing an intermediary role between environmental cues and downstream changes in morphology or behavior (Burmeister and Wilczynskia 2005; Leary et al. 2008; Choi et al. 2012).

C) Transgenerational plasticity

Maternal effects occur when a mother's phenotype or genotype influences the phenotype of her offspring through processes other than traditional genetic inheritance (Bernardo 1996; Mousseau and Fox 1998; Galloway 2005; Galloway and Etterson 2007). More recently, it has been recognized that fathers can contribute to this non-genetic influence through paternal effects (Adler and Bonduriansky 2013; Simmons and Lovegrove 2019), which has led to the use of the more general term, parental effect. Parental effects which result from alteration to parental environment have been referred to by researchers as parental environmental effects or, synonymously, transgenerational plasticity (TGP) (Bell and Hellmann 2019).

TGP can be further partitioned according to its adaptive value; if the TGP effect adjusts offspring phenotype to best match the environment anticipated by its parent(s), and thus increases both parental and offspring fitness, this effect is considered adaptive TGP (Bell and Hellmann 2019), or synonymously, an anticipatory parental effect (Burgess and Marshall 2014), or an adaptive environmentally induced parental effect (Lacey 1998). In contrast, selfish TGP effects occur when under certain environmental conditions, a parent reduces investment in some or all offspring. This reduces offspring fitness but makes additional resources available to support parental fecundity and/or survival. Thus selfish TGP has a net positive effect on parental fitness (Marshall and Uller 2007). TGP can also result from a 'condition transfer effect', where environmental-induced variation in parental condition results in correlated variation in offspring condition (Bonduriansky and Crean 2018). For example, several empirical studies have found that parental exposure to environmental stressors can be passively transmitted to offspring, often resulting in maladaptive offspring phenotypes (McCormick 2006; Sheriff et al. 2010; Beyer and Hambright 2017). Though condition-transfer TGP has negative effects for individuals of lower condition, if individuals of higher condition on average have more offspring, net selection could favor its maintenance (Bonduriansky and Crean 2018). These condition-transfer effects may occur incidentally due to parental resource limitation constraints (passive condition-transfer), or alternatively, may result from an evolved parental investment strategy (active condition-transfer) (Lacey 1998; Bonduriansky and Day 2009; Bonduriansky and Crean 2018; Bell and Hellmann 2019). More work is necessary to determine the relative prevalence of each of these types of TGP, but a recent meta-analysis emphasizes that there is currently a lack of evidence for the pervasiveness of adaptive TGP across taxa (Uller et al. 2013). Some argue that, because condition-transfer effects do not require a complex parental sensory system or tight correlation between parental and offspring environment, these effects should be more likely to evolve than adaptive TGP (Bonduriansky and Crean 2018). This thesis does not directly investigate the mechanism by which these forms of TGP influence offspring phenotype. However, it is important to consider their mechanistic bases to fully understand how they evolved and how they might shape the evolution of other traits (see Box 1).

Box 1: Proposed mechanisms of transgenerational plasticity*

Many pathways have been proposed to mediate the passage of nongenetic information across generations. One example is the inheritance of epigenetic markers such as DNA methylation or histone modifications. Because these modifications are sensitive to environmental cues, change patterns of gene expression, and can sometimes be transmitted across generations, they constitute a promising mechanism of TGP (Jablonka and Raz 2009). For example, the adaptive TGP response to parental drought conditions in annual plant *Polygonum persicaria* was reduced following treatment of seeds with a moderate demethylation agent, supporting the proposed epigenetic mechanism of this TGP (Herman and Sultan 2016). However, several studies have found the epigenome to be surprisingly stable in response to biotic or abiotic stressors, both within and across generations (Pecinka et al. 2009; Seymour et al. 2014; Eichten and Springer 2015). Accordingly, in their detailed analysis of the DNA methylone of *Arabidopsis* following drought stress, researchers found only four transgenerational drought stress-induced epialleles, three of which mapped to repetitive, heavily-methylated regions and none of which corresponded with methylation changes in the parental generation (Ganguly et al. 2017). Another potential mechanism of TGP involves differential provisioning of nutrients such as lipids, carbohydrates, minerals, or protein into the egg or seed (Herman and Sultan 2011). When provisioning is increased in response to particular environmental cues, offspring may receive a head-start in their development that will benefit them throughout their lifetime, an idea referred to as the 'silver spoon hypothesis' (Herman and Sultan 2011; Zas et al. 2013). Finally, mothers can also alter the concentrations of hormones or certain RNA transcripts in their eggs or seeds in order to influence offspring gene expression during development (Safran et al. 2010; Herman and Sultan 2011). Because many environmental conditions are known to cause fluctuations in hormone levels, this mechanism in particular has been the focus of much study (Groothuis et al. 2005). More work is needed to conclusively identify the contributions of each of these mechanisms to the transmission of nongenetic information from parents to offspring, especially in nonvertebrate systems where experimental manipulation is less restricted.

* Species with prolonged parental care may also transmit non-genetic information to their offspring through vertical social transmission (i.e., learning) (Krützen et al. 2005). If this vertical social transmission differs in response to parental environment, this could technically qualify as a mechanism of TGP. However, this mechanism is usually not included in literature on TGP, as TGP is most often considered in cases where species exhibit minimal post-natal parental care.

It is crucial that researchers continue to investigate mechanisms and consequences of TGP. Parental effects have been shown to contribute significantly to phenotypic variation measured in natural populations (Kruuk et al. 2000; Kruuk 2004). Therefore, in a purely practical sense, when pursuing

questions about phenotypic variation, it is important to consider the potential influence of TGP to avoid drawing incorrect conclusions. For example, leaving maternal identity out of models can strongly influence interpretations of trait heritability and of fitness as it relates to cooperative breeding (Kruuk 2004; Russell and Lummaa 2009). It is also important to take parental effects into account when designing common garden experiments, where individuals are raised in identical conditions to assess the contribution of genetic differentiation to phenotypic variation between populations (Roach and Wulff 1987). Criticisms have thus been made of work evaluating the relative contributions of genetic adaption and within-generation plasticity (WGP) to phenotypic change that fails to account for TGP (Urban et al. 2014). Further, several studies have emphasized that maternal effects have significant evolutionary consequences (*Reviewed in* Moore et al. 2019), for example, by altering demographic patterns (LaMontagne and McCauley 2001; Sheriff et al. 2010), increasing the responsiveness of offspring phenotype to selection (Kirkpatrick and Lande 1989), and facilitating evolutionary divergence (Pfennig and Martin 2009).

Because adaptive TGP should allow individuals to respond rapidly to new environments, it has also been implicated as a potential facilitator of biological invasion or range expansion (Herman and Sultan 2011). Initial populations resulting from biological invasions are often characterized by low genetic variation, as they are frequently derived from only a small number of founding individuals. This paucity of genetic variation might, at least initially, limit the ability of species to adapt to a new environment if not for phenotypic plasticity (*Reviewed in* Urban et al. 2014). Specifically, adaptive TGP may allow colonizing parents to produce pre-adapted offspring better able to persist in heterogenous, disturbed, and/or stressful conditions of a new range (Fenesi et al. 2014; Caño et al. 2016). For example, in the invasive annual grass, *Aegilops triuncialis*, poor maternal conditions led to both increased incidence of dispersal of propagules into higher quality patches and increased stress tolerance of seedlings through increased photosynthetic efficiency and phenological shifts (Dyer et al. 2010). Given the growing incidence of both nonnative introductions and climate change-induced range shifts, it will be vital to learn more about how pre-existing adaptive TGP shapes a species' ability to persist in new environments. This work might include comparisons of adaptive TGP across closely-related species that differ in their invasiveness, which has been attempted just twice (Sultan et al. 2009; Fenesi et al. 2014). These studies found that species with different degrees of invasion success exhibited different patterns of adaptive TGP in response to environmental factors such as drought stress and nitrogen availability (Sultan et al. 2009; Fenesi et al. 2014). This expanding field could also benefit from evaluation of the importance of adaptive TGP across the timescale of an invasion, to explore if the degree of adaptive TGP decreases once a species has settled in a new range. Further work on the relationship between adaptive TGP and invasion

will be especially important in the animal kingdom, which has been relatively under-studied in this context compared to plants.

Another important question in the study of TGP which warrants further exploration is its relationship with WGP, which may vary depending on the type of TGP being considered. First, when considering adaptive (i.e., anticipatory) TGP, it is important to evaluate the relative value of parental versus offspring cues in predicting offspring environment. In theoretical models that attempt to assess the relative adaptive potential conferred by these two processes, it is often concluded that WGP is more likely to evolve than adaptive TGP (Ezard et al. 2014; English et al. 2015). The evolution of adaptive environmental modification on phenotype requires high predictive accuracy of the environmental cue; it does an individual little good to adjust its phenotype using information about its environment that is no longer accurate (Leimar and McNamara 2015). Some models therefore point to WGP as a more efficient form of plasticity because the cues detected by progeny, rather than by parents, occur closer in time to selection on the resulting offspring phenotype, theoretically resulting in higher predictive accuracy (Ezard et al. 2014). Additionally, it is predicted that once capable of assessing their environment, offspring should rewrite parental cues with their own to maximize predictive accuracy (Auge et al. 2017). Less is known about interactions between TGP and WGP in forms of TGP which have not evolved to increase offspring fitness (e.g., selfish TGP and passive condition-transfer TGP). There are relatively few studies that assess parental effects in adult offspring (compared to those that assess parental effects in juvenile offspring) or that manipulate both parental and offspring environment to provide a direct comparison of the contributions of WGP and TGP to offspring's phenotype. Some work in plants, however, points to the possibility that plasticity is more complicated than previously anticipated (Auge et al. 2017). For example, in contrast to predictions about maternal effects declining with time, stressful or poor-quality maternal conditions have been shown to influence offspring traits such as growth rate, branch development, and leaf length for up to two years in several plant species (Latzel and Klimešová 2010; Walter et al. 2016). Further, although there are examples where progeny cues appear to override maternal cues, there are also cases of maternal cues masking progeny plasticity and of the two working additively in the same direction (Leimar and McNamara 2015; Bernareggi et al. 2016; Groot et al. 2016; *Reviewed in* Auge et al. 2017). Corresponding studies in animal systems are scarce, likely in part due to time constraints associated with measuring phenotypic responses across several generations (*but see* Lindholm et al. 2006; Shama et al. 2014). More work is therefore needed to draw any concrete conclusions about the relationship between these two modes of plasticity, and whether this relationship differs depending on what form of TGP is in place.

Finally, continued exploration of TGP is necessary to determine if effects on offspring phenotype are often limited to shifts in morphology (e.g., size, growth rate, allocation to particular tissue types), or

whether these effects can also extend to changes in offspring behavior. Some evidence for transgenerational effects on offspring behavior can be found in medical or psychology-based studies. This work explores the effects of artificial, human-related stressors, such as obesity or drug-use, in the parental generation, either prior to or during offspring development (Sullivan et al. 2014). For example, rats whose mothers were given morphine throughout adolescence showed an increase in anxiety-like behavior compared to offspring whose mothers were treated with saline (Byrnes et al. 2011). Less often have researchers explored transgenerational consequences on offspring behavior with an ecological perspective. Those that do have often focused on the effects of parental predator exposure on offspring antipredator behavior. For example, three-spined stickleback (*Gasterosteus aculeatus*) offspring of predator-exposed mothers exhibited tighter antipredator shoaling behavior compared to offspring of non-exposed mothers, though this result was not supported in similar experiments on live-bearing guppy (*Poecilia reticulata)* (Giesing et al. 2011; Cattelan et al. 2020). Similarly, female fall field crickets (*Gryllus pennsylvanicus*) exposed to a predatory spider produced offspring that showed an increase in antipredator immobility behavior compared to offspring of nonexposed mothers (Storm and Lima 2010). The later study also found that offspring from exposed mothers survived longer in the presence of the predatory spider than offspring of unexposed females, supporting the hypothesized adaptive nature of this transgenerational behavioral effect (Storm and Lima 2010). Though such studies of antipredator behavior are important, researchers must expand their investigations of TGP to include other behaviors relevant to offspring fitness, especially at the juvenile stage when mortality rates are high and a non-genetic parental influence on fitness would therefore have a larger effect. For example, researchers could examine effects on exploration and locomotive activity, which are predicted to influence ecologically important processes such as dispersal and foraging ability in nature (Bell 1990; Fraser et al. 2001). Despite significant interest in understanding the genetic factors contributing to variation in these behaviors, few studies have considered whether nongenetic factors influence exploration and locomotive activity (Bell 1990; Sarkar et al. 2006; Korsten et al. 2013; Tarès et al. 2013; Page et al. 2018). This is especially surprising in that exploration and activity levels are often associated with body condition, which itself has shown to be sensitive to parental environment in several species (McCormick 2006; Crocker and Hunter 2018a; Goosens et al. 2020).

These questions can all be explored through the study of transgenerational social plasticity. It is common for organisms to experience social heterogeneity in time and space throughout their lifetime. Therefore, individuals may benefit from adjusting their phenotype to relevant parameters of the social environment using WGP. Because this response often involves alteration of reproductive tissues and strategies, it is conceivable that an individual's social environment could also influence the phenotype of its progeny through TGP.

D) *Teleogryllus oceanicus* as a model of social plasticity within and across generations

To explore how organisms may respond to social heterogeneity through plasticity, I investigated the effects of maternal social (i.e., acoustic) environment on offspring phenotype in field cricket (*Teleogryllus oceanicus*) populations derived from the Hawaiian Islands. *T. oceanicus* is not eusocial and does not have a defined group structure. As a result, this species' social environment is defined by measures such as conspecific density, sex ratio, and quality. Because they are active at night and widely spaced (~meters) in the field, these crickets must rely on conspicuous, non-directional, and long-range acoustic signals (i.e., male song) to detect information about their social environment (Hack 1998). While they have contact pheromones and use tactile cues during physical interactions, for most crickets, song is the only known communication channel that can provide information about the abundance of sexually receptive males (to females) or rivals (to males) in the surrounding environment (Tregenza and Wedell 1997). To alter their social environment, I therefore manipulated their perceived conspecific density by exposing them to the absence or presence of conspecific song. Previous studies on this species and other closely-related crickets have manipulated social environment by altering both perceived conspecific density (presence or absence of conspecific song) and actual conspecific density (number of individuals in a container) (Bailey et al. 2011; Kasumovic et al. 2011; Bailey and Zuk 2012; Kasumovic et al. 2012; Balenger et al. 2018). These studies have shown that manipulation of both perceived and actual conspecific density affects an individual's phenotype, indicating that conspecific density is a relevant parameter of social environment in this system.

T. oceanicus breeds continuously, and the life cycle begins when a female deposits eggs in leaf litter or soil using her ovipositor, a long thin structure connected to her ovaries. These eggs hatch after approximately two to three weeks. *T. oceanicus'* juvenile development is hemimetabolous, characterized by a series of molts during which the old cuticular exoskeleton is replaced with a slightly bigger one. This allows the individual to attain a progressively larger body size. In this species and other hemimetabolous insects, the stage of an individual is often described based on the number of instars (phases between two molts) it has completed. For example, a juvenile which has undergone three molts would be classified as being in their fourth instar. The precise number of instars in *T. oceanicus* juvenile development is unknown, but based on closely related species, likely ranges between eight and nine achieved over a period of about 60 days (Staudacher 2009). After each molt, juveniles more closely resemble their adult counterparts, with wing buds (which later become full wings) and reproductive structures (ovipositors in females) appearing several instars before adulthood. Adulthood is achieved after the final molt (often referred to as eclosion) occurs. Because adults can no longer undergo molting, the size of their exoskeleton (referred to as their structural size) is fixed after their final molt, setting an upper limit on the mass of tissue they can accumulate. This structural size is often quantified by measuring the length or

width of the pronotum, which is the first body segment after the head. Structural size has been positively correlated with mating success in cricket species, indicating that this is important measures of an individual's fitness (Heinen-Kay, Urquhart, et al. 2019). Reproductively-mature males attract females by producing a calling song with specialized structures on their wings. If a female locates him after tracking his song (phonotaxis), the male produces a courtship song to elicit mounting by the female. The mounted male then transfers a spermatophore (a capsule containing spermatozoa) to the female, which may then fertilize her eggs.

Several features of this field cricket system suggest that adaptive transgenerational plasticity (TGP) could be a prominent, and adaptive, feature of this species natural history. First, like many insect species, *T. oceanicus* suffers high juvenile mortality. Therefore, any fitness-associated alterations to early juvenile phenotype as a result of TGP would be subject to intense selective pressure, which could lead to the rapid evolution of adaptive TGP. Second, based on model predictions about the integration of different cues to phenotypic determination, adaptive TGP is more likely to evolve when parental cues accurately predict offspring environment (Leimar and McNamara 2015). Though no work has formally investigated the temporal stability of social environment in this species, the fragmented distribution of *T. oceanicus* habitat on the Hawaiian Islands and the inability of juveniles to fly suggest that social environment is unlikely to vary substantially between parent and young offspring. Third, the same models predict that adaptive TGP is more likely to evolve when juveniles' environmental cues are inaccurate (Leimar and McNamara 2015). In *T. oceanicus*, individuals rely heavily on acoustic cues to interpret their social environment, as they are unlikely to interact frequently enough with conspecifics to gauge social parameters through tactile or olfactory modalities alone. A cricket's hearing is facilitated by a system of specialized structures on its prothoracic legs. To summarize briefly, sound, in the form of airborne vibrations, enters the tibiae and is amplified through hollow tubes called the acoustic trachea to the tympanal membranes (Nocke 1974). When the tympanal membrane vibrates, it activates the mechanosensitive auditory receptor neurons that make up the scolopidium organelles of the inner ear (Yack 2004). These scholopidium neurons then send impulses to auditory interneurons located in the prothoracic ganglion (Yack 2004). The combination of excitatory and inhibitory signals from these interneurons then get transmitted to the brain, where an ultimate "decision" is made on how to respond to the acoustic cue (Yack 2004). However, and perhaps crucially, this complex sensory system is not functional for much of the cricket's early life. In the closely-related *Teleogryllus commodus*, the scolopidium only begin to appear at the third instar, do not span the full distal-proximal axis of the tympanal organ until the seventh instar, and continue to increase in number until the adult stage (Young and Ball 1974). Additionally, though the two branches of the acoustic trachea are present as early as the second instar, they remain unconnected, and therefore nonfunctional, until instar seven (Young and Ball

1974). Finally, the tympanal membrane does not take on its translucent adult form until after the final molt (Young and Ball 1974). Work in the field cricket *Gryllus bimaculatus* DeGeer also shows that, though morphology of this auditory system in last-instar juveniles generally resembles that of an adult, their auditory threshold is 30-45 dB higher than in adults (Staudacher 2009). Taken together, this all suggests that juveniles of *T. oceanicus,* especially in early instars, are not likely capable of accurately assessing their own social environment, therefore supporting predictions of adaptive TGP.

In addition to adaptive TGP, it is possible that this species exhibits selfish TGP or condition-transfer TGP. Prior work has demonstrated that *T. oceanicus* displays considerable WGP in response to acoustic cues of social environment. When raised in silence, females exhibit relaxed preferences for song variants and increase phonotactic behavior compared to females raised in an environment with song (Bailey and Zuk 2008). Similarly, males of this species are more likely to participate in satellite behavior when raised in silence, with more time spent in proximity to a call-producing speaker and an overall increase in locomotive behavior (Bailey et al. 2010; Balenger and Zuk 2015). Further, females raised in song versus silence exhibit differential neural gene expression, which is suggestive of social plasticity at the neural level (Pascoal et al. 2018). The effects of social plasticity in this species also extend to reproductive investment. When raised in song, individuals of both sexes were found to have increased reproductive tissue mass compared to their counterparts raised in the presence of silence, though no evidence was found to indicate differences in number of eggs laid or hatched across maternal acoustic treatment (Bailey et al. 2010; Lierheimer and Tinghitella 2017; Heinen-Kay, Strub, et al. 2019). If individuals are capable of such extensive social plasticity, particularly alterations to reproductive tissues and behaviors, it is reasonable to suspect that such effects could contribute to phenotypic variation in the next generation. This contribution could result from a socially-induced parental shift in reproductive strategy which improves the parent's future fecundity or survival at the expense of current offspring's fitness (selfish TGP). Additionally, socially-induced changes in parental state could be incidentally inherited by offspring (passive condition-transfer).

Finally, this system is especially appropriate for exploring the effects of social plasticity because some populations have recently experienced rapid spread under selection of mutation(s) which silences male calling, thus dramatically changing the social environment. In Hawaii, the calling males of this species were subject to intense selective pressure from predation by an acoustically orienting parasitoid fly, *Ormia ochracea* (Zuk et al. 2006). As a result of this selective pressure, in fewer of than 20 generations, nearly 90% of males on the island of Kauai possessed an X-linked mutation that disrupted normal wing development, thus silencing calls (Pascoal et al. 2014). Following the loss of such an important sexual signal, researchers have investigated whether 'flatwing' males have undergone further behavioral or morphological adaptation to overcome the resulting challenges in locating mates, or

whether this mutation could be associated with effects on non-wing-related tissues. These studies have identified that, compared with normal-wing males, flatwing males demonstrate feminized cuticular hydrocarbon profiles, increased propensity to participate in satellite behavior, reduced testes mass, and greater socially-induced plasticity in neural gene expression (Bailey et al. 2010; Pascoal et al. 2018; Pascoal et al. 2020). Most of these studies have focused on identifying morph differences at the adult stage when the resulting change to wing structure is recognizable and should have the greatest fitness effect. However, gene expression analyses of developing wingbuds in late-stage juveniles and of whole embryos have indicated that flatwing and normal-wing genotypes show differences far before adulthood (Pascoal et al. 2020). Whether morphs also exhibit important behavioral or morphological differences at juvenile stages remains largely unexplored. Additionally, because this mutation changes the social environment of Hawaiian populations and is associated with increased social plasticity, whether it is also associated with transgenerational social plasticity is unknown.

Chapter II

EFFECTS OF SEX AND WING MORPH ON SIZE AND BEHAVIOR AT MULTIPLE JUVENILE STAGES

A) Background

To begin my investigation of TGP in *T. oceanicus*, I first sought to explore what variation exists in relevant juvenile morphology and behavior across wing morphs (flatwing and normal-wing), prior to maternal social manipulation. This allowed me to determine whether the rise of the flatwing morph might also have been accompanied by genetic changes in ecologically-relevant characteristics at the juvenile stage (e.g., growth rate and locomotive activity). An individual's rate of development likely has important consequences for its fitness, as juveniles who develop faster may get better access to resources as adults, or even cannibalize smaller, slower-growing individuals (Takasuka et al. 2004). On the other hand, juveniles growing at a slower rate may be less vulnerable to starvation and predation pressures (Gotthard et al. 1994; Gotthard 2000). I also measured an important behavioral characteristic, locomotive activity, using an open-field test (OFT). Lab-based measures of activity and exploration are often accurate indicators of ecologically-relevant processes such as dispersal and foraging in nature (Fraser et al. 2001; Dingemanse et al. 2003; Korsten et al. 2013). Further, locomotive activity could have especially important fitness consequences in this system, given the challenges of locating conspecifics in a silent, flatwing-dominated population.

I first compared these juvenile phenotypes across sexes. Adults of *T. oceanicus*, like many insect species, exhibit sexual dimorphism in size and growth rate (Kolluru et al. 2004; Stillwell et al. 2010). Specifically, females often reach adulthood earlier and weigh more as adults, despite being smaller structurally (Kolluru et al. 2004). Similarly, because of underlying differences in mating strategies, where females actively search for mates through phonotaxis while males rely more on stationary calling efforts, adults of different sexes likely differ in underlying activity and exploration behaviors. Sex, however, is not readily identifiable until late-juvenile penultimate instar. As a result, it is currently unknown in this species at what point individuals of different sexes begin to diverge in morphology and behavior. Studies on the ontogeny of sexual size dimorphism in other invertebrate species are also sparse, but some have found evidence for sex-based differences in growth rate, size at hatching, or number of instars prior to sexual maturation (Esperk et al. 2007; Abbott and Svensson 2008; Stillwell et al. 2010; Tammaru et al. 2010). Other studies, however, fail to find evidence for differences in either growth rate or behavioral traits such as boldness or exploration across juveniles of different sexes (Hedrick and Kortet 2012; Niemelä et al. 2012).

Through this experiment, I also compared juvenile phenotype across wing morphs. Though wing morphs exhibit differential gene expression at the embryonic stage, it is unknown if they also differ in developmental growth rate prior to adulthood (Pascoal et al. 2020). Similarly little is known about when individuals carrying the flatwing genotype and wildtype individuals begin to differ in behavior. Most studies comparing flatwing and normal-wing behavior have done so with phonotaxis trials, which is only

possible in hearing-capable adults (Zuk et al. 2006). One study did compare adult baseline locomotive activity without any acoustic stimulation and found no evidence for a difference between males of different wing morphs (Balenger and Zuk 2015). However, because this study's focus was to compare locomotive activity in response to acoustic rearing environment across several Hawaiian island populations with and without flatwings, the sample size comparing male wing morphs was limited (Balenger and Zuk 2015). Further, because wing morph cannot be readily identified in females, a large-scale comparison of behavior in wild-type females versus those carrying the flatwing allele has not been attempted. In contrast, I was able to make these comparisons in juveniles through the use of laboratory stock lines pure-breeding for either the flatwing or normal-wing genotype.

B) Methods

Cricket Populations and Rearing

Flatwing and normal-wing males cannot be differentiated until wingbuds begin to develop in the penultimate instar, while females that carry genotypes for the different wing morphs are not visually distinguishable at any stage (N. Bailey, personal observations). Therefore, to compare early juvenile morphology and behavior across these two wing morphs, I used six laboratory stock lines – three pure-breeding for the flatwing morph and three pure-breeding for the normal-wing morph – with each line serving as a biological replicate. These lines were established in 2016 from a series of controlled crosses of Kauai-derived individuals to ensure homozygosity at the locus or loci controlling the flatwing genotype (Pascoal et al. 2016). Stock populations were kept in 16-liter plastic containers and provided food (Burgess Supa Rabbit Exel Junior), moistened cotton, and cardboard egg cartons for shelter ad libitum. All populations were kept in identical conditions in a shared temperature and light-controlled growth chamber set to 25°C and a photo-reversed 12:12 hour light:dark cycle. All populations were maintained twice a week to replace food and water.

To obtain juveniles, I collected egg pads twice weekly for four weeks from each of the six pure-breeding lines. After approximately a fortnight, I monitored egg pads daily between 16:00 and 18:00 and removed all new hatchlings to individual 100 mL plastic pots with cardboard for shelter and *ad libitum* blended rabbit chow and water. I cleaned the containers and replaced food and water twice per week. Isolated individuals were kept in the same growth chamber and rearing conditions as described above.

Open-Field Test and Body Size Measurements

At 15 days and 45 days post-hatching, each isolated individual was assayed for locomotive behavior then measured for pronotum length. Locomotive behavior was quantified through an open field test (OFT). All OFTs were performed during the dark portion of the crickets' 12:12 light:dark cycle, when they're expected to be most active (8:00 to 20:00).

Temperature was kept between 23-25 °C and, as crickets cannot perceive red wavelengths of light, lighting consisted of two red incandescent bulbs on either side of the testing arena. The day of testing, all individuals were placed in small glass vials within their deli pot to reduce disturbance from handling immediately before testing. At the start of each trial, the glass vial containing the cricket was gently flipped onto the center of the 11x17cm clear plastic arena sitting on white poster paper. The vial remained atop of the cricket for two minutes to allow it to acclimatize. After this time, I began recording using a video camera mounted above the arena and then lifted the vial to allow the cricket to move freely. Recording lasted five minutes. The arena was wiped down with 70% ethanol before each trial to minimize residual chemical cues. To save time, two crickets were assayed at once in side-by-side arenas. It is unlikely that crickets tested at the same time were aware of one and other due to their inability to see in red wavelengths of light.

After the open field test, crickets were put on ice for two minutes to reduce movement then imaged one at a time overtop a micrometer using a Leica DFC295 digital camera affixed to a Leica M60 dissecting microscope. Crickets were also imaged at adulthood to measure final pronotum length. Version 1.8.0_112 of ImageJ was used to extract and record pronotum length from these images.

Coordinate Collection from OFT Videos via DORIS

Videos were trimmed to five minutes then run through a free movement analysis software DORIS (version 0.0.17) to extract coordinates for each frame, with videos containing 30 frames per second (Friard 2019). Initial trials revealed a software bug in which some individuals who, based on inspection of their videos, did not move were nonetheless assigned significant movement distances. Further inspection of coordinate files revealed that, when individuals stood still, the software often recorded a series of very small movements across the length of the cricket rather than one static point. When these coordinates were used to calculate distance, those many small movements added up to a substantially inflated distance traveled. To solve this, I averaged x and y coordinates over 24 frames to create a smoothed path, then removed coordinate pairs which were less than 1 mm from the position in the previous frame. To externally validate this fix, I randomly selected 100-second increments from 8 videos, extracted one JPEG for every 10 frames of the video, then manually measured the path across these images in version 1.8.0_112 of ImageJ. These manually collected coordinates produced distance values that were very highly correlated with distance values based on smoothed-DORIS coordinates (Pearson correlation: $r^2 = 0.999$, $n = 6$, $p < 0.001$), providing confidence in the validity of the smoothing methods (Figure 1).

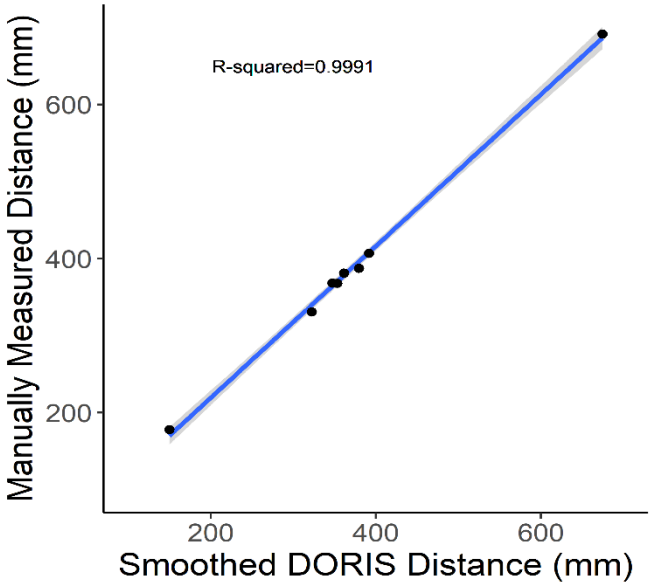

Figure 1 *Relationship between smoothed DORIS-calculated distance values and manually measured distance values in eight videos.*

Calculation of OFT parameters from Coordinates

Several movement parameters were recorded from the open field tests. First, I estimated general activity by measuring total distance traveled. I also calculated proportion of the arena entered as a measure of exploratory activity. I did this by overlaying coordinate data onto a 11x17cm raster grid containing 187 distinct 1x1cm squares, then extracting counts of how many of those squares had been entered and dividing by the total number of non-origin squares (186). Finally, I split the raster into three "zones": origin (the square where the individual started the test), edge (1 cm border around the perimeter of the arena), and middle (all other space in the arena) and calculated how much time was spent in each to investigate differences in space usage.

Statistical Analyses

All statistical tests were carried out using R version 4.0.2 (R Core Team 2020). I first compared movement behaviors across wing morphs (flatwing and normal-wing) and sex in 15-day old offspring by using separate linear models with the following response variables: distance traveled (mm), proportion of grid explored, time spent in edge region (s), time spent in middle region (s), and time spent in origin region (s). Any individuals who jumped during their assay (n = 2 in 45-day assay) or whose video was inadvertently deleted before movement analysis (n = 2 in 45-day assay) were excluded from movement analyses but were included in analyses of structural size. To satisfy assumptions of normality, data from the 15-day old assay for distance traveled and proportion of grid explored were square-root transformed, and data for time spent in middle region and time spent in origin region were log-transformed. For the 45-

day old assay, I log-transformed distance traveled and time spent in middle region, and square-root-transformed proportion of grid explored, time spent in edge region, and time spent in origin region.

For each movement response, I modelled *morph* and *sex* as categorical variables, with *line* nested within *morph* to account for variation among lines within morphs. Pronotum length, temperature, and time of day were included as covariates. 39 individuals died before their sex could be identified, so to verify that sex did not qualitatively affect the findings in either the 15-day old assay or the 45-day old assay, models including sex as a covariate were run on the subset of individuals for which sex could be identified. With the exception of the analysis of "origin time", sex was not significant in these models (all $p > 0.2$) and the qualitative outcome did not differ. Thus, models retaining all individuals, and excluding the sex covariate, were retained except in the case of origin time (Table 1). Distance and proportion of grid explored were strongly correlated (Pearson correlation: $r^2 = 0.709$, n = 252 $p < 0.001$), so models examining proportion of grid explored were run both with and without *distance* as a covariate. This was necessary to check whether proportion of grid explored was simply driven by variation in *distance*. All movement models were run first with all individuals, then with only individuals who moved during the assay. This was done to confirm that differences in distance traveled were not simply due to differences in likelihood of initiating movement.

I then compared pronotum length across morphs and sexes. In both 15- day old and 45-day old offspring, data was heavily bimodal and thus not amenable to basic transformation (square-root and log). Therefore, I used non-parametric Kruskal-Wallis and Wilcoxon-Mann-Whitney tests to evaluate the influence of *sex*, *morph*, and *line* (*line* was evaluated separately within each of the two *morph* groups). To compare final, adult pronotum length, I ran a linear model which included the interaction between *sex* and *morph,* with *line* nested within *morph*.

C) Results

Flatwing-carrying juvenile crickets at 15-days of age moved further than normal-wing juveniles, and these differences persisted in 45-day old juveniles (Table 1 and Figures 2A, 2C, 3A, 3C). In addition to moving further, flatwing (FW) individuals at both ages, entered a greater proportion of the arena, and spent more time along the edge of the arena than normal-wing (NW) individuals (Table 1 and Figure 2). When models were adjusted to include only individuals who moved, these morph differences persisted. Sex had no significant effect on distance traveled, proportion of grid explored, edge time, or middle time (ps > 0.2) in both 15-day and 45-day individuals, so it was excluded from the final versions of these models. However, it did significantly predict origin time at 15 days, with males spending more time in the origin than females (Table 1). In all models of proportion of grids explored, when distance was included as a covariate, there were no longer a significant effect of morph. This indicates that though FW-carrying individuals explore more grid squares, this difference is not independent of morph differences in distance

traveled. Statistical significance of these results should be interpreted cautiously due to the quantity of behavioral traits analyzed. On balance, however, a univariate approach (rather than analyses performed on principal component scores, for example) was favorable, as each trait has its own independent biological implications that are relevant to the emergence and rapid spread of silent flatwing males in nature.

Though NW juveniles had larger pronotum lengths than FW-carrying juveniles at 15 days (W = 6299.5, p = 0.003), this difference was relatively minor (Figure 2B). Additionally, at 45 days morphs did not differ significantly in pronotum length at 45 days (W = 5785, $p = 0.373$) (Figure 3B). At 15 days, according to Kruskal-Wallis tests, there was a significant effect of line within the FW morph (Kruskal-Wallis chi-squared = 6.987, df = 2, $p = 0.030$), and within the NW morph (Kruskal-Wallis chi-squared = 7.721, df = 2, $p = 0.021$). At 45 days, there was no significant effect of line within the FW morph (Kruskal-Wallis chi-squared = 2.833, df = 2, $p = 0.243$) or line within the NW morph (Kruskal-Wallis chi-squared = 0.175, df = 2, $p = 0.916$). Sex had no effect on pronotum length at 15 days (W = 5918, $p = 0.845$) or 45 days (W = 5493.5, $p = 0.295$), but in adults, males had larger pronotum lengths than females (Table 3).

Table 1 Linear models examining cricket behaviors in an open field test at 15 days of age

	Distance			Proportion Explored			Edge Time			Middle Time			Origin Time		
	df	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***
Morph	1, 245	12.9876	**0.0004**	1, 245	11.1104	**0.0010**	1, 245	18.2129	**<0.0001**	1, 245	0.9015	0.3433	1, 206	17.7308	**<0.0001**
Morph:Line	4, 245	2.8057	**0.0264**	4, 245	3.7843	**0.0053**	4, 245	1.1624	0.3291	4, 245	2.6797	**0.0323**	4, 206	2.3848	0.0525
Pronotum Length	1, 245	0.3595	0.5494	1, 245	0.1341	0.7145	1, 245	1.2680	0.2612	1, 245	0.6634	0.4162	1, 206	3.1301	0.0783
Assay Temperature	1, 245	0.6797	0.4105	1, 245	2.2669	0.1334	1, 245	0.5542	0.4573	1, 245	2.1608	0.1429	1, 206	0.1655	0.6846
Assay Time	1, 245	0.3106	0.5778	1, 245	1.6755	0.1967	1, 245	0.0202	0.8871	1, 245	0.4230	0.5160	1, 206	0.1810	0.6710
Sex	-	-	-	-	-	-	-	-	-	-	-	-	1, 206	4.1074	**0.0440**

Table 2 Linear models examining cricket behaviors in an open field test at 45 days of age

	Distance			Proportion Explored			Edge Time			Middle Time			Origin Time		
	df	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***
Morph	1, 210	16.5540	**<0.0001**	1, 210	14.3359	**0.0002**	1, 210	12.1958	**0.0006**	1, 210	0.4983	0.4810	1, 210	1.4262	0.2337
Morph:Line	4, 210	12.6962	**<0.0001**	4, 210	8.9201	**<0.0001**	4, 210	12.5767	**<0.0001**	4, 210	4.4372	**0.0018**	4, 210	10.5333	**< 0.0001**
Pronotum Length	1, 210	12.1224	**0.0006**	1, 210	10.8295	**0.0012**	1, 210	17.7070	**<0.0001**	1, 210	1.2848	0.2583	1, 210	10.2850	**0.0016**
Assay Temperature	1, 210	0.5612	0.4546	1, 210	0.2638	0.6081	1, 210	1.8087	0.1801	1, 210	0.1076	0.7433	1, 210	2.2224	0.1375
Assay Time	1, 210	33.0552	**<0.0001**	1, 210	44.4047	**<0.0001**	1, 210	12.4543	**0.0005**	1, 210	5.6665	**0.0182**	1, 210	4.4922	**0.0352**

Table 3 Linear models examining the effects of wing morph and sex on pronotum length at adulthood

Effect	***df***	***F***	***p***
Morph	1, 209	0.0427	0.8365
Morph:Line	4, 209	17.4480	**<0.0001**
Sex	1, 209	18.8300	**<0.0001**

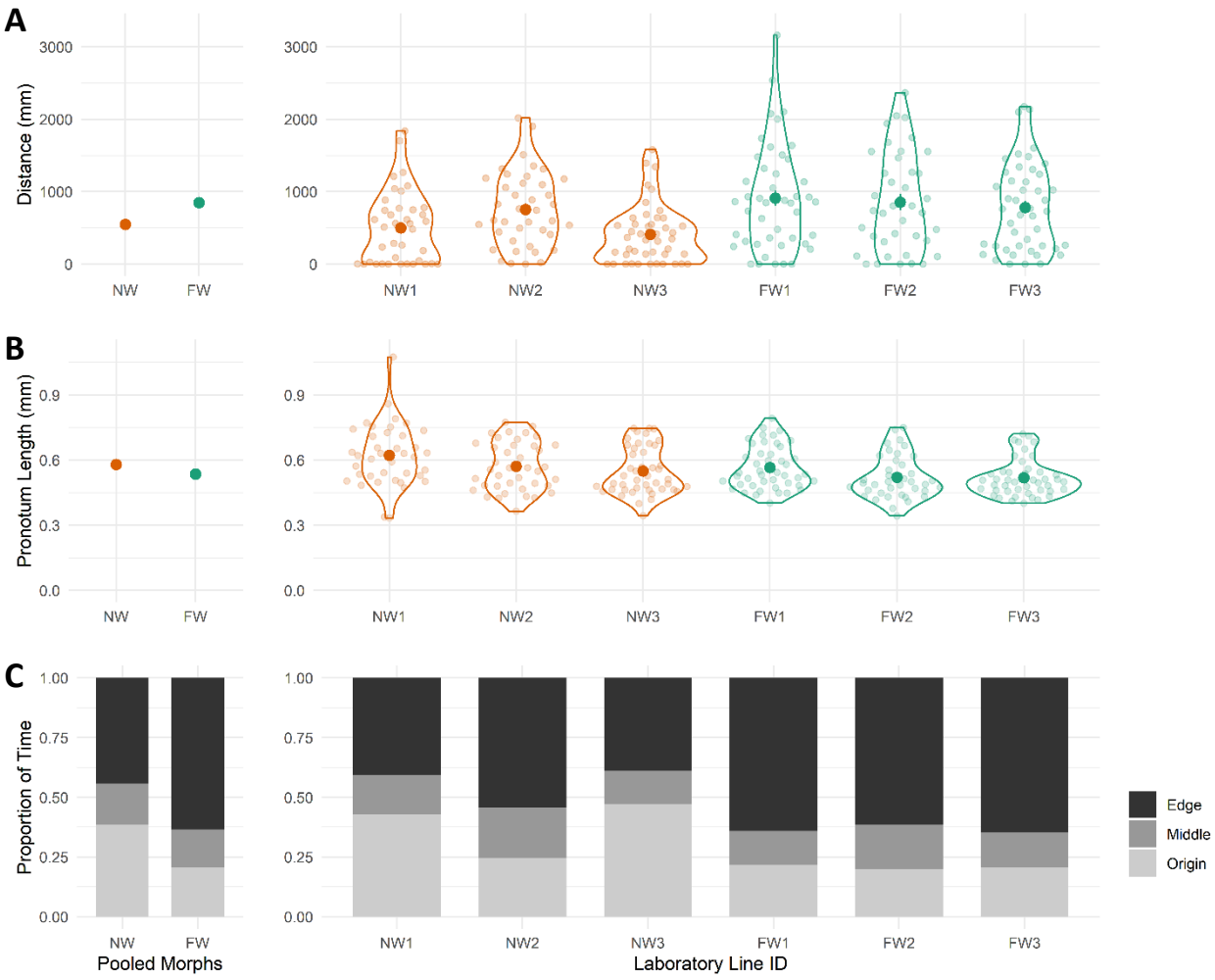

Figure 2 *In 15-day-old nymphs, the effect of morph (left) and line (right) on distance traveled during OFT (A), pronotum length (B), and proportion of time spent in different regions of the arena during OFT (C). Morph plots of distance traveled and pronotum length illustrate pooled morphs means. Line-specific plots of distance traveled and pronotum length give the mean (solid point) surrounded by all data points (slightly transparent points). Bars indicating +/- one standard error are not shown as these were too small to indicate graphically without being obstructed by the symbols for means.*

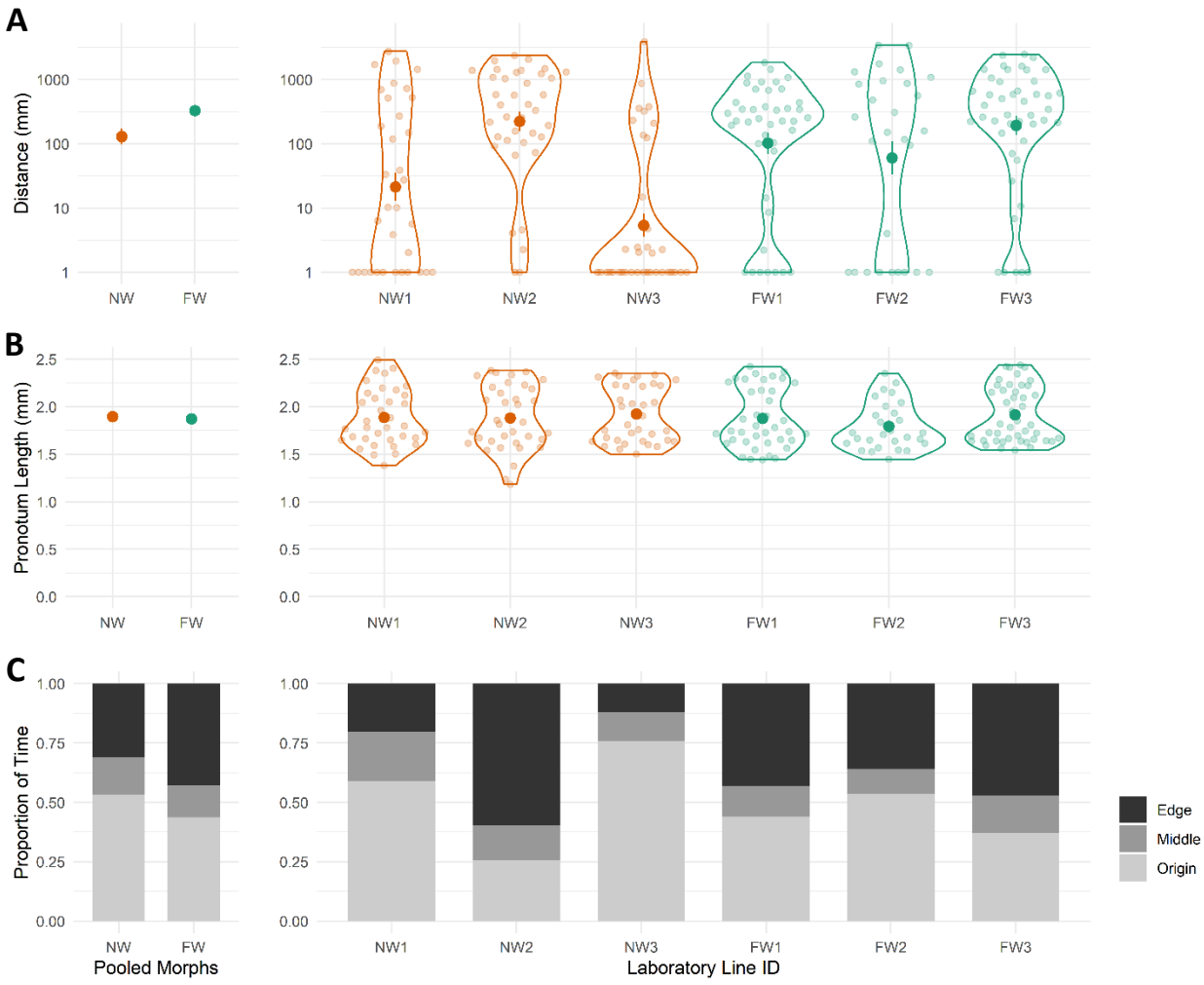

Figure 3 *In 45-day-old nymphs, the effect of morph (left) and line (right) on distance traveled during OFT (A), pronotum length (B), and proportion of time spent in different regions of the arena during OFT (C). Morph plots of distance traveled and pronotum length illustrate the mean with standard error bars. Line-specific plots of distance traveled and pronotum length give the mean (solid point) surrounded by all data points (slightly transparent points). Bars indicating +/- one standard error are not shown as these were too small to indicate graphically without being obstructed by the symbols for means.*

D) Discussion

Primarily adaptive mutations can often have off-target effects which modulate the direction and strength of resulting selection on that mutation. Accordingly, in the flatwing system, researchers have found that this mutant phenotype is associated with changes to morphology, behavior, and reproduction in addition to its primary effect on male wing morphology (Pascoal et al. 2018; Heinen-Kay, Urquhart, et al. 2019; Pascoal et al. 2020). Because the most apparent morphological effect of the flatwing phenotype is its call-silencing alteration of wing venation, its primary selective advantage is conferred only to adult male members of this species. Thus, research on the pleiotropic effects of this mutant genotype has understandably focused on investigating such effects in adult males. However, recent studies have found

that flatwing-carrier females also exhibit pleiotropic effects which might have influenced the trajectory of the flatwing genotype (Pascoal et al. 2018; Rayner, Pascoal, et al. 2019). Additionally, gene expression analyses of developing wingbuds in late-stage juveniles and of whole embryos have indicated that flatwing and normal-wing genotypes show differences far before adulthood (Pascoal et al. 2020). If wing morphs also exhibit important behavioral or morphological differences at juvenile stages, these effects could also influence the relative fitness of wing morphs, and such pleiotropy has long been understood to influence the fate of new mutations (Fisher 1958).

My study finds evidence of wing-morph related differences in juvenile morphology and behavior. First, in 15d juveniles, individuals carrying the flatwing allele were smaller than normal-wing nymphs. This difference was slight in absolute terms but still potentially biologically significant given the relative size of newly hatched crickets. Additionally, this difference did not persist in 45d juveniles or adults. One explanation for the ephemeral nature of this size difference is that flatwing-carrying mothers invest less in offspring than normal-wing parents, but throughout development, flatwing offspring are able to "catch up" in size. No study has compared size or nutrient reserves of eggs across wing morphs to directly test this possibility. However, there is evidence that flatwing-carrying females invest less in reproductive tissue (i.e., have lower ovary weight) than normal-wing females, suggesting that differential maternal investment could underly the morph-based size difference in early juveniles (Heinen-Kay, Strub, et al. 2019). Regardless of its cause, because this size difference did not persist with age, it is unlikely to create a disparity in fitness across wing morphs. Nevertheless, I cannot discount the possibility that, outside controlled laboratory conditions (i.e., ad libitum food and water), this early difference in size might influence fitness in late-juvenile and adult stages.

Second, at both ages, individuals carrying the flatwing allele exhibited greater activity, exploration, and boldness (measured through distance traveled, proportion of the grid explored, and time spent along edge, respectively) than normal-wing nymphs. This behavioral difference could result from pleiotropic or otherwise linked effects of the flatwing mutation, either in the offspring generation or in the parental generation via parental effects. It is possible that the observed difference in behavior is unrelated to the flatwing genotype. In other words, the three normal-wing laboratory lines could behave differently from the three flatwing-carrying lines for reasons other than their wing morph, for example through random changes in allele frequencies while being maintained in the lab. It seems unlikely, however that the three flatwing lines would so consistently exhibit increased activity by chance alone. The presence of such differences in juvenile locomotive behavior could influence population dynamics and suggest that the flatwing genotype may be exposed to selection at an earlier stage than previously anticipated. For example, in nature, greater locomotion and exploration could result in more efficient foraging for flatwing-carrying nymphs. Alternatively, such heightened activity may lead to greater predation risk.

Finally, if this difference does persist to adulthood, greater flatwing-associated activity could result in more efficient mate-searching, especially in the recently silent landscape of Hawaiian *T. oceanicus* populations. If so, this linked behavioral effect could have contributed to the initial rise of the flatwing mutation in Hawaiian males. A previous study on adult locomotion found no difference between flatwing and normal-wing males (Balenger and Zuk 2015). However, because this adult study's focus was to compare locomotive activity in response to acoustic rearing environment across several Hawaiian island populations with and without flatwings, the sample size comparing male wing morphs was limited (Balenger and Zuk 2015). Further, this study relied on indirect measures of activity (gridlines crossed, farthest grid reached, etc.). In contrast, I measured distance traveled directly using automated coordinate collection, giving me greater resolution to resolve differences in activity between wing morphs. Thus, it remains possible that adult-stage individuals of different wing morphs also differ in dispersal-related behavior. Further experiments would be necessary to determine if this behavioral difference affects the fitness of flatwing-carrying juveniles, and to confirm whether this difference persists to adulthood. Regardless, these results illustrate how pleiotropic effects during development may have important consequences for the trajectory of a mutant genotype which carries known fitness benefits at adult stages.

Sexual dimorphism in size (SSD) and behavior is observed in many species across taxa. Though much research has investigated how sexual dimorphism is influenced by sexual selection and natural selection, it remains unclear how and when the two sexes tend to diverge in their developmental trajectories, and whether the mechanism of this divergence is shared across different species (Lande 1980; Owens and Hartley 1998; Plavcan 2001). By measuring size and behavior in two early juvenile ages, I was able to address this gap in knowledge in my study. I found that sex did not influence size or behavior in nymphs of either age (15 or 45 days post-hatching), with the exception of 15-day old males spending more time in the origin of the open field test (OFT) arena than females of that age. At the adult stage, however, males were larger than females. Though in this species adult sexual dimorphism in size (i.e., SSD) and behavior have been observed before, my study is the first to explore how and when the two sexes of this species diverge in their developmental trajectories. Adult SSD can theoretically arise through several differences in juvenile growth schedule; sexes could differ in (1) size at birth, (2) instantaneous growth rate during juvenile development, and/or (3) length of juvenile development period (often called sexual bimaturation) (Esperk et al. 2007). Though I did not directly measure size at birth, a lack of difference at 15-days post-hatching indicates that the adult SSD likely does not arise via this mechanism. Further, because I did not find size differences in 15- or 45-day old nymphs, it is unlikely the sexes differ in growth rate during juvenile development, at least in early instars. Therefore, I predict the most likely cause of SSD in *T. oceanicus* is that the sexes differ in the length of their juvenile developmental period, either via changes to instar length or to the number of instars undergone. Such mechanisms for sexual

bimaturation are common across the insect class (Esperk et al. 2007; Tammaru et al. 2010). This prediction could be confirmed via active observation of the timing of each instar across sexes.

Regarding behavioral differences at adulthood, these are often attributed to sexual dimorphism in neural structures, and thus arise as early as embryonic development when the brain begins to take form (Belgacem and Martin 2002; Becker et al. 2005). However, whether the behavioral differences themselves, in addition to divergent neural structures, are present at the juvenile stage remains unclear. In my study, I found virtually no sex differences at either juvenile age in locomotive activity, exploration, or boldness. These results are supported by a previous study on field cricket, *Gryllus integer*, which also found no sex difference in juvenile boldness behavior, measured through a hiding assay, despite the occurrence of sexually dimorphic behavior in adults (Niemelä et al. 2012). It remains possible that sexually dimorphic behavior occurs in later juvenile instars, for example when external sexual structures (e.g., ovipositors) begin to develop. Thus, further study is necessary to determine when sexual dimorphism in behavior, especially in a non-sexual context, arises in ontogeny. Continued investigation of the mechanisms underlying sexual dimorphism in size and behavior will improve our understanding of how sex-specific phenotypes can arise from shared developmental processes.

Chapter III

SOCIAL PLASTICITY IN FEMALE REPRODUCTIVE INVESTMENT AND MATING BEHAVIOR

A) Background

I then asked whether Kauai-derived flatwing-carrying females of *T. oceanicus* exhibit socially-induced plasticity in reproductive investment, condition, or mating behavior. This experiment allowed me to confirm results of previous studies which have documented higher reproductive investment, and less responsive mating behavior in song-treated females (Bailey and Zuk 2008; Bailey and Zuk 2012; Swanger and Zuk 2015). I predicted the flatwing-carrier females of my study would exhibit the same trends in physiology and behavior in response to social heterogeneity. Additionally, this experiment was necessary to establish the extent of social plasticity in the maternal generation prior to my investigation of phenotypic effects in the offspring generation.

I chose to use only flatwing lines for my studies of social plasticity, both within and across generations, for several reasons. First, because flatwings now account for nearly all males on the island of Kauai, a flatwing lab population was the most relevant to current natural conditions (Zuk et al. 2018; Rayner, Aldridge, et al. 2019). Further, because the flatwing morph has dramatically changed the population's social landscape is associated with increased social plasticity, whether flatwing individuals also exhibit transgenerational plasticity is of great interest.

B) Methods

Cricket Populations and Acoustic Treatments

To produce individuals for this experiment, I reciprocally interbred the three laboratory stock lines homozygous for flatwing used in Chapter II experiments into an admixed flatwing stock population, which was spread across five 16L group-rearing containers. Following previous work, I isolated females at the juvenile stage when sex became apparent to keep them virgin and more easily manipulate their acoustic environment (Bailey and Zuk 2008; Pascoal et al. 2018). At this time, I also put a group of juvenile males in their own sex-specific 16-L box in the general incubator, to maintain their virginity. All group-rearing and isolated conditions were identical to those described in Chapter II methods.

Once isolated, all females were placed in a separate, temperature-controlled incubator with no male calling and checked daily for adult eclosion. Once eclosed, females were randomly assigned one of two acoustic treatments: silence or song. I chose to wait until adult eclosion to assign females to a specific treatment because I wanted to focus specifically on the influence of adult social experiences. Further, a cricket's hearing structures are not fully developed until after its final molt, and previous studies found no evidence that juvenile acoustic environment influences adult phenotype in this system (Young and Ball 1974; Staudacher 2009; Swanger and Zuk 2015). I 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 in silence prior to adult acoustic treatment. I kept each female in their acoustic treatment for 15 days post-eclosion to maximize the likelihood of phenotypic effects extending to offspring.

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 using a CEM DT-805 sound level meter) during the night portion of the crickets' light:dark cycle to best match settings in the wild. The song files played in the song treatment were used in a previous experiment examining the effects of acoustic environment (Pascoal et al. 2018). These files were made by recording 24 Kauai males, calculating average song parameters, and constructing two artificial calling files with these averaged parameters (Pascoal et al. 2018). Each of the two calls was played for a full hour at the beginning and end of the dark portion of the cycle to mimic natural conditions, where calling peaks just after dusk and right before dawn (8:00-10:00 and 18:00-20:00). In the middle of the crickets' night cycle (10:00-18:00), I played one call at a time at 15-minute intervals, with 5 minutes of silence between each alternating call. Each treatment occurred in a separate temperature-controlled incubator held at 25°C, and the same light:dark cycle as the general incubator. Twice a week, I switched which incubator housed each acoustic treatment, to prevent any incubator-related experimental confounds.

Mating of Acoustically Treated Females

Once they reached 15 days post-eclosion, isolated females were weighed and their pronotum width was measured using digital calipers. They were then placed in small plastic containers with cardboard, rabbit chow, and moistened cotton. I then randomly selected an adult virgin male from the mixed flatwing population, weighed it, measured its pronotum, and placed it in the container with the female. The pair was observed for 20 minutes, during which time I noted whether the female mounted the male and whether the male transferred a spermatophore. These mating trials were done between 20-23°C under a red light between 16:00h and 18:00h. After this time, the pairs were placed in a separate, silent temperature-controlled incubator, held at the same temperature and light:dark cycle as the general incubator. After 24 hours, the male was removed to reduce behavioral influences his continued presence might contribute to offspring phenotype. After another 24-48 hours, the female was removed, and the egg pad was collected.

Body Condition, Size, and Reproductive Tissue Measurements

To compare body condition across females from different acoustic treatment, I used pronotum width and total body weight to calculate the scaled mass index (SMI) of each individual. SMI was used in place of the residuals from a standard least square regression of body weight and length because it provides a more robust indicator of relative size of energy reserves and other body components (Peig and Green 2009). A subset of acoustically-treated females (n = 23) were dissected once they reached 15 days post-eclosion. Following euthanasia by freezing, I recorded their pronotum width using digital calipers, weighed them on a balance, and weighed the wet mass of their dissected ovaries. Somatic weight was calculated by subtracting ovary mass from total mass.

Statistical Analyses

All statistical tests were carried out using R version 4.0.2 (R Core Team 2020). First, I compared standard mass index (SMI) as a measure of body condition across acoustic treatments using a linear model with SMI as a response variable and the following predictor variables: *acoustic treatment*, *days isolated* (days a female was isolated before treatment), and *experimental replicate* (whether a female was treated in trial one or trial two of the experiment). SMI was log-transformed to increase normality.

Second, I compared mating behavior across acoustic treatments. I did this by first running a generalized linear model (GLM) with binomial error distribution on presence or absence of female mounting during the 20-minute mating trial. In this model, I included *acoustic treatment*, *female SMI*, and *male SMI* as predictor variables. Next, I ran a GLM with binomial distribution on presence or absence of spermatophore transfer. For this GLM, I only included the 49 mating trials (out of 65 total) where mounting had occurred (spermatophore transfer cannot occur without mounting), and *acoustic treatment*, *female SMI*, and *male SMI* were included as predictor variables.

Finally, I compared female reproductive investment across acoustic treatments in the subset (n = 23) of females that had been dissected by running a LM on log-transformed ovary mass. As in previous studies of reproductive investment, I attempted to control for body size by including log-transformed soma mass as a covariate (Tomkins and Simmons 2002; Bailey et al. 2010). Additionally, *acoustic treatment* and *days isolated* were included as predictor variables.

C) Results

Flatwing-carrying females raised in different acoustic environments showed differences in physiology, but not mating behavior (Figure 4). Specifically, females raised in song attained a higher condition (SMI) than those raised in silence (Table 4 and Figure 4A). Further, looking at propensity to mount during a 20-min mating trial, mounting was not influenced by female acoustic environment, but instead only significantly affected by male SMI (Table 5 and Figure 4C). Of the mating trials where mounting occurred (n = 49), likelihood of spermatophore was also not influenced by acoustic treatment, but instead only significantly explained by female SMI. (Table 5 and Figure 4D).

In the subset of females (n = 23) that were dissected, females reared in song had heavier ovaries when scaled to log-transformed somatic mass compared to females raised in silence (Table 4 and Figure 4B).

Table 4 Linear models examining the effects of acoustic environment on maternal condition (scaled mass index) and reproductive weight (g)

	Maternal Condition			Maternal Reproductive Weight		
	df	***F***	***P***	***df***	***F***	***P***
Acoustic Treatment	1, 110	6.0382	**0.0156**	1, 19	5.0148	**0.0373**
Days Isolated	1, 110	0.1289	0.7202	1, 19	1.0373	0.3213
Experimental Replicate	1, 110	0.9481	0.3324	-	-	-
Somatic Mass	-	-	-	1, 19	0.2107	0.6515

Table 5 Generalized linear models examining the effects of acoustic environment on maternal mating behavior

	Mounting			Spermatophore Transfer		
	df	***χ2***	***P***	***Df***	***χ2***	***P***
Acoustic Treatment	1, 61	0.3402	0.5597	1, 45	0.3319	0.5646
Male Condition	1, 61	6.7885	**0.0092**	1, 45	2.8635	0.0906
Female Condition	1, 61	2.7724	0.0959	1, 45	5.3611	**0.0206**

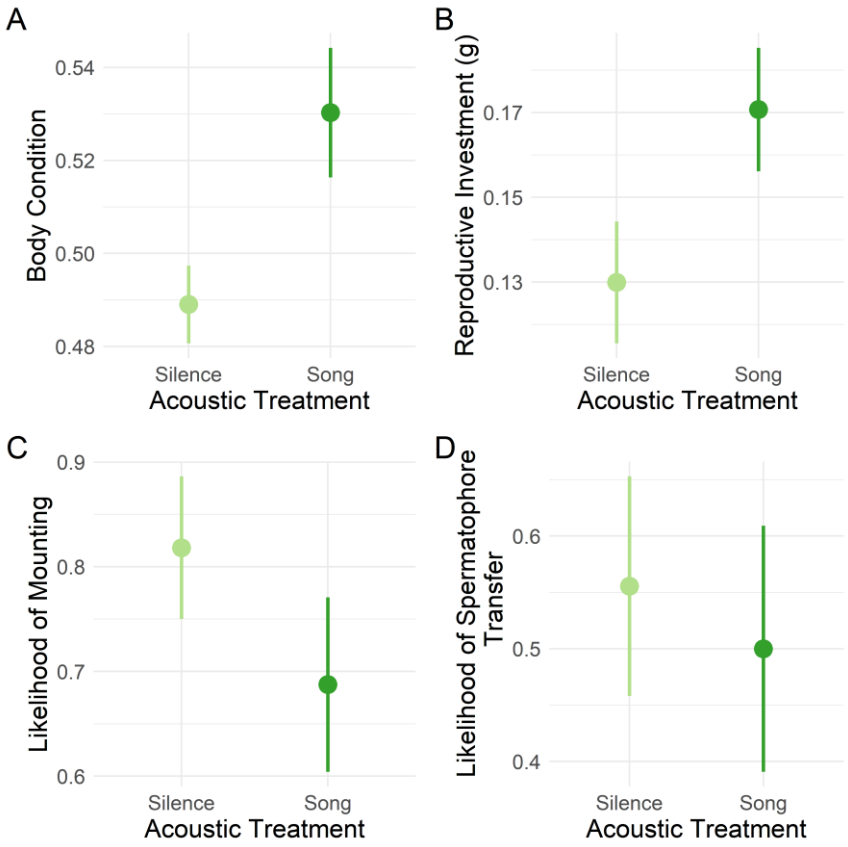

Figure 4 *The effect of acoustic environment on adult females' scaled mass index or body condition (A), reproductive investment (B), likelihood of mounting (C), and likelihood of receiving a spermatophore given the occurrence of mounting (D). All plots show mean and standard errors.*

D) Discussion

I found clear evidence that body condition is sensitive to the adult social environment in flatwing-carrying females. My results show that females adjust their condition in response to the perceived density of sexually mature calling males in their environment, with females reared in silence exhibiting lower body condition than females raised in the presence of male song. This difference in condition is likely due to a lower reproductive tissue investment in females raised in silence compared to those raised in song, which was supported by direct measurement of reproductive tissue weight in a subset of females. The finding that females with social experience invest more in reproductive tissue is consistent with studies in another cricket species, *Gryllus firmus* (Conroy and Roff 2018), in other populations of *T. oceanicus* from different islands (Bailey and Zuk 2012; Heinen-Kay, Strub, et al. 2019), and in males of *T. oceanicus* (Bailey et al. 2010). Such plasticity in reproductive tissue investment supports basic ideas underlying the theory of life-history trade-offs.

Resource allocation is a central component of life history theory; in a given species, the cumulative outcome of numerous resource allocation trade-offs forms the basis of that organism's life history strategy (Williams 1966; Gadgil and Bossert 1970). Because it is ubiquitous across taxa, much work in the field of life history evolution has been dedicated to understanding the trade-off between reproduction and survival (Kirkwood and Rose 1991; Harshman and Zera 2007; Schwenke et al. 2016). Many empirical studies have provided evidence for physiological explanations underlying this link. For example, when resources are limited, artificial manipulation of reproductive investment alters immune function, nutrient allocation to somatic tissue, and defense against starvation or oxidative stress and toxicity, all of which should support survival (Chippindale et al. 1996; Hosken 2001; Wang et al. 2001; French et al. 2007; Hatle et al. 2008; Cox et al. 2010).

It is clear from this body of work that reproductive investment comes with significant opportunity costs. Further, the extent of these costs should depend on the environmental conditions, and thus the selective pressures, experienced by the individual at the time of the trade-off. Therefore, if environmental conditions shift in a way that alters the optimal balance between reproduction and survival, selection should favor individuals who are able to in turn plastically shift their reproductive investment to maximize overall fitness (Heubel et al. 2008). This idea is partially supported in my study – individuals reduce their reproductive investment in response to an environmental signal that forecasts a lack of return on that investment (i.e., the absence of nearby males with which to mate), thus preventing resource waste. However, whether this decrease in reproductive investment in silent-reared females is also accompanied by increased investment in survival-promoting processes such as immune function or somatic tissue maintenance, and thus an increase in overall fitness, remains unknown.

In singing populations of crickets, social plasticity should allow females to avoid wasteful allocation of resources to reproduction when mates are unavailable. However, the females used in this experiment come from obligatorily silent cricket populations. In these populations, the environmental signal which induces plasticity has lost its predictive accuracy; a lack of song no longer reflects an absence of nearby males because silence is constitutive. It is therefore somewhat surprising that flatwing-carrying females still respond to this acoustic cue, especially given that the maintenance of plasticity is thought to be costly (Scheiner 1993). One possible explanation is that plasticity is in fact not as costly to maintain as previously anticipated and thus there is little selective pressure to remove it, even when environmental conditions are constant. This prediction is supported by a general lack of empirical evidence demonstrating the costs of plasticity (Scheiner and Berrigan 1998; Maughan et al. 2007; Van Buskirk and Steiner 2009) and by models which find that formally adaptive plasticity may be maintained even after many generations of environmental stasis (Masel et al. 2007). Further, work on natural populations of east killifish, *Heterandria formosa*, have found that populations which consistently

experience low densities still exhibit plasticity in response to manipulation of rearing density, suggesting this response may be evolutionary conserved (Leips et al. 2009).

If plasticity is in fact costly, another explanation for its maintenance is that not enough time has passed for selection to eliminate it in flatwing-dominated populations. The loss of plasticity in flatwing-carrying females might also have been delayed by its initial role in mediating the rise of the flatwing mutation. When the flatwing mutation first arose, the associated decline in male calling meant mating encounters were increasingly a result of chance. In theory the longer a female lived, the more likely she was to have such chance encounters and thus reproduce successfully with flatwing-carrying males. Therefore, it is possible that a plastic reduction in female reproductive investment in favor of survival-promoting processes might have helped to promote the rise of the flatwing mutation. If this were the case, this role could have contributed to the maintenance of the plasticity even after the flatwing reached near fixation in the population. I suggest that future studies monitor the occurrence and extent of acoustically-induced plasticity in populations which have become fixed for the flatwing allele such as those on Kauai (Rayner, Aldridge, et al. 2019). Such research would provide the opportunity to test model predictions that formally adaptive plasticity should be lost via genetic assimilation following the onset of environmental stasis (Lande 2009).

In addition to alteration of reproductive investment, females may shift aspects of their mating strategies in response to information on their social environment. This behavioral plasticity occurs in *T. oceanicus*, as females raised in silence exhibit more responsive phonotaxis behavior in response to a speaker broadcasting male call (Bailey and Zuk 2008; Swanger and Zuk 2015). Further, another study found that females of this species which have been reared in silence are more quick to mount a male in mating trials (Bailey and Zuk 2012). However, given two hours to mate, this difference in mounting latency did not result in an overall difference in mating rates in another study on this species (Lierheimer and Tinghitella 2017). My experiment demonstrates that females homozygous for the flatwing mutation also do not exhibit a difference in mating rate according to female acoustic environment. This lack of plasticity in mating rate likely results from that fact that the cost of mating is very limited when presented with a male in a small container. In contrast, searching for males is costly, which likely underlies females' plasticity in phonotaxis responsiveness according to acoustic rearing environment (Real 1990; Lierheimer and Tinghitella 2017).

It is worth noting that, though mating behavior was not influenced by female acoustic environment, it was affected by male and female body condition. Specifically, mounting was more likely to occur when males exhibited a higher body condition and, in cases where mounting did occur, spermatophore transfer was more likely when females possessed a higher body condition. Mounting in this species is performed by females, while spermatophore transfer is initiated by males. In theory this

system allows both participants to exert control over different components of the mating process, and thus influence the ultimate mating outcome. My results suggest that females' assessments of male condition (either directly through visual or tactile inspection or indirectly through cuticular hydrocarbons) influence their decision to mount a given male. Additionally, spermatophore transfer was influenced by female body condition, which supports the idea that males can in turn exercise mate choice either before or during mounting. Studies of sexual selection have found increasing evidence for male mate choice, even in the absence of male parental care (Bonduriansky 2001; Kokko and Johnstone 2002; Edward and Chapman 2011). Male choice is thought to arise more often when high costs associated with attracting mates or mating itself prevent males from mating with multiple females (Edward and Chapman 2011). If a male cannot mate with multiple females, he is more likely to avoid mating with females of lesser quality. In contrast, if he can mate with multiple females, there may be little reason for him to exhibit such preferences. In the crickets, male mate choice might arise from the high metabolic cost of spermatophore production, which limits how many females a male can mate with. This prediction is supported by a review which found male mate choice is more common in insect systems where males produce large spermatophores (Bonduriansky 2001). Further, the same review found that male mate choice is more likely to evolve when there is variation in female fecundity (Bonduriansky 2001). This condition is also met in in this species, as *T. oceanicus* females of larger size produce a higher number of offspring (Heinen-Kay, Strub, et al. 2019). One potential future avenue of research would be to explore whether orthopteran species which differ in calling effort and/or spermatophore size also exhibit different patterns of male mate choice.

Chapter IV

TRANSGENERATIONAL EFFECTS OF MATERNAL SOCIAL ENVIRONMENT

A) Background

Using the offspring of the acoustically-treated flatwing-carrier mothers from Chapter III experiments, I then investigated whether maternal acoustic environment influenced offspring phenotype, in both juvenile and adult stage offspring. First, as in my work comparing juvenile phenotype across morphs in Chapter II experiments, to explore transgenerational consequences of maternal social environment in juvenile offspring, I measured offspring phenotypes at 15 and 45 days of age. Juveniles of these two ages were ideal for the purposes of investigating adaptive TGP because they cannot yet hear their own social environment, and, as mentioned previously, models predict that adaptive TGP is more likely to evolve when juveniles' environmental cues are inaccurate (Leimar and McNamara 2015). Additionally, all types of maternal effects are generally stronger in juvenile-stage offspring than adults (Moore et al. 2019).

While some offspring from silent- or song-treated mothers were isolated at hatching and used for the investigation of maternal effects in early-juvenile offspring, others were pooled into 5 replicate boxes per treatment and then exposed to either a matched or mismatched social environment during their late-stage juvenile and early-adult life. Following offspring social treatment, I then measured structural size, somatic condition, reproductive investment, and locomotive activity and exploration via an open field test (OFT) to assess adult offspring phenotype.

Several studies on transgenerational social plasticity have found evidence of differences in offspring size based on maternal social treatments (McCormick 2006; Dantzer et al. 2013; Crocker and Hunter 2018a). However, across these studies, there is inconsistency in which of the maternal social treatments (crowded versus solitary) produces bigger offspring. Further it remains unclear whether this size difference is a result of an adaptive maternal signal of past conspecific density (adaptive TGP), an incidental signal of maternal condition (condition-transfer TGP) or a socially-induced shift in parental reproductive strategy (selfish TGP). Regardless of the direction of this transgenerational size effect, if present this effect could have important consequences for offspring fitness throughout development. To contribute to this discussion of socially-induced maternal effects on offspring growth, I measured size of the juvenile offspring at 15 and 45 days of age.

Though several studies have investigated transgenerational effects on offspring physiology, relatively fewer have extended their investigations to include behavioral measurements. Therefore, to explore maternal effects on behavior, I also subjected each individual to the same open field test (OFT) used in Chapter II experiments. Measurements collected from OFTs, such as distance traveled, proportion of the field explored, and time spent along the edge versus the center of the field are often used to quantify locomotive activity, exploration, and boldness, respectively. These behavioral traits in turn influence how an individual interacts with its environment – how far it disperses, how often it forages, how efficiently it forages, etc. I believe these behavioral traits might be influenced by maternal social

environment for several reasons. First, as mentioned before, several studies have linked maternal social environment with changes in offspring condition (size, weight, and/or growth rate) (McCormick 2006; Dantzer et al. 2013; Crocker and Hunter 2018b). This observation, combined with evidence that individuals of different condition also differ in dispersal-related behavioral traits, indicates that effects of maternal effects might extend to behavior (Aerts et al. 2000; Goosens et al. 2020). Second, an individual's social environment determines the level of competition for mates and resources it will experience, and many organisms are capable of adjusting their phenotype accordingly to increase their fitness (Stockley and Seal 2001; Relyea 2002). Maternally-derived cues of social environment would be similarly helpful by allowing nymphs to disperse away from conspecific-dense areas to avoid competition. Alternatively, cues of high conspecific density could indicate an environment with rich resources, dispersal away from which would be disadvantageous. It is crucial that we continue to improve our understanding of maternal-modification of offspring locomotive behavior because in theory it would allow mothers to not only influence the fitness of their offspring, but also have important effects on downstream population dynamics (Meylan et al. 2012).

By exposing mothers and offspring to matching or mismatching social cues before experiments on adult offspring, I was able to better understand the relationship between transgenerational plasticity (TGP) and within-generational (WGP). Models predict that WGP achieves better predictive accuracy than adaptive TGP because the offspring's cues occur closer in time to selection on the resulting offspring phenotype than cues experienced by parents (Ezard et al. 2014). Because of this higher predictive accuracy, it is expected that once capable of assessing their environment, offspring should rewrite parental cues with their own to maximize predictive accuracy (Auge et al. 2017). However, experimental studies in plants where both parental and offspring cues were manipulated have shown that, while offspring cues sometimes overwrite or mask parental cues, the opposite, where the expression of WGP depends on maternal environment, can also occur (*Reviewed in* Auge et al. 2017). These conflicting empirical trends may occur because non-adaptive TGP (e.g., selfish TGP effects, passive condition-transfer effects) interacts differently with offspring WGP than adaptive TGP effects. My study allowed me to investigate the interaction between parental and offspring cues in an animal system.

I was also able to compare the effects of maternal social environment across ontogeny of offspring, including stages before and after offspring are capable of assessing their own social environment. This comparison could demonstrate one of several trends. First, there could be no effect of maternal environment in either early-juvenile or adult-stage offspring, with only WGP contributing to variation in adult offspring phenotype. Second, TGP could exist in early-stage offspring but decline in strength or be completely absent in adult-stage offspring. Models predict that the evolution of adaptive TGP requires high predictive accuracy of parental cues (Leimar and McNamara 2015). As offspring age,

the time lag between parental cues and offspring environment increase, theoretically resulting in a decline in predictive accuracy of parental cues. Therefore, it is logical to predict that the strength of adaptive TGP declines with increased offspring age. Further, a recent meta-analysis found that all types of maternal effects are generally stronger in juvenile stage offspring than adults (Moore et al. 2019) and several empirical studies have demonstrated that maternal effects in early-stage offspring can be 'overwritten' or 'masked' in later-stage offspring that have experienced their own environmental cues (Lindholm et al. 2006; Groot et al. 2016). Third, a relatively small or even undetected maternal effect in early offspring phenotype could be compounded throughout development to produce a larger maternal effect in adult-stage offspring.

Finally, in addition to exploring effects of maternal acoustic environment, this experiment allowed me to better understand phenotypic changes resulting from offspring acoustic environment. Past experiments on this system have demonstrated within-generational social plasticity in reproductive investment and locomotive behavior (Bailey et al. 2010; Balenger and Zuk 2015; Heinen-Kay, Strub, et al. 2019). However, by including both males and females, my study allowed me to directly compare the extent of social plasticity across sexes. Further, previous studies have focused on normal-wing populations or mixed populations. By using pure-breeding flatwing populations in the current study, I was able to explore how flatwing individuals respond to a silent environment, and whether social plasticity could have supported the spread of this silencing-allele.

B) Methods

Cricket Population, Rearing, and COVID-19 Disruptions

All group-rearing and individual-rearing conditions and maintenance were identical to methods described in Chapter II methods unless otherwise indicated. Egg pads produced by the maternal generation used in Chapter III experiments were first kept in a separate incubator under the same temperature and light conditions as the general incubator. However, as the first individuals began to hatch, the first COVID-19 UK national lockdown (late March 2021) required that all experiments in the building be halted. To limit necessary maintenance for technicians with "essential worker" status, I pooled egg pads into five replicate 16-L boxes per maternal acoustic treatment (song and silence). Each box contained 6-7 egg pads (each egg pad from one pair of adults). I organized the pooling such that egg pads in Silent Box 1 were collected from matings that occurred at the same time as those that produced egg pads in Song Box 1, so that offspring from Silent Box 1 and Song Box 1 hatched at the same time. Thus, crickets in Silent Box 1 and Song Box 1 were older than crickets in Silent Box 2 and Song Box 2 and so on.

These 10 boxes were initially housed in the general incubator at 25°C for two weeks after the closure. After two weeks, the university granted me permission to bring the crickets back to my private apartment to relieve the workload of technicians, where I kept them in an undisturbed spare room. The

same level of precision in temperature-regulation as in the lab incubators could not be achieved. The room was kept at roughly 18-24°C throughout their development. The window in the room was blocked out using emergency blankets, and two lamps were plugged into timer-controlled outlets to match the photo-reversed light:dark cycle set in the general incubator.

Egg pads still containing un-hatched eggs at this time were placed in separate containers (to keep track of sibling relationships) and monitored daily for new hatchlings to use for the early juvenile offspring TGP experiment. These hatchlings were isolated and kept in the apartment rearing conditions described above for their entire development. Before I could bring these crickets home to the apartment, most eggs had already hatched, limiting the number of individuals available for the early juvenile offspring TGP experiment. Therefore, to supplement this original data, the experiment was repeated approximately two months later once the lab had re-opened. In this second trial, all rearing conditions were the same, except individuals were kept at a constant 25° in the general incubator (experimental trial was included as a random effect in all resulting models). In the end, for the early juvenile offspring TGP experiment, I tested a total of 309 offspring from 35 mothers (14 treated with song, 21 treated with silence). Number of offspring collected per mother ranged from 1-25, but on average, 8.8 were collected per mother.

Individuals which had already hatched before permission was granted to take them back to the apartment were kept in their original pooled boxes in apartment rearing conditions and later used for the adult offspring TGP experiment. Once I was allowed back in the lab, I returned the 10 boxes to the general incubator. These slight rearing temperature fluctuations were unavoidable. However, because crickets were switched to warmer conditions at different stages in development, these fluctuations likely resulted in variation in growth rate across the 5 pairs of boxes. To account for this variation, I have included an additional term in all models of this experiment: age class. For example, individuals from Song Box 1 and Silent Box 1 belong to age class 1. Because boxes of the same age class hatched at the same time and therefore experienced changes in rearing temperature at the same approximate stage in development, including this term in models should control for inconsistent rearing temperatures. Densities were kept consistent across these replicate boxes, except for Song Box 2, which experienced a maintenance-related die-off early on, and thus housed crickets at a much lower density. Only eight individuals from Song Box 2 were tested, thus the likelihood that this difference in density impacted overall results of this experiment is very unlikely. Once these individuals reached the stage at which sex is apparent, they were isolated and randomly assigned to either silence or song acoustic treatment (same incubators and acoustic treatment parameters as with the maternal treatment described in Chapter III methods). In total, 378 adult offspring were used for this experiment (189 females, 189 males), with

approximately equal assignment to each of the four treatment groups (maternal song, offspring song; maternal song, offspring silence; maternal silence, offspring song; maternal silence, offspring silence).

Open Field Test and Measurements

Open field test procedures for this experiment were identical to those in Chapter II experiments. For juvenile offspring, OFTs were performed at 15 and 45 days in trial one of this experiment. For trial one, the juvenile offspring OFTs were conducted in the spare room of my apartment at 23-25.5°C. For trial two of the experiment, the OFTs were conducted in the same video incubator as in Chapter II experiments. Due to a summer heat wave, during trial 2 of this experiment, assays had to be conducted at slightly higher temperature range of 24-28°C, as efficient cooling of the video incubator was impossible. Additionally, due to time constraints, during trial 2, OFTs were only performed on individuals 15 days post-hatching.

Adult offspring OFTs were performed in the video incubator at 23-28°C between the hours of 12:00 and 17:00 under dim red lighting. The OFT procedure for adult offspring was identical to that of juvenile offspring, except a slightly larger plastic arena was used with adults (41 cm wide, 37 cm long, and 28 cm high). Also, if the individual attempted to fly out of the arena during the assay, the recording was halted, and the individual was placed back in the silent incubator for 10 minutes. After this time, they were assayed again. The number of flight attempts was recorded for each individual.

Collection of coordinates from OFT videos of juvenile and adult offspring using DORIS was nearly identical to procedures used in Chapter II methods. Because a larger arena was used for the adult OFT, however, to calculate the latter two measurements, the arena was divided into a raster of 1,517 distinct 1 cm^2 grid squares. The 'edge' region of this arena was defined as a 2 cm^2 border along the perimeter of the arena. The 'origin' arena was defined as the 1 cm^2 grid in which the cricket began its trial. Lastly, the 'middle' region was all other space not defined as edge or origin.

Morphological Measurements

For juvenile offspring, following assaying, each individual was imaged overtop a micrometer using the same camera and dissecting scope as in Chapter II experiments. Later in ImageJ, these images were used to extract and record pronotum length. For adult offspring, after assays were completed for all individuals, crickets were euthanized in the freezer. Following euthanasia, I used a digital caliper to measure their pronotum length and weighed them to find their total mass. I then dissected out their gonads, blotted off excess water, and weighed them on the balance. Rather than using pronotum length and total weight when calculating scaled mass index, as I did in Chapter III experiments, for this experiment, I decided to use pronotum length and soma weight, which I calculated by subtracting gonadal weight from total weight measured at 6d of age. By doing this, SMI becomes a measure purely of somatic body condition, which allows me to investigate if differences in maternal or offspring acoustic environments influence

investment in somatic tissues, while still scaling to structural size by including pronotum length. Because male and female crickets differ in pronotum length, SMI was calculated separately for each sex. Gonadal weight was later compared directly.

Statistical Analyses

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

First, I sought to determine if juvenile offspring size differed between maternal acoustic treatments. I did this by running a linear mixed effect model (LMM) using square-root transformed pronotum length as a response variable. I included the following terms as predictor variables: *maternal treatment and experimental replicate*, with *maternal ID* as a random effect. *Experimental replicate* was included to account for slightly different rearing temperatures experienced by trial 1 and trial 2 individuals. The interaction between *maternal treatment* and *experimental replicate* was excluded from the final model due to insignificance ($p > 0.2$). The same models were run for pronotum length on 45-day old offspring data, except, because I only had 45-day nymph data for trial 1, *experimental replicate* was not included in models of pronotum length at 45 days.

Then, I compared juvenile offspring locomotive activity across maternal treatments. As in Chapter II experiments, I did this by creating a separate linear mixed model for each of the following response variables: distance traveled (mm), proportion of grid explored, time spent in edge region (s), time spent in middle region (s), and time spent in origin region (s). To improve normality in models of the 15-day assay, I square-root transformed time spent in middle region, and log-transformed time spent in origin region. To improve normality in models of the 45-day old assay, I square-root-transformed time spent in middle region and proportion of grid explored, time spent in edge region and time spent in origin region. All movement models included the following predictor variables: *maternal treatment, experimental replicate, assay temperature, assay time of day*, and *pronotum length*, with *mother ID* as a random effect. The same models were run for movement variables on 45-day old offspring data, except, because I only had 45-day data for trial 1, *experimental replicate* was not included in models comparing movement at 45 days old.

For adult offspring experiments, because of large sex differences in all physiological variables and the possibility of sex-specific maternal effects, all measurements of adult physiology were modeled separately in males and females.

First, I investigated whether adult individuals from different maternal or offspring acoustic treatments differed in structural size, as estimated by pronotum length. To do this, for each sex, I ran a linear model with pronotum length as the response variable and the following predictor variables: a two-way interaction between *maternal treatment,* and *offspring treatment,* with *age class* as a random effect. The interaction term was removed if found to be non-significant under a conservative assessment ($p >$

0.2). Next to compare adult offspring somatic body condition, I ran another pair of sex-specific linear models with somatic SMI as the response variable. In these models, I included *maternal treatment, offspring treatment*, and the *maternal treatment*offspring treatment* interaction term, with *age class* included as a random effect. If the interaction term was found to be non-significant ($p > 0.2$), it was removed from the final model. I then explored differences in adult offspring reproductive investment across acoustic treatments. To do this, I ran two sex-specific linear models using log-transformed gonadal weight as the response variable. For each sex, I began by comparing unscaled reproductive investment. Then I added pronotum length as a covariate to see if differences in reproductive investment between maternal or offspring treatments disappeared when differences in structural size were accounted for. Finally, I added an additional covariate of log-transformed somatic mass to see if treatment differences in reproductive investment disappeared when somatic weight was accounted for. *Age class* was included as a random effect in all models. The interaction between *maternal treatment* and *offspring treatment* was initially included in all models but was dropped from final models if found to be insignificant ($p > 0.2$).

Then to investigate whether adult individuals from different maternal and offspring acoustic treatments might also differ in behavior, I compared the following measurements from an open-field test (OFT): distance traveled (mm), proportion of field explored, time spent in edge region (s), time spent in middle region (s), and time spent in origin region (s). For each behavioral parameter, I ran a separate linear mixed effect model for each sex. For the female-specific models, to improve normality I square-root-transformed distance, edge time, middle time, and origin time, and log-transformed proportion of grids explored. For male-specific models, I square-root-transformed distance, edge time, middle time, and origin time. In all models, I included *maternal treatment*, *offspring treatment*, *assay temperature*, *assay time*, and *somatic SMI* as predictor variables, with *age class* included as a random effect. The *maternal treatment* and *offspring treatment* interaction was included at first but removed in any model where it was found to be non-significant under conservative assessment ($p > 0.2$). As in Chapter II experiments, I ran models of proportion of grid explored both with and without *distance* as a covariate.

Finally, I compared likelihood to attempt flight during the OFT across acoustic treatments and sexes in adult offspring. To do this, I ran a generalized linear mixed model (GLMM) with a binomial family distribution using flight attempts as a response variable (a score of 1 if flight was attempted and a score 0 if flight was not attempted). After removing insignificant interaction terms (those where $p > 0.2$), I was left with the following predictor variables: *maternal treatment, offspring treatment, sex, maternal treatment*sex,* and *somatic SMI*, with *age class* included as a random effect.

C) Results

I found no evidence for transgenerational effects of maternal acoustic environment on behavior or physiology of 15-day-old or 45-day old offspring (Tables 6-9).

Table 6 Linear models examining the effects of maternal acoustic environment on offspring open field test behaviors at 15 days of age

	Distance			Proportion Explored			Edge Time			Middle Time			Origin Time		
	df	*χ2*	*P*	*df*	*χ2*	*P*	*df*	*χ2*	*P*	*df*	*χ2*	*P*	*df*	*χ2*	*P*
Maternal Treatment	1, 300	0.6249	0.4292	1, 300	0.1353	0.7130	1, 300	0.2674	0.6051	1, 300	0.8028	0.3703	1, 300	0.5143	0.4733
Experimental Replicate	1, 300	45.0919	**<0.0001**	1, 300	21.5548	**<0.0001**	1, 300	0.1272	0.7214	1, 300	3.9589	**0.0466**	1, 300	0.0120	0.9126
Pronotum Length	1, 300	0.0863	0.7689	1, 300	0.0031	0.9558	1, 300	5.6987	**0.0170**	1, 300	2.4918	0.1144	1, 300	1.9094	0.1670
Assay Temperature	1, 300	0.5303	0.4665	1, 300	0.1982	0.6562	1, 300	1.8555	0.1732	1, 300	0.3590	0.5491	1, 300	2.2348	0.1349
Assay Time	1, 300	1.0725	0.3004	1, 300	3.9847	**0.0459**	1, 300	0.2453	0.6204	1, 300	2.2452	0.1340	1, 300	4.1024	**0.0428**

Table 7 Linear models examining the effects of maternal acoustic environment on offspring pronotum length at 15 days of age

	Pronotum Length		
	df	*χ2*	*P*
Maternal Treatment	1, 307	0.0464	0.8294
Experimental Replicate	1, 307	232.9527	**<0.0001**

Table 8 Linear models examining the effects of maternal acoustic environment on offspring open field test behaviors at 45 days of age

	Distance			Proportion Explored			Edge Time			Middle Time			Origin Time		
	df	*χ2*	*P*	*df*	*χ2*	*P*	*df*	*χ2*	*P*	*df*	*χ2*	*P*	*df*	*χ2*	*P*
Maternal Treatment	1, 191	0.0032	0.9546	1, 191	0.5405	0.4622	1, 191	0.1472	0.7012	1, 191	0.0001	0.9917	1, 300	0.3227	0.5700
Pronotum Length	1, 191	5.7143	**0.0168**	1, 191	2.9874	0.0839	1, 191	0.4541	0.5004	1, 191	0.2549	0.6137	1, 300	0.0457	0.8307
Assay Temperature	1, 191	2.2781	0.1312	1, 191	5.6950	**0.0170**	1, 191	2.4223	0.1196	1, 191	0.8385	0.3598	1, 300	3.8947	0.0484
Assay Time	1, 191	0.3376	0.5612	1, 191	2.7636	0.0964	1, 191	0.1283	0.7202	1, 191	4.4015	**0.0359**	1, 300	0.1626	0.6868

Table 9 Linear models examining the effects of maternal acoustic environment on offspring pronotum length at 45 days of age

	Pronotum Length		
	df	*χ2*	*P*
Maternal Treatment	1, 195	0.7322	0.3922

The acoustic treatment experienced by adult offspring influenced all measured morphological traits. In addition, and despite no evidence for transgenerational plastic effects on juvenile offspring phenotype, the acoustic environment that their mothers had experienced in the previous generation nevertheless affected several aspects of the adult offspring's phenotype. Moreover, these maternal effects often did not affect traits in the same direction as offspring treatment effects.

In adult females, individuals raised in different acoustic environments differed in pronotum length, but the direction of this difference was dependent on that individual's maternal treatment; in other words, there was a significant maternal treatment*offspring treatment interaction (Table 12). Specifically, individuals from a silent maternal treatment exhibited longer pronotum lengths when raised in silence compared to those raised in song (Figure 5A, left). In contrast, individuals from a song maternal treatment had shorter pronotum lengths when raised in silence compared to those raised in song (Figure 5A, left). Analysis of female somatic condition revealed a similarly significant interaction between maternal treatment and offspring treatment (Table 12). While individuals from the silent maternal treatment did not differ in somatic condition according to what acoustic environment they were reared in, individuals from the song maternal treatment had higher somatic condition when raised in song compared to those raised in silence (Figure 5B, left). In the female specific model of gonadal weight, the significance of the effect of *maternal treatment* was consistent regardless of which covariates indicative of size were included (Table 14). Across these models, there were significant effects of both *maternal treatment* and *offspring treatment,* with offspring raised in song having heavier ovaries than offspring raised in silence, and offspring from the silent maternal treatment having heavier ovaries than offspring from the song maternal treatment (Table 14 and Figure 5C, left).

In adult males, neither *maternal treatment* nor *offspring treatment* had a significant effect on pronotum length (Table 13 and Figure 5A, right). Only *offspring treatment* significantly influenced somatic condition, with males raised in song achieving higher somatic condition than males raised in silence, regardless of maternal treatment (Table 13 and Figure 5B, right). The significance of maternal and offspring environment on male reproductive weight depended on the inclusion of covariates in the model (Table 15). If no size-related covariates were included, models demonstrated significant effects of both *maternal treatment* and *offspring treatment* (Table 15 and Figure 5C, right). If pronotum length was included in the model as a covariate to account for differences in structural size, the significance of these effects was preserved (Table 15). However, if log-transformed soma weight was included, the effect of maternal treatment only approached significance while the effect of offspring treatment was no longer significant (Table 15). Finally, if both log-transformed soma weight and pronotum length were included, the effect of maternal treatment was significant, while the effect of offspring treatment only approached significance (Table 15).

Offspring treatment also had a strong influence on adult offspring behavioral traits, with almost no significant effects of *maternal treatment* (Table 10 and Table 11). In females, only *offspring treatment* had a significant effect on distance traveled, with females raised in song traveling farther than those raised in silence (Table 10 and Figure 5D, left). No significant effect of *maternal treatment* on female distance was found. In males, *offspring treatment* had significant effects on distance traveled, but the significance of the interaction between *offspring treatment* and *maternal treatment* only approached significance (Table 11 and Figure 5D, right). Specifically, males raised in song generally traveled farther than those raised in silence, but this difference was more exaggerated in males whose mothers had been raised in silence compared to those whose mothers had been raised in song. Female offspring raised in song explored a higher proportion of the arena than females raised in silence, but this effect was no longer present when distance was included as a covariate (Table 11). In female models of middle time, the interaction between maternal treatment and offspring treatment was significant (Table 11 and Figure 5E, left)). In males, neither offspring treatment nor maternal treatment explained significant variation in proportion of grids explored, edge time, middle time, or origin time (Table 11 and Figure 5E, right).

Finally, looking at propensity to fly during the activity assay, females attempted flight significantly more than males (Table 16 and Figure 6A). Additionally, adult offspring raised in silence attempted flight significantly more than offspring raised in song (Table 16 and Figure 6A). Somatic condition also had a significant effect on flight attempts; individuals with higher somatic condition were less likely to attempt flight (Table 16 and Figure 6B).

Table 10 Linear models examining the effects of maternal and offspring acoustic environment on open field test behaviors in adult females

	Distance			Proportion Explored			Edge Time			Middle Time			Origin Time		
	df	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***
Maternal Treatment	1, 181	2.0546	0.1517	1, 181	3.5632	0.0591	1, 181	0.2033	0.6521	1, 180	5.5802	**0.0182**	1, 181	0.0305	0.8613
Offspring Treatment	1, 181	6.2791	**0.0122**	1, 181	6.0796	**0.0137**	1, 181	2.6450	0.1039	1, 180	1.3802	0.2401	1, 181	2.8078	0.0938
Assay Temperature	1, 181	2.5352	0.1113	1, 181	1.6370	0.2007	1, 181	1.6115	0.2043	1, 180	1.5130	0.2187	1, 181	6.9780	**0.0083**
Assay Time	1, 181	1.9234	0.1655	1, 181	1.4866	0.2228	1, 181	2.0185	0.1554	1, 180	1.1520	0.2831	1, 181	5.2362	**0.0221**
Somatic Condition	1, 181	1.2110	0.2711	1, 181	0.8562	0.3548	1, 181	4.3603	**0.0368**	1, 180	0.4373	0.5084	1, 181	2.3320	0.1267
Maternal Treatment* Offspring Treatment	-	-	-	-	-	-	-	-	-	1, 180	5.6605	**0.0174**	-	-	-

Table 11 Linear models examining the effects of maternal and offspring acoustic environment on open field test behavior in adult males

	Distance			Proportion Explored			Edge Time			Middle Time			Origin Time		
	df	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***
Maternal Treatment	1, 182	0.7888	0.3745	1, 183	0.2906	0.5898	1, 183	0.2109	0.6460	1, 183	0.1138	0.7358	1, 183	0.0000	0.9979
Offspring Treatment	1, 182	6.9852	**0.0082**	1, 183	3.2575	0.0711	1, 183	2.0562	0.1516	1, 183	2.2936	0.1299	1, 183	0.1075	0.7430
Assay Temperature	1, 182	6.6200	**0.0101**	1, 183	5.3825	**0.0203**	1, 183	1.5325	0.2157	1, 183	0.0056	0.9403	1, 183	0.6296	0.4275
Assay Time	1, 182	0.7240	0.3948	1, 183	2.0589	0.1513	1, 183	0.2440	0.6213	1, 183	0.0589	0.8083	1, 183	1.9795	0.1594
Somatic Condition	1, 182	3.5665	0.0590	1, 183	3.5077	0.0611	1, 183	0.0052	0.9424	1, 183	0.2838	0.5942	1, 183	0.4141	0.5199
Maternal Treatment* Offspring Treatment	1, 182	3.6951	0.0546	-	-	-	-	-	-	-	-	-	-	-	-

Table 12 Linear models examining the effects of maternal and offspring acoustic environment on several parameters of physiology at adulthood in females

	Pronotum Length			Somatic Condition		
	df	***χ2***	***P***	***df***	***χ2***	***P***
Maternal Treatment	1, 187	0.6266	0.4286	1, 187	3.0601	0.0802
Offspring Treatment	1, 187	1.4540	0.2279	1, 187	0.0786	0.7792
Maternal Treatment* Offspring Treatment	1, 187	4.3428	**0.0372**	1, 187	4.6701	**0.0307**

Table 13 Linear models examining the effects of maternal and offspring acoustic environment on several parameters of physiology at adulthood in males

	Pronotum Length			Somatic Condition		
	df	***χ2***	***P***	***Df***	***χ2***	***P***
Maternal Treatment	1, 187	0.1993	0.6553	1, 187	1.2649	0.2607
Offspring Treatment	1, 187	0.0005	0.9826	1, 187	8.1693	**0.0043**

Table 14 Linear models examining the effects of maternal and offspring acoustic environment on reproductive investment in females, depending on the inclusion of size-related covariates

	Reproductive Investment			Reproductive Investment			Reproductive Investment			Reproductive Investment		
	df	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***
Maternal Treatment	1, 188	5.8906	**0.0152**	1, 187	5.0449	**0.0247**	1, 187	4.0106	**0.0452**	1, 186	3.9104	**0.0480**
Offspring Treatment	1, 188	21.7189	**<0.0001**	1, 187	23.1712	**<0.0001**	1, 187	16.2163	**<0.0001**	1, 186	16.8603	**<0.0001**
Pronotum Length	-	-	-	1, 187	6.4423	**0.0111**	-	-	-	1, 186	0.8936	0.3445
Somatic Weight	-	-	-	-	-	-	1, 187	22.3020	**<0.0001**	1, 186	15.6014	**<0.0001**

Table 15 Linear models examining the effects of maternal and offspring acoustic environment on reproductive investment in males, depending on the inclusion of size-related covariates

	Reproductive Investment			Reproductive Investment			Reproductive Investment			Reproductive Investment		
	df	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***	***df***	***χ2***	***P***
Maternal Treatment	1, 187	5.4653	**0.0194**	1, 186	5.5356	**0.0186**	1, 186	3.7163	0.0539	1, 185	3.9748	**0.0462**
Offspring Treatment	1, 187	5.3000	**0.0213**	1, 186	5.6128	**0.0178**	1, 186	2.2861	0.1305	1, 185	2.7997	0.0943
Pronotum Length	-	-	-	1, 186	12.8055	**0.0003**	-	-	-	1, 185	1.6977	0.1926
Somatic Weight	-	-	-	-	-	-	1, 186	19.4575	**<0.0001**	1, 185	8.6745	**0.0032**

Table 16 Results of binomial model examining the effects of maternal and offspring acoustic environment on flight attempts during the OFT

	Flight		
	df	***χ2***	***P***
Maternal Treatment	1, 371	0.0853	0.7703
Offspring Treatment	1, 371	8.788	**0.0030**
Sex	1, 371	4.2127	**0.0401**
Somatic Condition	1, 371	4.1608	**0.0413**
Maternal Treatment* Sex	1, 371	2.14	0.1435

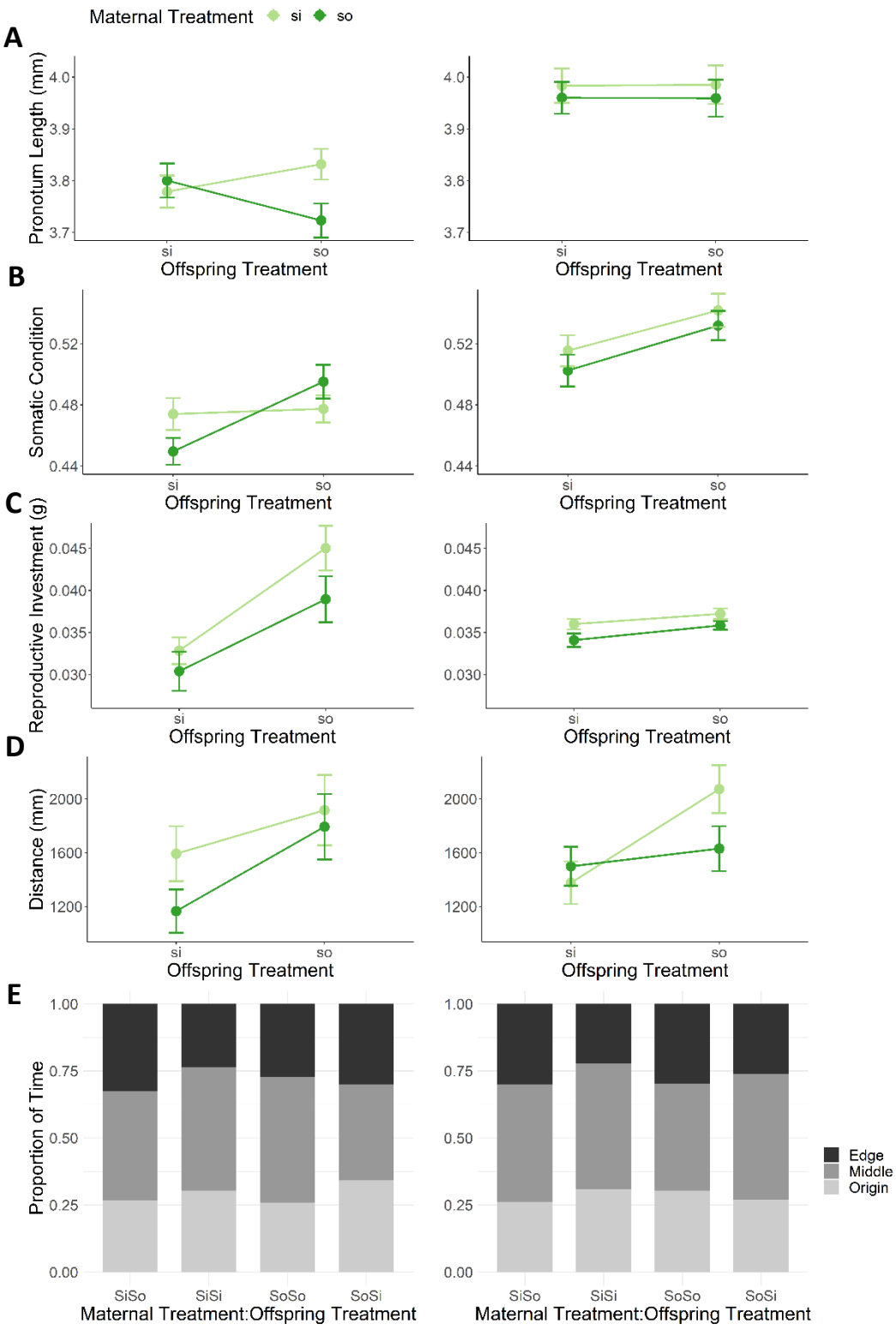

Figure 5 *The effects of maternal acoustic treatment and offspring acoustic treatment in adult females (left) and males (right) on pronotum length (A), somatic condition (B), reproductive investment (C), distance traveled (D), and proportion of time spent in the three regions of the OFT arena (E). In A-D, the mean and standard error of each subgroup are given.*

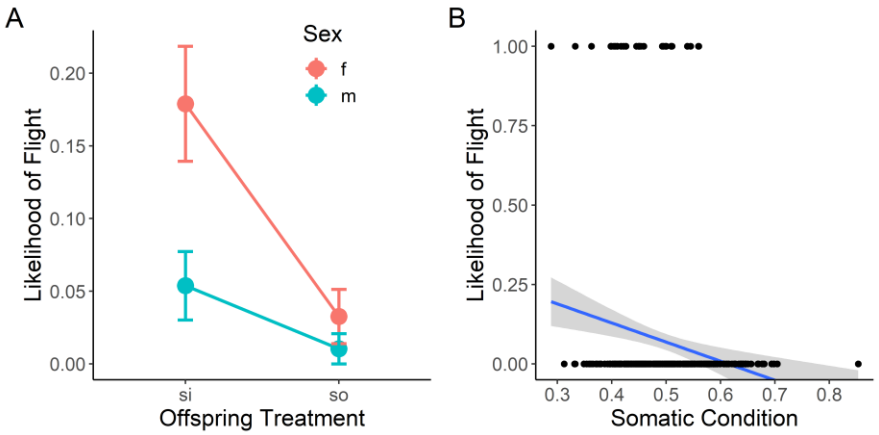

Figure 6 *The likelihood of flight during OFT based on sex and offspring treatment (A) and somatic condition (B). Means and standard errors are presented for each subgroup in A.*

D) Discussion

It is often argued that the strength of maternal effects declines throughout offspring development. This assertion is supported by empirical studies which find that both the relative and absolute contribution of maternal genetic effects to offspring phenotype decrease with age (Lindholm et al. 2006; Wilson and Réale 2006; Krist 2011), as well as a recent meta-analysis examining all types of maternal effects (Moore et al. 2019). Despite these predictions and findings, I found evidence that maternal social environment affects adult, but not juvenile, offspring phenotype for all measured traits except locomotive activity. My results contrast with studies which found that manipulation of maternal conspecific density resulted in changes to juvenile offspring size across a range of taxa (McCormick 2006; Allen et al. 2008; Leips et al. 2009; Dantzer et al. 2013; Crocker and Hunter 2018b) and in some cases dispersal potential (Allen et al. 2008). There is also evidence that juveniles alter activity or dispersal in response to maternal exposure to predator cues (Storm and Lima 2010; Cattelan et al. 2020) and parental parasite load (Tschirren et al. 2007).

One explanation for the lack of TGP effect in 15- and 45-day old juvenile offspring is that maternal social environment influenced offspring phenotype very early in development, but that these effects dissipated prior to first measurement of juvenile phenotype in my experiment. For example, maternal treatment could have influenced time to hatching (embryonic growth rate) or size at hatching. This scenario is consistent with results from a study on an amphibian, *Ambystoma talpoideum*, which found that individuals with artificially reduced maternal provisioning were initially smaller at hatching but caught up with control individuals in body size within the first month of the larval stage (Moore et al. 2015). If this were the case, cues of low competition and low nutritional stress in isolated laboratory conditions could have 'overridden' cues of high social density from mothers, resulting in the absence of a

maternal effect at the juvenile ages measured in this experiment. Regardless of whether there was a maternal effect prior to measurement of offspring phenotype, the absence of such an effect at 15 and 45-days post-hatching indicates that this maternal effect was ultimately inconsequential for offspring phenotype at these ages, at least in the environmental rearing conditions of this experiment.

In contrast, maternal social environment did influence offspring phenotype at the adult stage. One reason for this emergence of maternal effects later in development might be that individuals used for the adult experiments were stored in communal boxes at relatively high (but consistent) densities rather than isolated at hatching as with the juvenile stage experiment. As a result, they might not have received the same cues of low competition and/or nutritional stress as with juveniles, and therefore did not override maternal effects in the same way early in development. However, a more parsimonious explanation may be that the maternal social environment influenced phenotype via mechanisms that would not cause observable phenotypic differences until late in development. For example, many insects exhibit significant plasticity in the number of instars they undergo prior to sexual maturation and this plasticity in developmental trajectories is thought to underly the evolution of sexual size dimorphism at adulthood (Esperk et al. 2007; Stillwell et al. 2010). It is possible then that maternal social environment influenced offspring development through alteration of instar number, as such an effect would not necessarily result in differences in size at early instars but would influence size at adulthood.

To understand why and how TGP affected adult phenotype in this experiment, it is essential to consider how offspring integrate parental cues with cues from their own environment. It is often expected that, for adaptive TGP, within-generational plasticity (WGP) is more efficient than TGP because progeny cues more accurately predict offspring environment than parental cues (Ezard et al. 2014). Therefore, once capable of assessing their environment, offspring should rewrite parental cues with their own (Auge et al. 2017). By exposing adult offspring to social environments that created either a match or mismatch with their maternal social environment (i.e., a fully factorial experimental design), my experiment allowed me to investigate the relative contributions, and potential interactions, of WGP and TGP to adult offspring phenotype. I found that maternal social environment influenced several important offspring traits by altering the direction and/or strength of WGP in response to offspring acoustic environment. This trend was evident by the significance of the interaction term between maternal and offspring acoustic environment for several offspring traits. For example, mothers reared in silence produced females that grew larger in the presence of song when compared to female offspring reared in silence, whereas mothers reared in song produced females that grew smaller in the presence of song when compared to female offspring reared in silence. Thus, maternal social experience caused opposing effects on how their offspring developed in different social environments. Further, in females, though offspring environment did not influence somatic condition in offspring from the silent maternal treatment, amongst offspring

from the song maternal treatment, individuals raised in song achieved higher somatic condition than individuals raised in silence. Overall, these results emphasize that the expression of TGP is often dependent on offspring environment, which is consistent with findings in other fully-factorial TGP studies (Groot et al. 2016; Auge et al. 2017).

Male offspring did not exhibit a significant effect of maternal acoustic environment on either pronotum length or somatic condition. This contrast is interesting, as it suggests maternal acoustic environment has sex-specific consequences for offspring phenotype. Sex-specific TGP has been observed in mammals with respect to parental stress but remains relatively unexplored in other taxa and in response to other environmental cues (Bale 2011; Glover and Hill 2012; Bell and Hellmann 2019). Maternal stress-related hormonal shifts can have sex-specific effects on offspring methylation and hormone receptor patterns (Bale 2011), so it is conceivable that sex-specific TGP arises due to alteration of offspring's responses to hormone shifts during development. Regardless of the underlying mechanism, such a process could have important evolutionary consequences, as it gives mothers the ability to differentially alter the phenotype, and ultimate fitness, of their sons and daughters (Bell and Hellmann 2019).

Offspring reproductive investment in both sexes was generally higher in individuals derived from silent-reared mothers. Females of many insect species exhibit significant plasticity in reproductive investment in response to numerous environmental cues (Hodin 2009). This plasticity can occur at the adult stage via alteration of oocyte maturation rate, oocyte size, the number of active ovarioles, and oviposition patterns (Hodin 2009). However, the maximum number of ovarioles a female develops, and thus her maximum potential reproductive output, is fixed before the adult stage in nearly all insects (Hodin 2009). Several environmental factors can influence plasticity in ovariole number prior to its fixation at adulthood, but generally, abundant food and uncrowded conditions lead to an increase in ovariole number (Boulétreau-Merle et al. 1982; Stewart et al. 1991; Hodin 2009). In this experiment, mothers reared in silence produced daughters with higher reproductive investment, and this maternal effect was stronger amongst female offspring who themselves had been reared in song. Future work would benefit from exploring this effect by measuring ovariole number in female offspring derived from mothers of different acoustic environments. This would determine whether silent-reared mothers produce daughters with more ovarioles, which could increase their maximum potential reproductive output. This effect could also be explored in males to determine whether male offspring from silent-reared mothers have greater testes weight due to maternal manipulation of developing testes tissue prior to adulthood. Confirmation of such an effect would be significant, as maternal alteration of ovariole number represents a potential mechanism by which TGP can influence the degree of plasticity possible in offspring (i.e., WGP). It is important to note that, in males, the effect of maternal environment was no longer significant following the inclusion of log-transformed soma mass (but not pronotum length) as a covariate in models

of reproductive investment. This may indicate that although maternal treatment does seem to influence reproductive weight in male offspring, this effect may occur through maternal influences on somatic mass.

An important remaining question is whether the observed TGP effects in this experiment are adaptive, either for mothers, offspring, or both parties. If adaptive for offspring (and therefore also for mothers), a match between parental and offspring environment should result in higher offspring fitness when compared to mis-matched offspring (Marshall and Uller 2007; Uller et al. 2013). I found no evidence supporting this expectation for offspring performance traits (i.e., larger size at adulthood, greater somatic condition, greater reproductive investment). For example, if maternal acoustic environment served as an adaptive, anticipatory cue of conspecific density for offspring, one might expect offspring of song-reared mothers to obtain greater reproductive weight (i.e., for maternal environment to operate on reproductive weight in the same direction as offspring environment). The opposite is observed in this case; reproductive investment of both sexes was highest when offspring were raised in song and mothers were raised in silence (i.e., a mis-matched group). Therefore, I find it unlikely that the observed TGP was an anticipatory effect to increase offspring fitness (i.e., adaptive TGP). This conclusion is supported by a recent meta-analysis which found weak evidence for the prevalence of anticipatory parental effects (i.e., adaptive TGP) across taxa (Uller et al. 2013). Alternatively, this TGP effect may result from condition transfer, where parental condition is positively correlated with offspring condition either passively or actively (Bonduriansky and Crean 2018). This explanation is also unlikely, as the maternal group which achieved greater condition and reproductive investment (the song-reared mothers) produced offspring which had lower reproductive investment overall compared to the silent-reared mothers. Therefore, I find it most likely that the observed TGP is this experiment was an incidentally-transmitted physiological consequence of mothers responding to their social environment in a way that may increase their own fitness (i.e., a selfish TGP effect) (Marshall and Uller 2007). For example, mothers reared in song may have interpreted this acoustic cue as a high probability of future reproduction with other males. As a result, despite their greater reproductive weight, they might have invested less in their first clutch of offspring in favor of saving resources for future offspring with other males (i.e., a trade-off between current and future fecundity). Alternatively, mothers reared in silence may perceive future reproduction to be unlikely and thus invest more in current reproduction. This difference in maternal investment could explain why offspring from silent-reared mothers exhibited greater reproductive investment than those from song-reared mothers. This hypothesis is supported by a study in common gobies (*Pomatoschistus microps*) which found that females respond to social cues of mate limitation (specifically, female-biased sex ratio) by increasing the size of their first clutch (Heubel et al. 2008). Though previous studies in *T. oceanicus* found equivalent hatching success across eggs from different maternal acoustic environments,

this does not eliminate the possibility that overall offspring quality is higher in silent-reared mothers (Lierheimer and Tinghitella 2017).

This experiment also revealed several interesting trends regarding the effect of acoustic environment within a generation. First, I observed that while both sexes increased reproductive investment when raised in song, the extent of this increase was much higher in females. Greater ovary and egg weight likely result in an increase in female fecundity when males are present, but increased testes size may only increase male reproductive success to a point. Instead, males respond to cues of high competition by increasing sperm content, as demonstrated in a previous study in this species (Gray and Simmons 2013). This likely explains the relatively narrow range of testes weight found in this experiment.

Second, I found that offspring of both sexes were more active when reared in song than when reared in silence. This could be for several reasons. One explanation is that this increased activity is driven by state-dependent differences in boldness. Size or body condition often predict between-individual variation in boldness or exploration, with individuals of higher condition or larger size exhibiting more bold, exploratory behavior (Kelleher et al. 2017; Goosens et al. 2020). In this experiment, individuals raised in song generally attained higher body condition and thus perhaps this difference in condition led to increased boldness and thus heightened locomotive activity in the open field test (OFT). This explanation is unlikely, though, due to the observation that individual condition did not explain significant variation in distanced traveled in either sex. Alternatively, this increased locomotive activity could reflect an increase in mate-searching. Though the primary method to find mates in most cricket species is through acoustic cues, more short-range location of conspecifics may be achieved through chemical signaling using cuticular hydrocarbons (Bailey 2011). When reared in song, members of both sexes may therefore increase walking activity in an attempt to locate conspecifics which they perceive to be abundant nearby, even in the absence of an immediate phonotactic cue. In contrast, crickets raised in silence have been given no indication of nearby conspecifics. As a result, they may decrease short-range mate-searching via walking and instead wait for an acoustic cue. This trade-off is likely motivated by a high metabolic cost of mate-searching (Hack 1998) and the resulting increase in predation risk (Bell 1990). I also observed that individuals reared in silence were more likely to attempt flight during the OFT. One interpretation of this trend is that individuals reared in silence may use flight as an un-directed, long-range method of dispersal to increase their chances of reaching an area of greater conspecific resources. By increasing propensity for long-range dispersal (i.e., flight) in silent flatwing-dominated subpopulations, such a response to silence could have in theory increased the speed over which the flatwing allele spread across the fragmented distribution of *T. oceanicus* in the Hawaiian Islands.

It is important to note that these results contrast with previous studies in this species that have found that males raised in song are less active than those raised in silence (Balenger and Zuk 2015) and that females exhibit limited flexibility in locomotive behavior in response to acoustic environment (Heinen-Kay et al. 2018). One explanation for this apparent contradiction is that these studies relied on indirect measures of activity (gridlines crossed, farthest grid reached, etc.). In contrast, I measured distance traveled directly using automated coordinate collection, giving me greater resolution to resolve differences in activity between individuals reared in different acoustic environments. Additionally, previous experiments have used mixed study populations which contain both flatwing and normal-wing carrying individuals. Because I used a pure-breeding flatwing population for my experiment, my results may therefore reflect a flatwing-specific behavioral response to acoustic environment.

Chapter V

CONCLUSIONS

To fully understand how plasticity evolves and how it influences subsequent evolution of other traits, it is essential to consider the adaptive value of its effects beyond just one generation. I explored this topic by investigating the role of socially-induced plasticity in the rapid adaptation of *Teleogryllus oceanicus* to a novel predator via loss of an important signal. Consistent with previous studies on this system, I found that socially-induced within generational plasticity (WGP) appears to have adaptive consequences; it allows adults to reduce investment in reproductive tissue in response to acoustic cues indicative of low mating potential (i.e., lack of conspecific song). If transgenerational plasticity (TGP) effects of maternal social environment had evolved to be adaptive to offspring, one might expect juvenile offspring whose mothers had been reared in song to grow at a faster rate, allowing them to better compete in a crowded environment. Additionally, juvenile offspring from mothers reared in song might be expected to have greater locomotive activity to increase dispersal away from crowded areas. In contrast to these expectations, my results demonstrated that there was no effect of maternal social environment on either juvenile growth rate or locomotive behaviour. However, these juvenile traits were affected by wing morph genotype. Specifically, flatwing-carrying juveniles exhibited increased locomotive activity and transiently slower growth than normal-wing juveniles. It is possible then that the constitutive wing-morph associated effects on locomotive behavior and growth limited the degree to which these traits were plastic to maternal acoustic environment in flatwing-carrying individuals. Further experiments investigating the effects of maternal social environment in normal-wing crickets unaffected by the flatwing genotype should be conducted to determine if the lack of social TGP is common to both morphs, or is instead specific to flatwings.

Adult offspring did show evidence of transgenerational effects of maternal social environment. If the observed transgenerational plasticity (TGP) effects in adult offspring had evolved to extend the adaptive consequences of WGP across multiple generations, one might expect silence in the maternal generation to result in reduced reproductive investment in offspring. I find the opposite to be true; adult offspring produced by mothers raised in silence had greater reproductive investment than those produced by mothers raised in song. I therefore hypothesize that the observed socially-induced TGP in this species has not evolved to be adaptive for offspring, but instead may be an incidental consequence of the maternal generation adaptively adjusting their reproductive strategy to their perceived social environment. In other words, adaptive WGP in the maternal generation has resulted in non-adaptive TGP in the offspring generation.

Because this TGP does not appear to be directly adaptive for offspring, it is unclear whether these effects influenced the rise of the flatwing mutation in Hawaiian populations of *T. oceanicus*. However, it remains possible that the observed TGP was an incidental result of the rapid adaptation to a novel predation pressure in this system. Theory predicts that, following rapid environmental change,

populations will exhibit a transient increase in plasticity as a means of rapidly shifting the population's mean phenotype closer to the new optimum (Lande 2009). As mentioned previously, non-adaptive TGP (e.g., selfish TGP or condition-transfer) can often occur as an incidental inheritance of parental state (Lacey 1998; Bonduriansky and Day 2009; Bell and Hellmann 2019). Increases in WGP during an adaptive evolutionary response to a novel environment could therefore result in a spillover of these effects to the offspring generation, causing the increase in WGP to be paralleled by an increase in TGP. Though this TGP may initially be non-adaptive or even mal-adaptive for offspring, over time, selection could act to either eliminate this incidental transfer of information across generations or shape it to become adaptive. Previous work in the flatwing system supports model predictions of increased WGP following rapid environmental change, in this case a shift towards a silent social environment (Pascoal et al. 2018). Specifically, this study found that flatwing males and flatwing-carrying females exhibit a higher degree of socially-mediated plasticity in neural gene expression when compared to normal-wing individuals (Pascoal et al. 2018). It is possible then that an increase in socially-mediated plasticity among flatwing-carrying individuals resulted in a parallel increase in TGP via incidental inheritance of parental state. Further studies should perform similar experiments on strictly normal-wing populations to better understand whether the signal of TGP found in this experiment is common to both morphs or instead a result of general increased plasticity documented previously in flatwing individuals (Pascoal et al. 2018). Additionally, theory predicts genetic assimilation of previously plastic traits to occur following environmental stabilization (Lande 2009). It would be interesting then to explore the extent of WGP and TGP found in populations which have since become fixed for the flatwing allele and therefore experience constitutive silence (Zuk et al. 2018). Overall, my findings indicate that it is crucial to consider the potentially conflicting effects of WGP and TGP when making predictions about how plasticity as a whole will influence the ability of a population to rapidly respond to environmental change.

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