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LIFE HISTORY TRADEOFFS AND PHENOTYPIC PLASTICITY:
THE TALE OF A FLIGHT POLYPHENIC FIELD CRICKET

by

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A DISSERTATION

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Under the Supervision of Professors William E. Wagner Jr. and Anthony J. Zera

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LIFE HISTORY TRADEOFFS AND PHENOTYPIC PLASTICITY:

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University of Nebraska, 2011

Advisers: William E. Wagner Jr. and Anthony J. Zera

Most organisms are resource limited. Such limitations can result in tradeoffs between life history traits - any traits that affect survival or reproduction. Flight polyphenic field crickets are thought to be a classic example of such a life history tradeoff, in which individuals tradeoff investment in flight capability and investment in reproduction. This polyphenism results from the interaction of two morphological traits: wing morphology (short or long) and flight muscle morphology (functional pink or non-functional white), and is affected by both genes and the environment. I examined life history traits of a flight polyphenic field cricket, *Gryllus lineaticeps*. First, I investigated whether females and males of flight capable and flightless morphs express alternative reproductive tactics congruent with their alternative life histories. I hypothesized that individuals in poor environments invest in flight capability, making it easier to locate mates, at the cost of early reproduction, while individuals in good environments invest in early reproduction, at the cost of flight capability. My results supported these hypotheses in both females and males. Next, I investigated costs, benefits, and constraints on the environment specific expression of life history traits. Having found that individuals with

developed flight muscles pay a reproductive cost, I asked whether they gain flight capability. I found that only individuals with both long wings and developed flight muscles can fly. In addition, I found that flight muscle development and breakdown have correlated effects on other traits such as jumping ability, a trait used to escape predators and therefore likely to have survival consequences, leading to the conclusion that, how resources are allocated between flight capability and reproduction may be constrained from tracking environmental shifts due to selection for/against correlated traits. Lastly, I examined this life history tradeoff in the field. I found that different morphs of field caught individuals tradeoff flight capability and reproduction, by varying investment in reproductive tissues and lipids used for energy storage and egg production. This series of experiments provides a comprehensive look at life history evolution in a phenotypically plastic species.

DEDICATION

“You cannot harm me, You cannot harm one who has dreamed a dream like mine”

- Sioux warrior (Densmore, 1926, *American Anthropologist*, 28 (2): 447-44)

For Eric, Ma, Baba, Sara and Tobie

Eric, thank you so much for all your love, patience and humor. I could not have done this without you, and I truly appreciate your putting your life on hold while I worked my way through this temporary (one hopes) insanity. Thank you, most of all, for making me laugh when I wanted to howl and throw things.

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CHAPTER ONE: Females in a flight polyphenic field cricket express alternative reproductive tactics: behavioral and physiological variation among morphs

ABSTRACT

Females show extensive variation in their reproductive behavior, the adaptive significance of which is still poorly understood. We examined variation in female reproductive behaviors of a flight polyphenic field cricket. This polyphenism results from the interaction of two morphological traits: wing morphology (short or long) and flight muscle morphology (functional pink or non-functional white). Previous studies have shown that these crickets tradeoff early reproduction with flight capability. Here, we hypothesized that females of flight capable and flightless morphs express alternative reproductive tactics congruent with their alternative life histories. Some females may invest in flight capability, improving their ability to locate mates, at the cost of early reproduction. Their fitness may be strongly affected by male-provided fecundity benefits, and they may be able to sample more males. These females should be more choosy. Other females may invest in early reproduction, to maximize their fecundity at the cost of flight capability. Their fitness may be less strongly affected by male-provided fecundity benefits, and they may be able to sample fewer males. These females should be less choosy. Our results supported these hypotheses. While we found no effect of wing morph, flight muscle morph affected all measured traits. Females with developed pink flight muscles invested less in current reproduction, were less responsive to male song, and were more choosy. As density of available mates is temporally variable, different

tactics may result in higher fitness at different times, and environmental variability may result in the maintenance of this polyphenism.

Key words. Life history tradeoffs, wing-polymorphism, phenotypic plasticity, sexual signals, female responsiveness, female choosiness.

INTRODUCTION

Alternative reproductive tactics have been studied extensively in males of many different species (reviewed by Gross 1996; Shuster and Wade 2003). However, while diverse reproductive behaviors and breeding tactics clearly exist in females, studies of such variation among females are still rare (reviewed by Henson and Warner 1997; Jennions and Petrie 1997; Brockmann 2001). The study of individual variation among females in reproductive behavior is important because it can affect the strength and nature of sexual selection on male traits, and may therefore help explain the observed variation in male sexual traits (Jennions and Petrie 1997). In addition, individual variation in female reproductive behavior may be favored by selection if females adaptively adjust their behavior to extrinsic or intrinsic factors (Jennions and Petrie 1997; Widemo and Sæther 1999; Cotton et al. 2006). For example, previous studies have found that female reproductive behavior can be affected by age (e.g., Mautz and Sakaluk 2008; Morris et al. 2010), nutritional condition (e.g., Hebets et al. 2008; Vitousek 2009), mating status (Wilder and Rypstra 2008; Judge 2010), social experience (Hebets 2003), abiotic conditions (Velez and Brockmann 2006; Milner et al. 2010), and non-genetic maternal effects (Forstmeier et al. 2004). Such variation among females may be caused by

individual level differences in the costs and benefits of expressing a behavior, and these differential costs and benefits may result in different females pursuing alternative mating tactics, or alternative behavior patterns (Brockmann 2001) that result in increasing their fitness.

Variation among females in reproductive behavior should be particularly likely when females vary in their life history strategies, as differences in life history traits may change the costs and benefits of expressing any given reproductive behavior. For example, predation risk may affect maturation time and average body size (e.g., Reznick and Endler 1982). In low predation populations, where individuals delay sexual maturation, females might be able to afford the costs of sampling multiple males, which may allow them to be choosy. Here, choosiness is defined as effort/energy that a female invests in mate assessment (Jennions and Petrie 1997). In contrast, in high predation populations, where individuals accelerate sexual maturation, females might not be able to afford the costs of sampling multiple males, which may preclude being choosy. Therefore, different adaptive combinations of behavioral and life history traits may be selected for in different environments (e.g., Breden and Stoner 1987; Houde and Endler 1990). Tradeoffs between traits may likewise limit the reproductive behaviors that females can express. Such tradeoffs are often a result of resource limitations, when allocation of resources to one trait reduces the resources that can be allocated to other traits (Stearns 1976; Schlichting and Pigliucci 1998; Zera and Harshman 2001). They can constrain the evolution of traits since an evolutionary increase in one trait can lead to an evolutionary decrease in correlated traits. Tradeoffs can occur between different life history traits (e.g., Stearns 1976; Partridge and Harvey 1988), between different sexually

selected traits (e.g., Basolo 1998; Wagner et al. in review), or between sexually selected traits and other life history traits (e.g., Griffith and Sheldon 2001), and may lead to individuals within a population using different reproductive behaviors and strategies. For example, females in high predation populations may invest less time in mate assessment than females in low predation populations, since mate sampling is riskier, delayed reproduction is riskier, and mate sampling may reduce anti-predator vigilance.

Correlations between reproductive behavior and life history traits may be particularly strong in polymorphic/polyphenic species, in which females occur as discrete morphological variants differing in their life history strategies. Such species are therefore particularly amenable for testing hypotheses about adaptive covariation in reproductive behavior and other life history traits. For example, female morphs in damselflies (Hammers et al. 2009), swordtail fish (Morris et al. 2003; Robinson and Morris 2010), and salmon (Morbey and Guglielmo 2006) differ in reproductive behavior. In these species, the authors hypothesized that differences between the morphs in life history traits, such as length of time spent as juveniles (Hammers et al. 2009), the level of aggressiveness (Robinson and Morris 2010), and body size (Morbey and Guglielmo 2006), may explain the morph-specific reproductive behaviors.

Flight polymorphism/polyphenism in insects involves tradeoffs between multiple life history traits (reviewed by Harrison 1980; Zera and Denno 1997; Zera and Brisson in press), where tradeoff is defined as a negative correlation between traits. In these insects, there are discrete morphs that differ in traits related to flight capability and reproduction. Flight polymorphic/polyphenic field crickets, in particular, have been intensively studied (reviewed by Zera 2009). In these animals the two flight phenotypes result from the

interaction of wing length (long wing [LW] or short wing [SW]), and flight muscle development (functional pink [P] or non-functional white [W]). Individuals with short wings and/or non-functional white flight muscles are flightless, while individuals with both long wings and functional pink flight (LW_P) muscles are capable of flight. Flight capable LW individuals produce and maintain energetically expensive pink flight muscles and lipid flight fuels, and delay reproduction (Roff and Fairbairn 1991; Mole and Zera 1993; Zera et al. 1994). Pink flight muscle tissue has more and larger fibers, higher respiration rates, and higher in vitro enzyme activity than the non-functional white flight muscle tissue (Zera et al. 1997). However, individuals can histolyze their flight muscles, which turn from pink to white in color, and which causes them to become flightless (e.g., Zera et al. 1997; Roff and Gelinas 2003). Morph expression (wing length and flight muscle morph) is affected by both genes and environment, and is phenotypically plastic in many species of field crickets (e.g., Fairbairn and Roff 1990; Roff 1996; Lorenz 2007; Zera 2009; Zera and Brisson in press). In examining the tradeoff between flight capability and reproduction, previous studies have found that males of different morphs differ in the amount of time they spend producing song, as well as in their calling song characteristics (Webb and Roff 1992; Crnokrak and Roff 1995; 1998; Mitra et al. 2011), two factors likely to affect male reproductive success. In addition, SW and LW_W females have been shown to have larger ovaries than LW_P females (Roff et al. 1997; Zera et al. 1997; Zera and Cisper 2001); ovary size is correlated with egg number (Roff 1994).

Our study species is the variable field cricket, *Gryllus lineaticeps*, which is flight polyphenic: adults occur as SW, LW_P and LW_W . In working with this species, we found a fourth morph: SW individuals with pink flight muscles that resemble the muscles of

flight capable LW individuals (SW_P). While SW individuals with pink muscles have been reported in another field cricket, *Gryllus firmus*, they have either been described as an extremely rare morph (Roff 1989), or reported as occurring only in individuals during the first three days post final molt (Zera et al. 1997). Unexpectedly, we have found this morph at relatively high frequencies in lab populations (see below) and in several field populations (Mitra personal observation).

The mating behavior of this species is well studied (e.g., Wagner and Basolo 2007). In *G. lineaticeps*, males produce calling songs to attract females from a distance (Wagner 1996). Females prefer songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner and Basolo 2007; Beckers and Wagner 2011), and under some environmental conditions, females receive fecundity and life span benefits from mating with males with these traits (Wagner and Harper 2003; Tolle and Wagner 2011). Females may mate repeatedly with a given male within a night and with multiple males over several nights (Wagner et al. 2001a).

In this study, we tested the hypothesis that females of the flight capable and flightless morphs of *G. lineaticeps* express alternative reproductive tactics. Flight capable females (LW_P) may trade off early reproduction for the ability to fly, allowing them to move from areas with few or no males to areas with more males. Their enhanced mobility may allow them to be choosier, and because of their reduced initial investment in reproduction, male-provided direct benefits may have a large effect on their reproductive success. Therefore, these females may benefit more from being choosy (e.g., South et al. 2011), which includes lower responsiveness to an average song type and stronger choosiness for high chirp rate songs (which are correlated with fecundity benefits). Thus,

flight capable females may adopt a high cost, low risk reproductive strategy. In contrast, flightless females (SW_W and LW_W) may trade off flight capability and increased mobility for enhanced early reproduction, at the risk of not mating if few males are nearby. Their reduced mobility may limit their ability to be choosy, and because invest in reproduction instead of investing in expensive flight muscles, male-provided direct benefits may have less of an effect on their reproductive success. Therefore, these females may benefit less from being choosy, which includes higher responsiveness to an average song type and weaker choosiness for high chirp rate songs. Thus, flightless females may adopt a low cost, high risk reproductive strategy in which being choosy is not beneficial. Because there is temporal variability in density of available mates both within and between seasons (Mitra personal observation), adopting different tactics may result in higher fitness at different times. Therefore, hypothetically, environmental variability may result in the maintenance of this polyphenism and alternative reproductive tactics in this species.

Lastly, the existence of the SW_P morph provides us with the unique ability to examine the tradeoff between traits related to flight and traits related to reproduction, because it allows us to separate the effects of wing morph from the effects of flight muscle status. However, this makes the predictions for the SW_P morph less clear: should they behave like the flight capable LW_P morph because they also have developed flight muscles, or should they behave like the flightless SW_W and LW_W morphs because they too are likely flightless. If the production of this morph is a consequence of developmental constraints (i.e., a maladaptive by-product of phenotypic plasticity; Dewitt et al. 1998), SW_P females may behave like the flight capable LW_P females, paying a cost

in terms of lower early reproduction for having developed flight muscles, while gaining no apparent benefit in terms of increased mobility.

METHODS

General Methods

Individuals used for the laboratory studies were reared from field-caught crickets collected at Sedgwick Reserve, Santa Ynez Valley, California. We captured adult females (presumed to have mated in the field) during the summers of 2006-2008, and transported them to University of Nebraska–Lincoln. Subsequent matings set up in the laboratory were designed to minimize inbreeding, and genealogies of all lab bred animals were known (see Wagner and Basolo 2007 for details).

We reared crickets in plastic containers (38 x 24 x 15 cm). During the penultimate stadium (second to last stage before molting as an adult), we moved the crickets individually from the rearing containers to smaller containers (17 x 10 x 11 cm). All containers contained a paper towel substrate, cardboard egg crate shelters, *ad libitum* cat chow and a water vial plugged with cotton, and were maintained at approximately 23°C in a reversed light dark cycle (L:D - 16:8 h). We monitored the individual containers daily and recorded the day when the crickets molted into adults. We kept all individual containers holding females acoustically isolated from any mature males to prevent experience with male song from affecting the responsiveness or female choosiness trials (e.g., Wagner et al. 2001b; Beckers and Wagner 2011). Females used in trials were within the age range of 7 - 15 days post final molt.

SW_P : A fourth morph

As this unexpected morph has not been described in any detail in the past, we wanted to examine its prevalence in the lab population. To do this we monitored lab crickets to assess the proportion of nymphs which emerged as SW_P adults. We followed 150 nymphs daily until they completed their final molt into adults. The day after this molt, we determined wing morph of each individual by comparing the length of the hindwings to the forewings; individuals were marked as LW if the hindwings were longer than the forewings, and SW if the hindwings were shorter than the forewings. We determined flight muscle morph by lifting a hind leg and recording the color of the muscle patch behind the thin membrane between the body and the leg. On crickets with developed, pink flight muscles, the patches are pink in color. On crickets with undeveloped or histolyzed flight muscles, the patches are either not visible or are a bright white in color. We tested this method of determining flight muscle morph by cold anesthetizing and dissecting animals we had previously classified as pink or white, and found that muscle morph identification via patch color was accurate 100% of the time (Mitra personal observation). Of the 150 nymphs monitored, 101 emerged with short wings on the day after their final molt into adults. Of these 150 animals, 24 (16%) were found to have developed, pink flight muscles.

Next, in order to determine whether SW_P individuals, like LW_P individuals, histolyze their flight muscles with age, we monitored the flight muscle morph of 21 LW_P and 15 SW_P individuals from the day after they molted into adults until the muscle patches between their bodies and hind legs were white in color. There was no significant

difference in average age of histolysis between the two wing morphs (LW_P : 5.2 ± 0.77 (mean \pm SE), $n = 21$; SW_P : 5.9 ± 1.21 , $n = 15$; Mann Whitney $U = 149.5$, tied $P = 0.796$).

Experiment 1: Morph specific differences in ovary size

To examine differences in ovary size between females of the different morphs, we cold anesthetized and dissected 178 females (51 LW_P , 49 LW_W , 23 SW_P and 55 SW_W). After dissecting a female, we removed and weighed her ovaries to the nearest 0.1 mg, and noted her flight muscle color, wing morph and age. Although this assay has been conducted in flight-polyphenic crickets in the past (e.g., Zera et al. 1997; Zera and Cisper 2001), this is the first study to compare SW_P females to the other three morphs.

Experiment 2: Morph specific differences in female responsiveness to male song

We examined variation in female responsiveness to male song by measuring the amount of time a female spent around a speaker broadcasting male calling song.

Females were tested with a synthetic male calling song with an intermediate chirp rate (3.0 chirps/s) and an intermediate chirp duration (120 ms; after Wagner and Reiser 2000). To create the song stimulus, we selected a single pulse from a natural chirp (pulse duration = 11 ms, dominant frequency = 5.17 kHz), and copied this eight times to create a single chirp with eight pulses (the interpulse interval was kept constant at 4 ms; see Wagner and Basolo 2007).

We tested females in a 2.2 x 2.2 x 2.7 m chamber, equipped with dim red lights and lined with acoustic foam to reduce echoes (see Wagner and Basolo 2007). We observed crickets during tests via a Panasonic WV - BP100 video camera mounted on the

ceiling of the chamber and monitored using a Panasonic CT - 1384Y monitor outside the chamber. A KLH 970 speaker was placed at one corner of the room, 0.31 m from any wall. We drew a circle around this speaker, with an arbitrary radius of 0.26 m, leaving a minimum gap of 5 cm between the wall and the edge of the circle. The area within this circle around the speaker was 4.4 % of the area of the room. The artificial calling song stimulus was broadcast from this speaker using SoundEdit 16 version 2, a Macintosh Quadra 840 AV computer, and an Optimus SA - 155 amplifier. The song was presented at 75 dB SPL (re: 20 μ Pa at 30 cm from the speaker). We calibrated the sound pressure level of the broadcast using a Casella CEL - 254 Digital Impulse Sound Level Meter (impulse RMS) prior to each trial. Room temperature was maintained at approximately 24°C.

We tested a total of 395 females: 125 LW_P, 84 LW_W, 51 SW_P and 135 SW_W. Before each trial, we recorded the morph and age of the test female. To start the trial, we placed the test female under a cup at the center of the arena and started broadcasting the calling song stimulus. After a 10 min acclimation period, we lifted the cup and monitored the female for a further 10 min, measuring the total time the female spent in the circle around the speaker or on the speaker. As there was a minimum distance of 5 cm between the circle and the wall, a female following the wall around the chamber would never enter the circle.

Female responsiveness was measured in two ways: (1) whether or not the female approached the male song (entered the circle around the speaker), and (2) the time a female spent searching for the simulated male (the time spent in the circle around the speaker or on the speaker). We used a Fisher's exact probability test to measure

differences between morphs in the probability of entering the circle around the speaker, and nonparametric tests to examine differences in amount of time spent around the speaker as these data were not normally distributed.

Experiment 3: Morph specific differences in female chirp rate choosiness

We used a two-speaker choice test design to test for female choosiness (see Wagner 1996). Females were tested with synthetic male calling songs that varied in chirp rate. We chose to test female choosiness for this song character because previous research has shown that females choose high chirp rates over lower ones (Wagner 1996; Wagner and Reiser 2000), and that females on a low nutrition diet receive fecundity benefits from mating with higher chirp rate males (Wagner and Harper 2003; Tolle and Wagner 2011).

Three calling song stimuli with different chirp rates were used: (1) low (1.8 chirps/s), (2) intermediate (3.0 chirps/second), and (3) high (4.2 chirps/second). We ran two sets of choice tests: (1) high chirp rate vs. intermediate chirp rate, and (2) intermediate chirp rate vs. low chirp rate. We created the stimuli as described in the responsiveness trials. To create the three stimuli varying in chirp rate, we only varied the interchirp intervals, thus ensuring that other properties of the song, such as pulse duration, pulses per chirp, chirp duration and dominant frequency, remained identical across the three stimuli.

We tested females in the chamber described in Experiment 2. Here, however, the two song stimuli were broadcast simultaneously from two KLH 970 speakers placed at opposite corners of the room (0.31 m from any wall). Each song was presented at 75 dB SPL (re: 20 μ Pa at 30 cm from the speaker). All other setup details were identical to that

described in Experiment 2. Which speaker broadcast the higher chirp rate song was alternated between trials.

Before each trial, we recorded the morph and age of the test female. During the trial, a test female was acclimated under a cup in the center of the chamber for 10 min, with the speakers broadcasting the stimuli from the two speakers. After acclimation, the cup was removed and the female was allowed 10 min to make a decision (physically touch a speaker). Females were only tested once.

We tested a total of 243 females in these trials, 101 of which did not touch a speaker within the allotted 10 min and were excluded from further analyses. Of the 142 females who did touch a speaker, 40 were LW_P, 41 were LW_W, 18 were SW_P and 43 were SW_W.

We used Stata/IC 10.0 for Macintosh to conduct a logistic regression to compare the probability of a female choosing the stimulus with a higher chirp rate. Our independent variables were test type (high vs. intermediate, or intermediate vs. low), wing morph and muscle morph. In this design, a significant effect of morph would suggest that different morphs differ in the strength of their choosiness, or have different probabilities of choosing a higher chirp rate stimulus. In turn, a significant effect of test would suggest that females discriminate more strongly between one pair of stimuli than between the other pair. Finally, a significant interaction between test and morph would suggest that different morphs have differently shaped choice functions (e.g., one morph might strongly discriminate between high and intermediate, whereas the other morph might most strongly discriminate between intermediate and low).

RESULTS

Wing morph (LW vs. SW) had no significant effects on ovary size, female responsiveness, or female choosiness, and no significant interactions with flight muscle morph. Thus, we have not included it as a factor in any of the analyses below.

Experiment 1: Morph specific differences in ovary size

We used ANCOVA to examine the effect of flight muscle morph and age on ovary mass. We square root transformed the ovary mass data, as they were positively skewed. White muscled females had significantly larger ovaries than pink muscled females ($F = 87.1, P < 0.001$; Figure 1.1). In addition, older females had larger ovaries ($F = 15.82, P < 0.001$). There was not a significant difference in ages of the pink and white muscled females used (Pink = 11.59 ± 0.24 days, White = 11.82 ± 0.20 days, tied $Z = -0.74, P = 0.458$)

Experiment 2: Morph specific differences in female responsiveness to male song

First, we examined the effect of flight muscle morph on the probability that a female responded to a male song with an intermediate chirp rate and chirp duration. As female age did not significantly affect their responses, age was not included in the analysis. White muscled females were significantly more likely to approach the speaker than pink muscled females ($P = 0.014$; Figure 1.2a).

Second, we examined the effect of flight muscle morph on the strength of female responses to male song. Females who never entered the circle were scored as zeros. White muscled females spent significantly more time in the circle around the speaker

than pink muscled females (Mann-Whitney U test: tied $Z = -2.93$, $P = 0.003$, Figure 1.2b).

Experiment 3: Morph specific differences in female chirp rate choosiness

We examined the effect of flight muscle morph on female choices between low and intermediate chirp rate song and on female choices between intermediate and high chirp rate song. A logistic regression was then used to examine the effect of test type and flight muscle morph on the probability that a female would choose the higher chirp rate. As female age did not affect the probability that a female would choose the higher chirp rate, age was not included in the analysis. There was a significant effect of the interaction between test type and flight muscle morph on the probability of a female choosing the higher chirp rate ($Z = 2.32$, $P = 0.021$; Figure 1.3), indicating that females of the two flight muscle morphs have differently shaped choice functions. White muscled females discriminated more strongly between low and intermediate chirp rates than between the intermediate and high chirp rates. In contrast, pink muscled females discriminated more strongly between intermediate and high chirp rates than between low and intermediate chirp rates. There was no effect of muscle morph on the probability of a female not touching either speaker (i.e., being removed from the analyses; $Z = 0.88$, $P = 0.378$).

DISCUSSION

Wing length vs. flight muscle morph

In *G. lineaticeps*, individuals can be short or long winged and have functional pink or non-functional white flight muscles. We found no effects of wing morph on any

female trait measured. In contrast, we found that white muscled females had larger ovaries, were more responsive to male song, but expressed weaker choosiness for high chirp rates than pink muscled females. Wing morph expression in crickets is a polyphenism affected by both genes and rearing environment, and is likely determined by an environmentally sensitive switch (Zera and Denno 1997; Guerra 2011; Zera and Brisson in press). In several species, the sensitive development period for this switch occurs as late as the last stadium (reviewed in Zera and Denno 1997), and remains fixed in adults. Flight muscle morph is also determined in the nymphal stage. However, unlike wing morph, the flight muscle morph of some adults can change: pink flight muscles can be histolyzed to white (Zera and Denno 1997). The construction and maintenance of flight muscles is energetically expensive, and their maintenance costs comprise a significant proportion of the animal's total energy budget (Zera and Denno 1997). This is because they are not only larger in size, but also have significantly higher respiration rates than white flight muscle (Zera and Denno 1997; Zera et al. 1997; Crnokrak and Roff 2002). Therefore, our finding that flight muscle morph has a greater effect on reproductive traits than wing morph is not surprising, and is consistent with past studies of other field crickets (first emphasized by Zera et al. 1997, and subsequently found by Crnokrak and Roff 1998; Guerra and Pollack 2007; Mitra et al. 2011).

Differential investment in early reproduction

We found that females with white flight muscles had significantly larger ovaries than females with pink flight muscles (Figure 1.1), a finding consistent with previous studies (Zera et al. 1997; Stirling et al. 2001). As ovary size and egg number are highly

correlated (Roff 1994; Zera et al. 1997), ovary size predicts female fecundity. This observed tradeoff between investment in flight muscles and ovaries is possibly caused by competition between the two traits for limited energy resources within individuals (Zera and Denno 1997; Zera and Harshman 2001). Such tradeoffs between competing life-history traits or reproductive traits have been found in several systems (e.g., Harrison 1980; Basolo 1998; Griffith and Sheldon 2001; Wagner et al. in review). In addition, it appears that SW_P are paying similar costs in terms of investment in early reproduction as LW_P, due to the high energetic costs of constructing and maintaining flight muscles.

Differential costs and benefits of mate finding and choosiness

In *G. lineaticeps*, females use male calling song to locate and assess males, and females select males based on variation in song characteristics such as chirp rate (Wagner 1996; Wagner and Basolo 2007). Females mating with males that produce high chirp rate songs receive fertility and fecundity benefits, particularly in low nutrition environments (Wagner and Harper 2003; Tolle and Wagner 2011). In the above experiments, we found that white muscled females are more responsive to an average male song type than pink muscled females: they are significantly more likely to approach a speaker playing the song, and they spend significantly more time in the vicinity of the song (Figure 1.2a and 1.2b). In addition, we found that although females of both flight muscle morphs choose higher chirp rate songs, they have differently shaped choice functions for male calling song chirp rate (Figure 1.3). While white muscled females strongly discriminated against low chirp rate calls, they did not discriminate as strongly between intermediate and high chirp rate calls. In contrast, pink muscled females strongly preferred high chirp rates to

intermediate chirp rates, but did not discriminate as strongly between low and intermediate chirp rate calls. This interaction suggests that while both morphs reject low chirp rate songs, white muscled females are more accepting of average chirp rate songs than are pink muscled females. These differences in responsiveness and choosiness to song can potentially be explained by morph specific costs and benefits of mate finding and mate choice.

First, the lower mobility of white muscled females may result in higher costs of bypassing a nearby male: this may cause them to delay mating, increase their probability of not mating, or increase their risk of being killed by terrestrial predators while searching for a different male. For example, as flightless females have larger ovaries, they may have to pay increased costs of carrying a large egg load. Females with large egg loads in many species incur increased energetic and predation costs (e.g., Seigel et al. 1987; Kullberg et al. 2005; Johnson et al. 2010). In addition, older unmated *G. lineaticeps* females dump unfertilized eggs (Wagner personal observation), which may partly be due to egg viability decreasing with time (e.g., Unnithan and Paye 1991; Proshold 1996; Moore et al. 2007). Therefore, flightless females may be more responsive to average male song due to morph-specific differential costs of delaying mating or mate searching. In addition, flightless females may incur high costs of being very choosy because their limited mobility reduces the pool of available mates.

Second, as white muscled females devote substantial resources to early reproduction and do not have to invest in expensive flight muscles, male-provided fecundity benefits may have little effect on the fitness of these females (just as they have little effect on the fitness of females that have experienced good nutritional environments;

Wagner and Harper 2003). Therefore, the benefit of being very choosy may be small. In contrast, being very choosy may confer higher benefits to flight capable females. As their initial investment in reproduction is lower, male-provided fecundity benefits may have a large effect on the fitness of these females (just as they have an effect on the fitness of females that have experienced poor nutritional environments; Wagner and Harper 2003; Tolle and Wagner 2011). Therefore, flight capable females may have to be more choosy than flightless females due to morph-specific differential benefits of receiving male-provided direct benefits.

The anomalous SW_P morph

In the above experiments, we found that short-winged females with pink flight muscles (SW_P) resemble long-winged females with pink flight muscles (LW_P) more than they resemble short-winged females with white flight muscles (SW_W). Females with pink flight muscles, regardless of their wing type, had smaller ovaries, were less responsive to average male song, and were more choosy than females with white flight muscles. Therefore, both pink muscled morphs appear to pay a reproductive cost for their increased investment in flight muscles, but only those with long wings receive the mobility benefit. This supports our hypothesis that this morph may be a result of developmental constraints, and a possible cost of phenotypic plasticity (Dewitt et al. 1998; Auld et al. 2010).

One important mechanism for dealing with environmental variation is phenotypic plasticity, as any organism that can adjust its phenotype to environmental conditions will have the advantage of minimizing the cost of expressing a suboptimal phenotype for the

environment (e.g., Lewontin 1957; Schlichting and Pigliucci 1998). Past work has established that plasticity can be adaptive and may be selected for in natural populations (e.g., Fischer et al. 2004; Huber et al. 2004; Lyytinen et al. 2004). Recently, costs of plasticity have been explored as explanations for the maintenance of genetic variation in plasticity, for reducing the degree of plasticity that evolves, or for allowing the coexistence of both fixed/canalized and plastic morphs (Lively 1986; Van Tienderen 1991; DeWitt et al. 1998; Auld et al. 2010). One type of cost may be that of phenotype-environment mismatching (Auld et al. 2010).

In field crickets, if wing and flight muscle morph are determined by switches cued by different environmental conditions, or by environmental conditions during different developmental stages (Zera and Tanaka 1996), some individuals may end up with low fitness wing and flight muscle morph combinations for their environment. In poor environments, individuals would benefit from greater mobility and should invest in flight capability, developing both long wings and developed flight muscles. In good environments, individuals would benefit from starting to reproduce as early as possible, and should develop short wings and not develop their flight muscles. When both flight muscles and wing morph are cued by the same environmental conditions, an individual improperly assessing that cue may produce a lower fitness phenotype for its environment (e.g., SW_W in a poor environment, or LW_P in good environment), but it will not produce very low fitness mismatched trait combinations. In contrast, if the flight muscles and wing morph are cued by different environmental conditions, an individual correctly assessing both opposing cues of environmental quality may produce a mismatched trait combination of very low fitness. For example, if low adult density cues flight muscle

development and an individual develops functional pink flight muscles, but high food quality cues wing morph development and the same individual develops short wings, the cricket in question will mature as a SW_P in an environment with very few available mates. Similarly, if the flight muscles and wing morph are cued by the same environmental condition at different developmental stages (Zera and Tanaka 1996), if the environment changes between when wing morph was determined and when muscle morph was determined, an individual correctly assessing both the cues at both times may produce a mismatched trait combination of very low fitness. For example, if low adult density early in development cues flight muscle development, an individual may develop functional pink flight muscles. However, if later in development adult density increases, perhaps as the mating season progresses, and cues wing development, and the same individual may develop short wing. The cricket in question will therefore mature as a SW_P in a good environment and will have delayed reproduction with no perceivable benefits. If the production of the SW_P morph is the result of a developmental constraint, it would be an extreme type of cost of phenotype-environment mismatching (Auld et al. 2010), in which plastic organisms could produce very low fitness phenotypes not likely produced by canalized organisms. This hypothesis would receive further support if more plastic families produce a higher proportion of such morphs.

If the production of SW_P is the result of a developmental constraints, the question arises, why do these females behave like LW_P females? There are two possible explanations. The first explanation is that these females express maladaptive behavior. A virgin female with limited mobility should probably not bypass a male with an intermediate song type, particularly in a species with extensive female multiple mating.

The second explanation is that being choosy is adaptive. When two males are nearby, and one produces a high chirp rate song, SW_P females might strongly benefit from going to the higher quality male because male-provided fecundity benefits will have a large effect on their fitness.

Alternative reproductive strategies and morph specific reproductive behavior in females

The results of these three experiments support our hypothesis that pink and white muscled females adopt alternative reproductive tactics. We had predicted that flight capable females (LW_P) would be less responsive to an average male song type, and would be more choosy, in addition to having smaller ovaries, in comparison to flightless females (SW_W and LW_W). These predictions were based upon flight capable females adopting a high cost, low risk reproductive strategy in which being choosy is beneficial, and flightless females adopting a low cost, high risk reproductive strategy in which being choosy is not beneficial. We had hypothesized that flight capable females trade off investment in early reproduction for greater mobility, allowing them to move to areas with more males and find high quality mates. Due to the high costs of maintaining developed flight muscles, they would have smaller ovaries. In addition, as they do not invest in early reproduction, male-provided direct benefits would have a significant effect on their fitness. Therefore, they would be less responsive to average male songs, while strongly preferring high quality males. In contrast, flightless females would tradeoff lower mobility for enhanced early reproduction. As they do not pay the costs of maintaining developed flight muscles, they would have larger ovaries and be less affected

by male-provided direct benefits. And as their reduced mobility would place them at risk of not mating if few males are nearby, they would be more responsive to average male songs, and be less choosy with regard to chirp rate. As density of available mates is temporally variable, the relative fitness of each tactic may be temporally variable.

Our results highlight the importance of examining variation in behavior among females. Such variation is important because it can affect the strength of sexual selection on male traits, and help explain the maintenance of heritable variation in female reproductive behaviors (Jennions and Petrie 1997; Widemo and Sæther 1999; Cotton et al. 2006). As proportions of flight muscle morphs within a population can change both temporally and spatially, with changing environmental conditions, our results suggest that patterns of sexual selection on males also may change. For example, in a population with more white muscled individuals, selection on males to produce high chirp rate songs may be weaker than in a population with a larger proportion of pink muscled females. Secondly, as the costs of not mating may be higher in some female morphs than others, being more choosy may have a higher cost for some female phenotypes. Lastly, as benefits gained by female choice may also be morph specific, being more choosy may be more beneficial for some female phenotypes. A recent examination of correlated evolution across 32 species of fireflies found significant correlations between female neoteny and male accessory gland number, as well as between female flightlessness and loss of male spermatophores (South et al. 2011). The authors suggested that these results may be explained by spermatophores having greater benefits (i.e., causing proportionally higher fecundity increases) in species with flight capable versus flightless females (South

et al. 2011). Our results suggest that flight capable versus flightless morphs within the same species may likewise gain differential benefits from male spermatophores.

Alternative reproductive strategies and tactics have long been thought to be exclusively male behaviors (Andersson 1994), seen in females only in rare polyandrous breeding systems (Shuster and Wade 2003). However, as seen in this study, female alternative mating tactics do occur in other mating systems. These tactics would evolve not because competition for mates is intense, as is the case for most male tactics (Shuster and Wade 2003), but because of variation in habitat quality. Females in poor quality habitats, such as habitats that contain few males, could gain higher fitness by delaying reproduction in favor of greater mobility. These females may have more opportunities to be choosy because greater mobility would allow them to encounter more males, and may benefit more from being choosy because they would have devoted fewer of their own resources to reproduction. Since females of most species vary in their relative investment in early reproduction and in mobility, these results are just as relevant for non-polymorphic/polyphenic animals. With further examination, we might expect to see similar conditional alternative reproductive tactics, based on external conditions, in either sex of many other species.

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FIGURE LEGENDS

Figure 1.1

Variation between females of different flight muscle morphs in ovary mass: females with white flight muscles have significantly larger ovaries than females with pink flight muscles. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 1.2

Variation between females of different flight muscle morphs in responsiveness to male song. (a) Proportion of tested females of each flight muscle morph who entered the circle around the speaker playing male calling song: females with white flight muscles (N = 182 of 219) were significantly more likely to approach the speaker than females with pink flight muscles (N = 128 of 176). (b) Boxplot of time spent by females of each flight muscle morph within the circle around the speaker playing male calling song: females with white flight muscles spent significantly longer near the speaker than females with pink flight muscles. Different letters designate statistically significant differences ($P \leq 0.05$).

Figure 1.3

Variation among females of different flight muscle morphs in choosiness based on chirp rate of male calling song. This illustrates the results of a two-speaker choice test in which the alternative calls differed in chirp rate: females were given a choice between either (1)

low and intermediate chirp rate songs, or (2) intermediate and high chirp rate songs. The y-axis shows the proportion of females who chose the higher chirp rate song over the lower chirp rate song in both sets of tests. Results for the two flight muscle morphs are plotted separately: dark circles represent pink muscled females, and open circles represent white muscled females. There is a significant interaction between test type and flight muscle morph. White muscled females more strongly prefer average chirp rate calls when presented with stimuli of low and average chirp rates, than do pink muscled females. In contrast, pink muscled females more strongly prefer high chirp rate calls when presented with stimuli of average and high chirp rates, than do white muscled females.

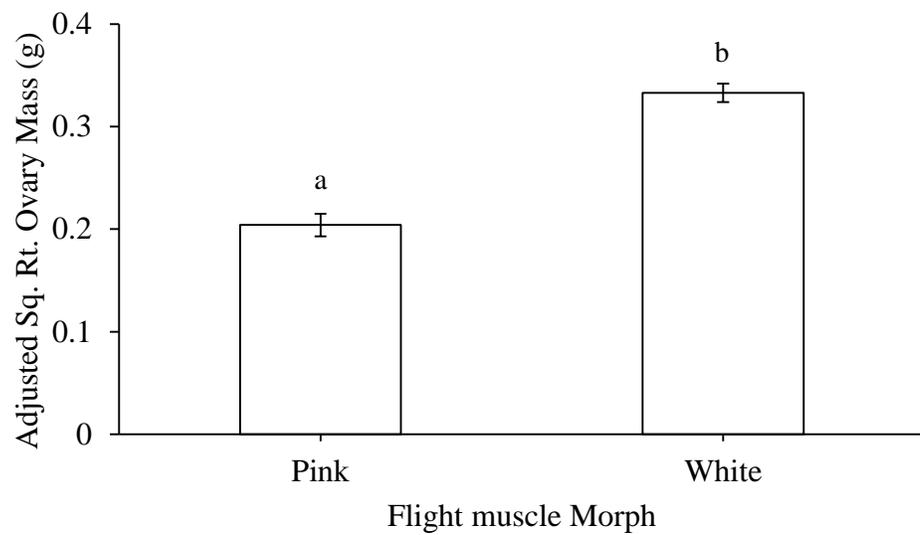
Figure 1.1

Figure 1.2

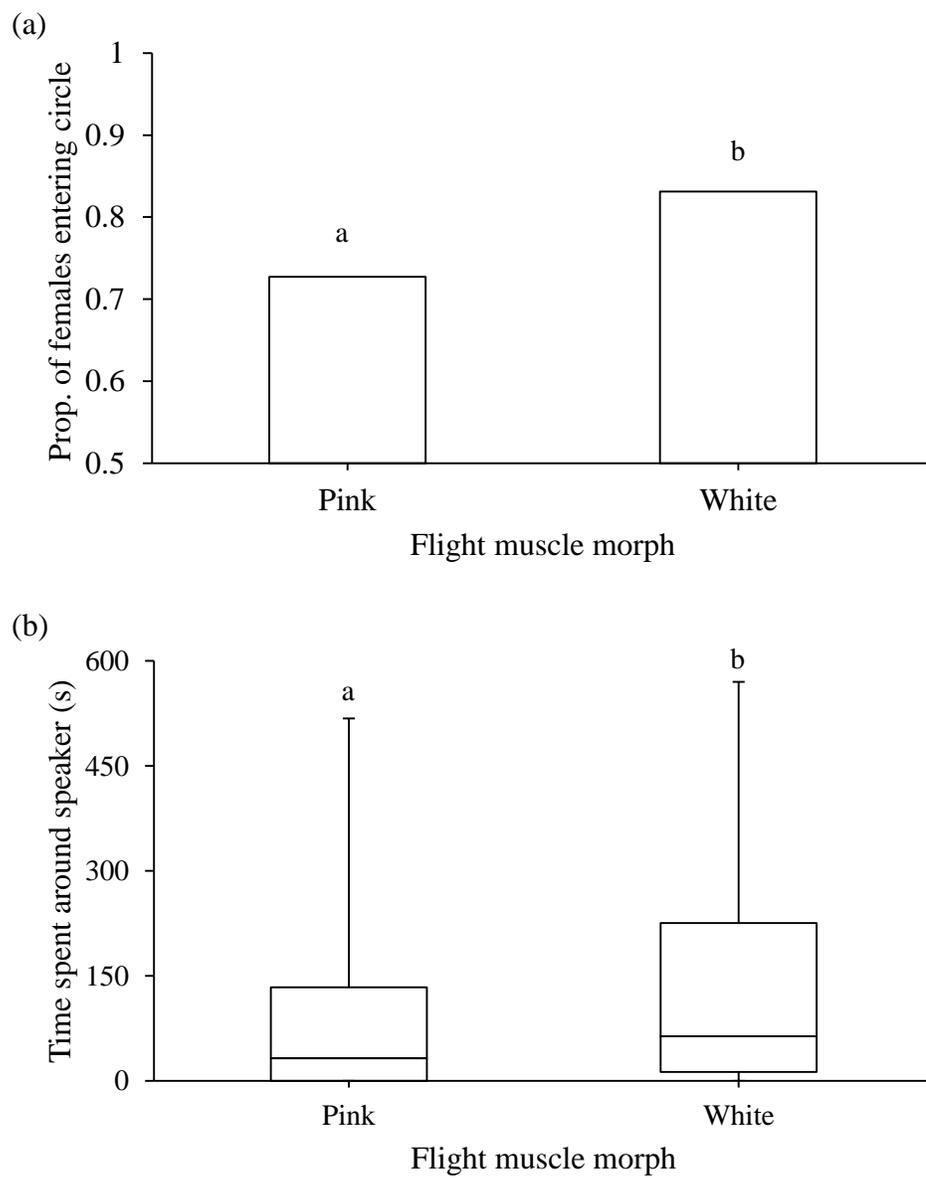
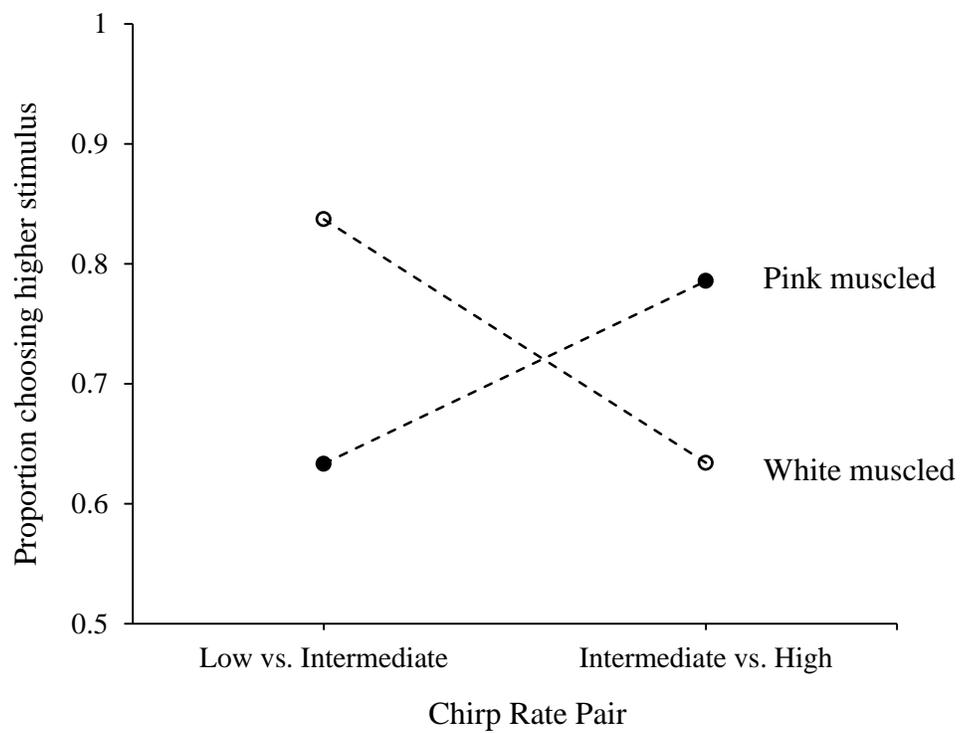


Figure 1.3



CHAPTER TWO: The problem of trait correlations: costs, benefits and constraints on life history traits in a flight polyphenic field cricket

ABSTRACT

Life history tradeoffs can occur when traits affecting survival or reproduction are negatively correlated with each other due to competition for limited resources within a single individual, and are common. When fitness benefits of investing in one trait over another change between environments, differential resource allocation to traits should be environment dependent, or phenotypically plastic. While the evolution and physiology of such resource allocation tradeoffs have been extensively studied, factors that constrain allocation patterns have been explored to a lesser degree. Flight polyphenic crickets are a classic example of such a life history tradeoff between investment in reproduction and flight capability. We used the flight polyphenic field cricket, *Gryllus lineaticeps*, to examine costs, benefits, and constraints on the environment-specific expression of life history traits. In this species, the polyphenic morphs differ in wing morphology (short or long) and flight muscle morphology (functional pink or non-functional white). We have previously shown that females who do not invest in flight muscles gain reproductive benefits. In this study we asked whether, (1) morphs with developed flight muscles can fly, and thereby perhaps gaining dispersal benefits, and (2) flight muscle development or breakdown have correlated effects of other life history traits, specifically jumping capability. We found that only individuals with both long wings and developed flight muscles flew. Secondly, we found that individuals who emerge as adults with developed flight muscles have larger hind leg femoral muscles and can jump further than individuals

who emerge with undeveloped flight muscles. In addition, we found that individuals that breakdown their flight muscles as adults, thereby reallocating resources to reproduction, decrease their hind leg femoral muscles, and probably their jumping ability. These results suggest that flight muscle development and breakdown have correlated effects on other traits such as jumping ability, a trait used to escape predators, and therefore likely to have survival consequences. How resources are allocated between flight capability and reproduction may therefore be constrained from tracking environmental shifts, due to selection for/against correlated traits.

Key words. Phenotypic plasticity, polyphenism, wing polymorphism, flight muscle histolysis, locomotion.

INTRODUCTION

Most organisms are resource limited. When two traits share a common resource pool, increasing the resources allocated to one trait can result in decreases to the other (Van Noordwijk and De Jong, 1986). Traits within an individual may thus be negatively correlated with each other (Zera and Harshman, 2001). Life history traits are defined as traits that directly affect survival or reproduction (Ricklefs and Wikelski, 2002). Costs and benefits of investing in one life history trait versus another in different environments, and the physiology underlying these tradeoffs, have been extensively studied over many decades and in many systems (reviewed in Partridge and Harvey, 1988; Stearns, 1976; 1989; Zera and Harshman, 2001). Although it is well established that the physiological mechanisms underlying these tradeoffs are complex (Zera and Harshman, 2001), whether

life history traits are constrained by anything other than direct resource limitation have been less explored (Ricklefs and Wikelski, 2002). Such constraints could include physiological or behavioural control mechanisms affecting multiple life history traits, which in turn could lead to individuals appearing to have non-optimal phenotypes for their environment. For example, if within a species there is a tradeoff increasing body size and investing in current reproduction, there may be some environments in which larger body size increases lifetime fitness more than increasing current reproduction, and vice versa. However, if the mechanism controlling increased investment in current reproduction has a correlated effect on increasing immunological function, selection on immunity may constrain the allocation pattern between growth and current reproduction from optimally tracking changes in the environment. Therefore, we may observe individuals, apparently sub-optimally, investing in reproduction in environments where investment in growth would yield higher fitness benefits. We examined the existence of such constraints in a flight polyphenic insect that trades off investment in flight and reproduction.

Polyphenisms are a type of phenotypic plasticity in which a single genotype produces multiple, discrete phenotypes in response to environmental variation (Schlichting and Pigliucci, 1998). Many insects are flight polyphenic: some individuals within a given population can fly while others are incapable of flight (reviewed in Zera and Denno, 1997). These discrete morphs commonly differ in wing and flight muscle morphology (Denno, 1994; Harrison, 1980; Roff, 1986). Such polyphenisms likely evolve because different morphs are favoured under different environmental conditions (Denno et al., 1996; Denno, 1994; Roff, 1990), and morph production is affected by

environmental variation (Denno, 1994; Denno et al., 1991). Flight polyphenisms in field crickets have been used as a model system for studies of life history tradeoffs (reviewed by Harrison, 1980; Roff, 1990; Zera, 2009). These insects show plasticity in two traits associated with flight capability: wing length and flight muscle morphology. First, individuals may mature with long wings (LW) or short wings (SW). Second, individuals may vary in flight muscle morphology, which is plastic at two stages. They may mature with functional pink (P) or non-functional white (W) flight muscles. In addition, after maturation, those that have functional flight muscles may breakdown (histolyze) their flight muscles and become flightless; during histolysis the flight muscles turn from pink (P) to white (W) in colour (e.g., Roff and Gelinas, 2003; Zera et al., 1997). Functional flight muscles are energetically expensive to produce and maintain, and as a result, individuals with functional flight muscles invest less in reproduction (Roff and Fairbairn, 1991; Zera et al., 1994). Wing and flight muscle development are affected by both genetic and environmental factors, and are most likely determined by environmentally sensitive switches during development (Zera and Denno, 1997; Zera and Tanaka, 1996).

We examined costs, benefits and constraints of investing in different life history traits in the variable field cricket, *Gryllus lineaticeps*. In this species, there are four adult flight morphs that vary in wing length and flight muscle status: long winged pink (LW_P), long winged white (LW_W), short winged pink (SW_P) and short winged white (SW_W; Chapter 1). The SW_P morph has been found at relatively high frequencies in laboratory populations (Chapter 1) and several field populations (Mitra, personal observation), but its existence is paradoxical. Like LW_P females, SW_P females have smaller ovaries than LW_W and SW_W females (Chapter 1). Therefore, SW_P females pay a reproductive cost for

flight muscles that they may not be able to use for flight. One potential explanation for this morph is that it results from developmental constraints associated with phenotypic plasticity. If wing and flight muscle development are determined by switches cued by different environmental conditions, or by environmental conditions during sensitive periods in different developmental stages, mismatched environmental cues, or mistakes in environmental assessment might result in mismatched phenotypes (Zera and Tanaka, 1996, Chapter 1). However, adaptive explanations for the existence of this odd morph need to be further explored.

In the current study, we examined possible costs, benefits and constraints on investment in flight apparatus versus investment in reproduction. We have previously found that individuals with undeveloped flight muscles invest more in reproduction in comparison to individuals with developed flight muscles (females: Chapter 1; males: Chapter 3). Here we tested whether individuals with developed flight muscles can fly, hypothetically attaining increased dispersal benefits in comparison to individuals with undeveloped flight muscles. We tested this by examining whether individuals of different wing and flight muscle combinations can fly. We hypothesized that neither wing morph with white muscles (SW_W and LW_W) would be able to fly, since they lack the necessary musculature. We also hypothesized that individuals with short wings and developed flight muscles (SW_P) would not be able to fly and thereby gain dispersal benefits, since they lack the necessary wing surface area.

If SW_P individuals gain neither reproductive benefits nor flight capability for their developed flight muscles, there may be other benefits related to having developed flight muscles that are unrelated to flight capability. Such benefits may help explain the

existence of this morph, and may exist if the mechanisms controlling muscle development are not completely localizable—if, for example, flight muscle development during the nymphal stages is correlated with the development of other muscles in the body. We focused on jumping ability because one of the major ways that many saltatory Orthoptera escape predators is via jumping using their enlarged hind legs and femoral muscles (e.g., Bennet-Clark, 1975; Burrows and Morris, 2003). We examined (1) average jumping distance, and (2) hind leg femoral muscle mass of individuals of different wing and flight muscle morphs, hypothesizing that if flight muscle development is correlated with the development of other muscles, individuals with developed, pink flight muscles will be able to jump further and will have heavier hind leg femoral muscles than individuals with undeveloped, white flight muscles. Furthermore, if the mechanisms controlling muscle development are not completely localizable, the mechanisms controlling muscle breakdown or histolysis may likewise not be completely localizable. To test this we examined whether hind leg femoral muscle mass is correlated with flight muscle histolysis in adults. We hypothesized that if flight muscle histolysis has correlated effects on other muscles, individuals who emerge with developed, pink flight muscles which are histolyzed with age will have smaller hind leg femoral muscles than individuals who emerge with developed, pink flight muscles which are not histolyzed with age.

If leg muscle mass and jumping ability are correlated with flight muscle development and histolysis, the evolution of these life history traits may be affected not only by the fitness tradeoffs between flight capability and reproduction, but also by the fitness effects of other correlated traits.

METHODS

General Methods

Test subjects were laboratory-reared descendants of field-caught crickets collected at Sedgwick Reserve, Santa Ynez Valley, California, during the summers of 2006-2008 (see Chapter 1 for rearing methods).

Crickets were used in trials when they were 6 - 12 days post final moult. Every tested cricket had their mass, sex, age, wing and morph recorded. We determined the wing morph of test crickets by comparing the length of the hindwings to the forewings; individuals were scored as LW if the hindwings were longer than the forewings, and SW if the hindwings were shorter than the forewings. We determined flight muscle morph by lifting a hind leg and recording the colour of the muscle patch behind the thin membrane between the body and the leg. On crickets with developed, pink flight muscles, the patches are pink in colour. On crickets with undeveloped or histolyzed flight muscles, the patches are either not visible or are a bright white in colour. This is known to be an accurate method for assessing flight muscle status (see Chapter 1).

Experiment 1: Morph specific differences in flight

To test whether each of the morphs can fly, we attached individuals to a wooden skewer using beeswax. We used a retort clamp to hold the skewer in place, and lowered the clamp until the cricket could stand on a smooth plastic container in front of a small table fan. To begin the trial we slowly removed the plastic container from under the test cricket to encourage flight (see Guerra and Pollack, 2009). At this point the animal would either spread its forewings and start moving its hind wings to fly, or it would dangle from

the wooden applicator making no attempt to spread its wings and fly. We should note that this assay does not separate ability to fly from motivation to fly. We tested a total of 64 animals: 19 LW_P (12 females and 7 males), 13 LW_W (8 females and 5 males), 13 SW_P (5 females and 8 males), and 19 SW_W (13 females and 6 males).

Experiment 2: Morph specific differences in jumping distance

To test whether the different morphs differ in their jumping abilities, we measured the jumping distances of individuals. We tested individuals in a 2.2 x 2.2 x 2.7 m room. The floor of the room was covered in brown paper, and divided into a 10 x 10 cm grid drawn with a black marker. During the trials, the room was lit with dim red lights, and the trials were recorded using a Panasonic WV-BP100 video camera mounted on the ceiling of the chamber, attached to a Panasonic CT-1384Y monitor and a Sanyo VWM-668 VCR outside the chamber. The trials were recorded on Maxell Standard Grade T-160 VHS tapes.

We tested 102 individuals: 33 LW_P (4 females and 29 males), 13 LW_W (8 females and 5 males), 23 SW_P (5 females and 18 males) and 33 SW_W (13 females and 20 males). To start the trial, we placed the test cricket under a cup at the centre of the arena for a 10 min acclimation period. After this period, we lifted the cup and lightly touched the cerci of animal with a size zero, soft-bristled paint brush (after Killian et al., 2006), causing them to jump. This process was repeated three times with each animal. Trial tapes were later analysed to calculate distances jumped by each cricket, and average jump distance for each individual was used for analyses. We measured repeatability of jumping distance using the intraclass correlation coefficient (Hayes and Jenkins, 1997). We used

ANCOVA to test effects of wing morph, muscle morph and sex on average jumping distance, using age and body mass as covariates.

Experiment 3: Morph specific differences in mass of hind leg femoral muscles

In order to test whether the morphs differ in the mass of hind leg femoral muscles, and whether flight muscle histolysis affected this mass, we monitored individuals daily and recorded their flight muscle morph on the day the crickets moulted into adults. On the test date, we recorded the age, body mass, wing morph and muscle morph of test individuals. Then, we held the test crickets by one hind leg causing them to autotomize it. Because individuals held by their hind legs readily autotomize them as an escape response (Bateman and Fleming, 2005), we did not need to sacrifice animals in order to measure leg muscle mass. We dissected the femurs of these autotomized hind legs immediately, scraping and weighing all muscle fibres.

First, we used ANCOVA to test effects of wing morph, muscle morph and sex on mass of hind leg femoral muscles, using age and body mass as covariates. Next, to test whether flight muscle histolysis in adults is correlated to leg muscle mass, we used a subset of the data. We examined whether hind leg femoral muscle mass of individuals who moulted into adults with developed, pink muscles, varied between individuals who histolyzed their flight muscles before the test date (pink was histolyzed to white: P-W) and those who maintained developed flight muscles until the test date (pink remained pink: P-P). To test this, we used ANCOVA to test effects of wing morph, muscle morph change (P-P and P-W), and sex on mass of hind leg femoral muscles, using age and body mass as covariates.

RESULTS

Experiment 1: Morph specific differences in flight

While a few of the SW_W, LW_W or SW_P crickets tested were able to horizontally spread their forewings (2 SW_W, 1 LW_W, and 4 SW_P), none moved their hind wings and flew during the flight trials (Table 2.1). In contrast, 12 of the 19 LW_P tested horizontally spread their forewings, flapped their hind wings, and flew for at least 1 minute (Table 2.1; Pearson $\chi^2 = 34.98$, $p < 0.001$). Female and male LW_P were equally likely to fly (Pearson $\chi^2 = 2.10$, $p = 0.147$). Tested crickets of the different morphs did not significantly differ in age (Kruskal-Wallis: $H = 7.08$, tied $P = 0.069$).

Experiment 2: Morph specific differences in jumping distance

Jumping distance measures were highly repeatable within individuals ($r = 0.505$). Individuals with pink flight muscles jumped significantly further than individuals with white flight muscles (ANCOVA: $F = 18.94$, $P < 0.001$; Figure 2.1). However, there was no effect of wing morph (LW vs. SW: $F = 1.63$, $P = 0.205$), sex ($F = 0.54$, $P = 0.463$), the two-way interactions between wing and muscle morph ($F = 0.52$, $P = 0.473$), wing morph and sex ($F < 0.01$, $P = 0.987$), muscle morph and sex ($F = 0.06$, $P = 0.804$), or the three-way interaction between sex, wing and muscle morph ($F = 1.24$, $P = 0.269$) on jumping distance. While body mass was not a significant covariate ($F = 0.77$, $P = 0.384$), age had a significant effect ($F = 10.05$, $P = 0.002$), with average jumping distance decreasing with cricket age. However, there was no significant variation among the morphs in cricket age (Kruskal-Wallis: $H = 1.36$, $P = 0.716$).

Experiment 3: Morph specific differences in mass of hind leg femoral muscles

First, in order to examine whether the differences in jumping ability that we found above could be explained by differences in hind leg femoral mass, we examined the effects of wing morph, muscle morph on test date, and sex on mass of hind leg femoral muscles, using age and body mass as covariates. We found that individuals with pink flight muscles had significantly heavier hind leg femoral muscles than individuals with white flight muscles (ANCOVA: $F = 7.96$, $P = 0.005$; Figure 2.2a). There was no effect of the three-way interaction between sex, wing and muscle morph ($F = 0.03$, $P = 0.858$), and no effect of the two-way interactions between muscle morph and wing morph ($F = 0.02$, $P = 0.900$), and muscle morph and sex ($F = 1.30$, $P = 0.255$) on hind leg femoral muscle mass. There was a significant effect of the interaction between sex and wing morph ($F = 6.35$, $P = 0.012$) on hind leg femoral muscle mass, with short winged females and long winged males having heavier hind leg femoral muscles. Age was not a significant covariate ($F = 0.77$, $P = 0.381$). Not surprisingly, hind leg femoral muscle mass increased with body mass ($F = 250.48$, $P < 0.001$).

Next, in order to examine effects of flight muscle histolysis on hind leg femoral mass, we examined just the individuals who emerged with developed, pink flight muscles. We found an effect of change in muscle morph hind leg femoral muscle mass ($F = 8.42$, $P = 0.004$; Figure 2.2b): individuals that emerged with developed, pink flight muscles which were histolyzed before the test date (P-W) had significantly lower hind leg femoral muscle mass than individuals that emerged with developed, pink flight muscles which were not histolyzed. As in the previous analysis, there was no effect of the three-way interaction between sex, wing morph and change in muscle morph ($F = 0.23$, P

= 0.636), and no effect of the two-way interactions between change in muscle morph and wing morph ($F = 0.01$, $P = 0.915$), and change in muscle morph and sex ($F = 0.83$, $P = 0.364$) on hind leg femoral muscle mass. Once again, while there was a significant effect of the interaction between sex and wing morph ($F = 7.94$, $P = 0.005$) on hind leg femoral muscle mass, with short winged females and long winged males having heavier hind leg femoral muscles. Age was not a significant covariate ($F = 1.01$, $P = 0.316$). Not surprisingly, hind leg femoral muscle mass increased with body mass ($F = 194.48$, $P < 0.001$).

DISCUSSION

Life history tradeoffs are ubiquitous. Most organisms are resource limited at some point of their life cycle, and when traits within a single individual compete for limiting resources, increases of resources to one trait leads to a decrease to others (Van Noordwijk and De Jong, 1986). The most studied tradeoffs involve the costs of reproduction, such as survival or growth vs. current reproduction, current vs. future reproduction, and number of offspring vs. size of offspring (Stearns, 1989; Zera and Harshman, 2001). In flight polyphenic field crickets, individuals differentially allocate resources to reproduction (e.g., ovary tissue and energy spent producing mate attraction signals) or flight capability (e.g., growth and maintenance of flight muscles, and accumulation of flight fuels; Zera, 2009). We examined this tradeoff in the flight polyphenic *G. lineaticeps*, specifically assaying flight ability/motivation of individuals with developed flight muscles, and whether shared control mechanisms between different life history traits can constrain investment in one over another.

Flight capability

Most studies examining the tradeoff between flight capability and reproduction in flight polyphenic crickets have assumed that individuals with either reduced wings or reduced flight muscles are flightless. In order to establish this in our species, we ran tethered flight trials of individuals of all four morphs (LW_P , LW_W , SW_P and SW_W), and found, not surprisingly, that only individuals with long wings and developed, pink flight muscles (LW_P) flew. As expected, no short winged individuals who emerged with non-functional white muscles (SW_W), and no long winged individuals who were tested after histolyzing their flight muscles (LW_W) flew. Likewise, no short winged individuals with developed, pink flight muscles (SW_P) flew. However, 63% of long winged individuals with developed flight muscles (LW_P) tested, flew for at least one minute. Therefore, only individuals of the LW_P morph gain flight capability, a likely dispersal benefit, from investing in developed flight muscles. In the future, we need to assess whether short winged individuals with developed flight muscles invest in lipid flight fuels, like long winged individuals with developed flight muscles. If SW_P do invest in energetically expensive flight fuels, in addition to investing in producing and maintaining the energetically expensive, developed, pink flight muscles, this morph would appear even more paradoxical.

Correlated effects of flight muscle development

Myogenesis in insects is largely controlled by the interplay between juvenile hormone (JH) and ecdysteroids during nymphal stages, and both muscle proliferation and differentiation are affected by the levels of one in comparison to levels of the other

(Gilbert, 2009; Marden, 2000). These hormones are involved in the development of various muscles in insects, ranging from flight muscles (e.g., Zera and Tanaka, 1996), to leg muscles (Hegstrom and Truman, 1996; Luedeman and Levine, 1996), to ovipositor muscles (Rose, 2004; Rose et al., 2001). As the action of these hormones may not be completely localizable, the development of muscles in one part of the body may be correlated with the development of muscles in other parts of the body. In order to examine this hypothesis, we looked at the effect of flight muscle status on jumping ability and hind leg femoral muscle mass. We found that individuals with developed, pink flight muscles jumped significantly further, and had significantly heavier hind leg femoral muscle mass than individuals with undeveloped, white flight muscles. As the major muscles used in jumping are in the hind legs (Bennet-Clark, 1975; Burrows and Morris, 2003), these results suggest that individuals who have developed flight muscles also have more developed leg muscles, which allow them to jump further. Tested individuals never spread their wings when jumping, making a direct effect of flight muscles on jumping distance unlikely. Jumping is one of the major ways saltatory Orthoptera escape predators, and therefore differences in jumping ability may affect the survival of individuals.

If the hormones involved in mediating the tradeoff between flight muscle development and reproduction, in this case likely JH and edysteroids, have correlated effects on other life history traits, in this case leg muscle mass and corresponding jumping ability, resource allocation patterns for flight muscle development and reproduction may be constrained from optimally tracking environmental changes. An individual maturing in an environment conducive to early reproduction from which they

do not need to disperse, may be cued to divert resources from flight capability to reproduction, and may emerge with short wings. However, predation pressure may independently cue increased leg muscle development and better jumping ability. Due to the correlated effect on flight muscle development, these opposing environmental cues may result in the development of what appear to be non-optimal phenotypes for the environment: e.g., individuals with pink flight muscles developing in environments conducive to reproduction, and, perhaps, the production of the seemingly paradoxical SW_P morph.

Correlated effects of flight muscle histolysis

Studies in many species of insects have found that increasing JH levels in adults can trigger flight muscle histolysis (Dingle and Winchell, 1997; Oliver et al., 2007; Shiga et al., 2002). As discussed above, if the action of these hormones is not completely localizable, breakdown or histolysis of muscles in one part of the body may have correlated effects on muscles in other parts of the body. In order to examine this hypothesis, we looked at the relationship between change in flight muscle status between the day an individual moulted into an adult and the test date, and hind leg femoral muscle mass. We had hypothesized that if flight muscle histolysis had correlated effects on leg muscles, individuals who emerged with developed, pink flight muscles and histolyzed them with age (P-W) would have smaller hind leg femoral muscles than individuals who emerged with developed, pink flight muscles and did not histolyze them (P-P). Our results supported this hypothesis: individuals who moulted with pink flight muscles and histolyzed them with age had significantly smaller hind leg femoral muscle mass than

individuals who moulted with and maintained pink flight muscles. This suggests that the process of flight muscle histolysis may have caused a correlated decrease in the mass of the hind leg femoral muscles.

As with flight muscle development, if the hormones involved in mediating the tradeoff between flight muscle maintenance and histolysis and, in this case likely JH, have correlated effects on other life history traits, in this case leg muscle mass and likely jumping ability, flight muscle status (maintenance and histolysis) may be constrained from optimally tracking environmental changes. Once a flight capable adult disperses to an environment conducive to reproduction from which they do not need to disperse further, they may be cued to divert resources from flight capability to reproduction, and histolyze their flight muscles. However, if flight muscle histolysis causes a correlated decrease in leg muscles, and likely jumping ability, these individuals may face greater predation related mortality.

Correlated traits as constraints

Flight polyphenism in many insects has been cited as one of the reasons for the evolutionary success of insects (Zera and Denno, 1997), as it allows individuals to adapt to changing environments and track shifting resources. In an environment conducive to reproduction, individuals should emerge as flightless and invest most of their resources into increased reproduction. In contrast, in an environment not conducive to reproduction, individuals should emerge as flight capable and disperse to better environments. Once they have dispersed to a good environment, flight capable individuals should break down their flight muscles, becoming flightless, and shift available resources from flight to

reproduction. However, this adaptive shift between investing on flight capability and reproduction may be constrained by trait correlations with other life history traits affecting survival or reproductive success. As we have shown here, individuals who emerge as flightless may be less capable of escaping predators by jumping, and individuals who breakdown their flight muscles as adults may have correlated decreases in other muscles. Such constraints, caused by correlated effects of the mechanism mediating the tradeoff, may be far more common than previously thought. More studies are needed to examine such constraints in different systems, and to gauge their effects of the evolution, maintenance and expression of resource allocation patterns of life history traits in different selective environments.

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Table 2.1 Number of crickets of each wing/flight muscle morph in the flight trials who flew vs. did not fly

Morph	Flew	Did not fly
LW _P	12	7
LW _W	0	13
SW _P	0	13
SW _W	0	19

FIGURE LEGENDS

Figure 2.1

Differences in average jumping distance, controlling for body mass and age, between wing and muscle morphs. While there was no effect of wing morph (LW vs. SW), individuals with developed, pink flight muscles jumped significantly further than individuals with undeveloped, white flight muscles. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 2.2

(a) Differences in hind leg femoral mass, controlling for body mass and age, between individuals of different flight muscle morphs (P vs. W). Individuals with developed, pink flight muscles had significantly larger leg muscle mass than individuals with undeveloped, white flight muscles. (b) Differences in hind leg femoral mass, controlling for body mass and age, between individuals based on change in flight muscle morph between day of moulting into an adult and test day (pink remained pink: P-P, or pink was histolyzed to white: P-W). P-W individuals had significantly smaller hind leg femoral mass than P-P individuals. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 2.1

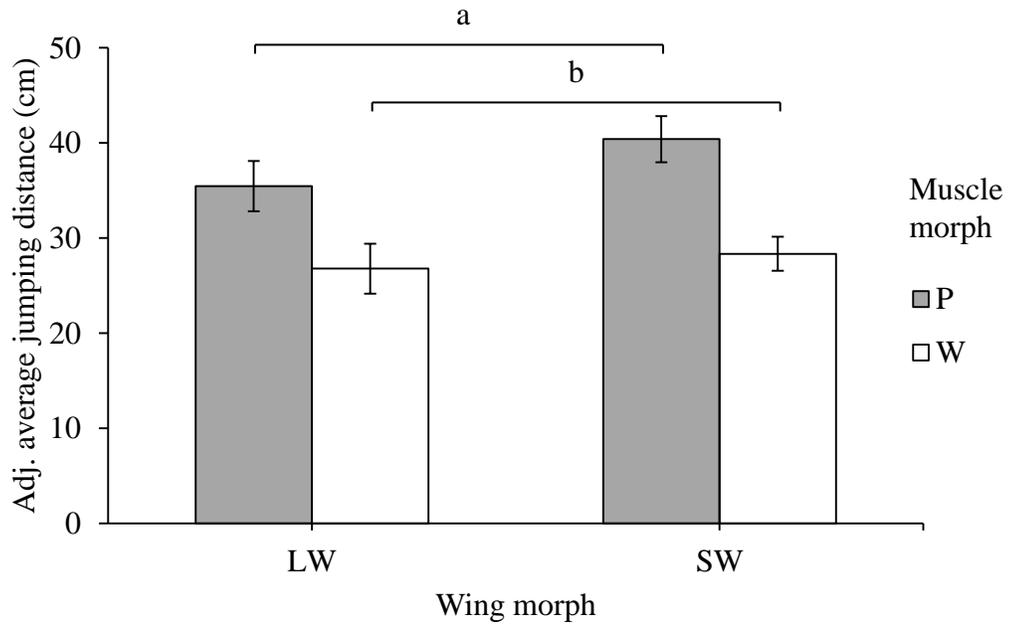
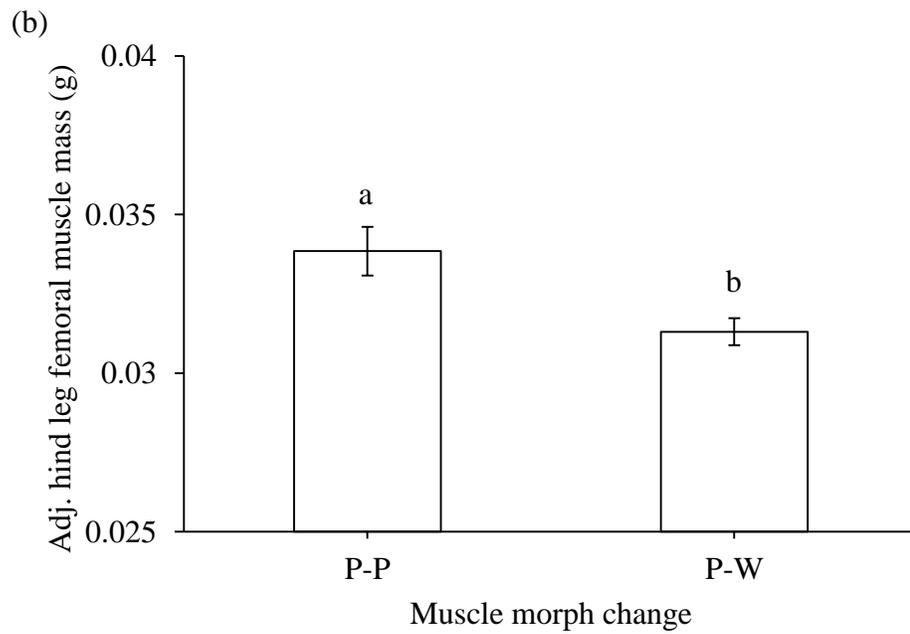
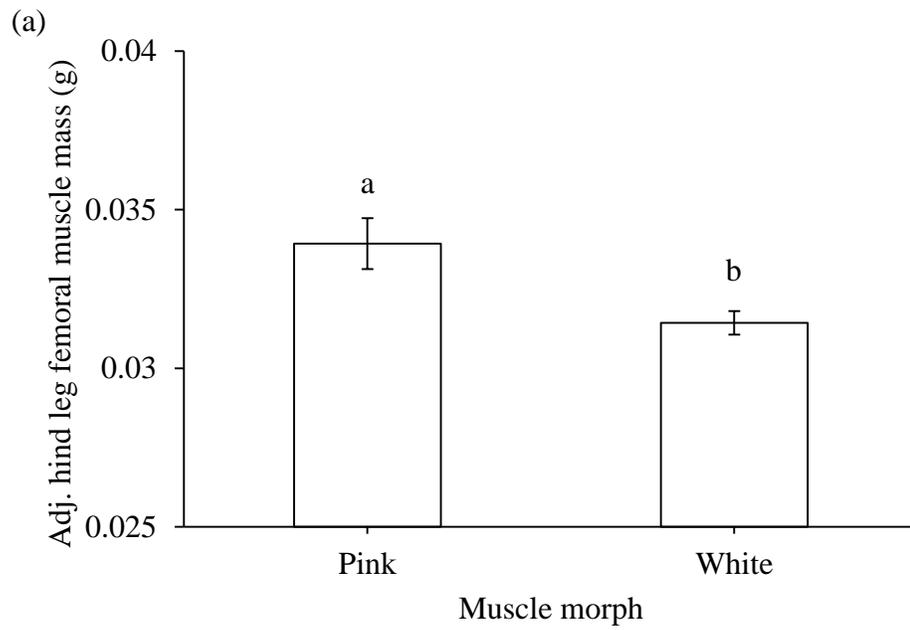


Figure 2.2

CHAPTER THREE: Males in a flight polyphenic field cricket express alternative reproductive tactics: behavioral and physiological variation among morphs

ABSTRACT

Individuals should express environment-specific alternative reproductive tactics, if specific combinations of behavioral and physiological traits yield greater fitness in different environments. We examined the variation in reproductive behavior and physiology of different male morphs of a flight polyphenic field cricket, hypothesizing that there may be morph-specific alternative reproductive tactics congruent with morph-specific alternative life histories. In these polyphenic species, individuals can vary in wing length (short or long) and flight muscle morphology (functional pink or non-functional white). We hypothesized that in good environments, individuals should emerge as flightless and, as they do not invest in energetically expensive flight muscles and fuels, should invest heavily in reproduction. In contrast, in poor environments, individuals should emerge as flight capable and able to disperse, and should delay investment in reproduction and invest in flight capability. Our results supported these hypotheses. We found that flight capable males sing less and have smaller accessory glands, used to produce seminal fluids, than do flightless males. Interestingly, we found that the two wing morphs of flightless males invest in reproduction differently. Long-winged flightless males invest in larger relative testes size and larger spermatophores, but are slower to remate. In contrast, short-winged flightless males remate quickly, but produce smaller spermatophores. As environmental quality, such as the density of available mates, vary temporally, matching reproductive tactics to environmental

variation may result in higher fitness. Such variability may help maintain this polyphenism in the wild.

Key words. Life history tradeoffs, wing polymorphism, phenotypic plasticity, calling song, spermatophore, accessory gland, testes, latency to remating.

INTRODUCTION

Alternative reproductive tactics, when reproductive behavior, morphology and physiology vary discontinuously between individuals of a species, have fascinated evolutionary biologists since Darwin (reviewed in Brockmann, 2001; Gross, 1996; Oliveira et al., 2008; Shuster and Wade, 2003). This fascination stems largely from the desire to explain the incredible diversity of such tactics found within a wide cross section of species from insects, to fish, to birds and mammals (reviewed in Gross, 1996; Shuster, 2010). The alternative morphs can result from genetic effects on phenotypic traits, called strategies, or from environmental effects on phenotypic traits, called tactics (Brockmann, 2001). Tactics have been further subdivided into “mixed”, when an individual’s tactic is set by a random decision rule, and “conditional”, when an individual’s tactic is set by internal or external environmental cues (Brockmann, 2001; Gross, 1996). While examples of genetic strategies (Ryan et al., 1992; Shuster and Wade, 1991) and mixed tactics (Widemo, 1998) are rare, conditional tactics have been found in many systems (reviewed in Gross, 1996). Interestingly, although conditional tactics are defined as based on external or internal environmental cues (Brockmann, 2001), the majority of studies thus far have focused on variation in internal environmental cues (e.g., body condition,

size, age, etc.). How external environmental conditions, such as social group, population density, etc. affect tactics have been far less studied (but see Formica et al., 2004; Formica and Tuttle, 2009; Ribeiro et al., 2010).

Conditional tactics based on external environmental conditions are particularly likely in species that trade off different life history traits in response to environmental variation. Such life history tradeoffs occur when resources are limiting, and when increasing allocation of the limiting resource to one trait has the effect of decreasing resources to other traits (Partridge and Harvey, 1988; Stearns, 1976; 1989; Zera and Harshman, 2001). When differential investment in life history traits in response to changing environmental variables changes the costs and benefits of different investment patterns, we should see the evolution of specific combinations of behavioral and physiological traits in different environments. Such combinations may be particularly likely in polyphenic species. In these species, individuals develop as discrete morphological variants differing in life history strategies, based on environmental differences. This makes them an ideal study system for examining hypotheses about conditional alternative reproductive tactics, as well as adaptive covariation between behavioral and physiological reproductive traits and other life history traits.

Many species of insects trade off investment in flight capability with investment in reproduction (reviewed by Harrison, 1980; Zera and Denno, 1997; Zera and Brisson in press). In these flight polymorphic/polyphenic insects, individuals occur as discrete morphs that are either flightless or flight capable, depending on variation in wing length (long wing [LW] or short wing [SW]), variation in flight muscle development (functional pink [P] or non-functional white [W]), or both (Zera and Denno, 1997). This life history

tradeoff has been intensively studied in several species of field crickets (reviewed by Zera, 2009). In these animals, individuals with short wings and/or undeveloped flight muscles are flightless (SW_W , SW_P and LW_W), while individuals with both long wings and developed flight muscles (LW_P) are flight capable (Chapter 1 and 2). Previous studies have found that individuals with developed flight muscles invest less in current reproduction (Crnokrak and Roff, 1998; Mole and Zera, 1993; Roff and Fairbairn, 1991; Chapter 1). This is likely due to increased allocation of resources to producing and maintaining functional flight muscles, which have more and larger fibers, higher respiration rates, and higher in vitro enzyme activity than the non-functional flight muscle tissue (Zera et al., 1997), as well as the cost of producing and maintaining lipid flight fuel stores (Zera et al., 1994). However, individuals can redirect resources from flight capability to reproduction by breaking down (histolyzing) their flight muscles; when these muscle tissues are histolyzed, they turn from pink to white in color, and the animal becomes flightless (e.g., Roff and Gelinas, 2003; Zera et al., 1997). Wing length and flight muscle development in these animals is phenotypically plastic, affected both by genes and the environment (e.g., Fairbairn and Roff, 1990; Lorenz, 2007; Zera, 2009; Zera and Brisson in press).

In the flight polyphenic field cricket, *Gryllus lineaticeps*, males signal from burrow entrances, producing calling song to attract females from a distance (Wagner, 1996). Females may mate repeatedly with the same male over a single night, and may mate with multiple males over her lifetime (Wagner et al., 2001). Adults occur as LW_W , LW_P , SW_W and SW_P (Chapter 1). The paradoxical SW_P morph produces and maintains developed flight muscles, and pays a reproductive cost (females: Chapter 1) while

gaining no dispersal benefits as they cannot fly (Chapter 2). We used this study system to test the hypothesis that flightless and flight capable male morphs express alternative reproductive tactics. As decreasing rearing density increases the proportion of individuals emerging as flight capable (*G. lineaticeps*: personal observations by CM; other cricket species: Olvido et al., 2003; Zera and Tiebel, 1988), males emerging in low density populations may be trading off early reproduction for flight capability, which allows them disperse to areas with more conspecifics. Males of many species produce mate attraction signals while in close proximity, and signaling in groups may be an advantage because females are more attracted by aggregated signaling, because females pay lower costs of mate assessment, or because both males and females face lowered predation risks due to risk dilution or predator-satiation (Gerhardt and Huber, 2002). Consequently, males who mature in low density populations may benefit from delaying reproduction and dispersing to areas with more males with whom they can signal, and more potential mates per capita. Therefore, flight capable males may adopt a high cost, low risk reproductive tactic. Flightless males (SW_w and LW_w), on the other hand, may trade off flight capability for early investment in reproduction. As they have lower mobility, they risk not mating if few conspecifics are nearby. Therefore, they may be adopting a low cost, high risk reproductive tactic.

Males may invest in reproduction in a number of different ways. First, they may increase the amount of time, and therefore energy, they spend producing mate attraction signals, which has been found to strongly affect mating success in many species (e.g., Butlin et al., 1985; Eiriksson, 1994; French and Cade, 1989; Greer and Wells, 1980; Wagner and Sullivan, 1995). Second, they may increase investment in reproductive

tissues. Increasing testes size has been shown to affect male reproductive success in many species, because testes size has been shown to positively affect sperm number and, in some systems, success in sperm competition (e.g., Bangham et al., 2002; Demary and Lewis, 2007; Schulte-Hostedde and Millar, 2004). Third, in many insects, increasing investment in male accessory glands, which produce seminal fluids, increases male reproductive success, because seminal fluid products may help in sperm competition, may increase time to remating in females, and may be a form of male-provided direct benefit to females (e.g., Bangham et al., 2002; Demary and Lewis, 2007; Leopold, 1976; Wolfner, 1997). Fourth, males may increase investment in spermatophore production, by increasing spermatophore size or increasing speed of spermatophore replacement. The size of the spermatophore a male transfers during mating has been shown to affect male reproductive success in a number of insect species, because spermatophore size may be related to increased sperm numbers or increased amounts of seminal fluids transferred (McNamara et al., 2009; Oberhauser, 1989; South et al., 2011). Fifth, in species in which females are receptive to mating with an individual male repeatedly within a short time, or in which a male may encounter multiple receptive females within a short time, his speed of spermatophore replacement should affect his reproductive success. Previous work in a number of species of insects, has shown that energy limited individuals take longer to generate new spermatophores (Gwynne, 1990; Jia et al., 2000; Proctor, 1992; Wagner, 2005).

We tested the hypothesis that flight capable and flightless males in *G. lineaticeps* express morph-related alternative reproductive tactics. We measured morph-specific differences in, (1) the amount of time males spend signaling, (2) testes and accessory

gland mass, (3) size of spermatophore transferred during mating, and (4) speed of spermatophore replacement. We hypothesized that if flight capable individuals invest less energy in reproduction, they would spend less time singing, have smaller testes and accessory glands, transfer smaller spermatophores, and take longer to generate new spermatophores. In natural populations of *G. lineaticeps*, we have observed temporal as well as spatial variability in population density (personal observation by CM and WEW). Therefore, since different reproductive tactics may result in higher fitness at different times, environmental variability may favor the evolution and maintenance of morph-related reproductive tactics and, perhaps, this polyphenism.

But how about the SW_P morph? In previous work, we have shown that SW_P individuals do not gain flight capability (Chapter 2), but in females, do pay reproductive costs (Chapter 1) for their developed flight muscles. If SW_P males, like SW_P females, resemble individuals of the flight capable LW_P morph, we would expect them to pay similar reproductive costs for their developed flight muscles.

METHODS

General Methods

Test animals were reared from field-caught crickets collected at Sedgwick Reserve, Santa Ynez Valley, California. Adult females, who we presumed had mated in the field, were captured during the summers of 2006-2008, and brought to University of Nebraska–Lincoln. In the laboratory, we set up matings designed to minimize inbreeding (see Wagner and Basolo, 2007) for details). Genealogies of all lab bred animals were known. For details of cricket rearing see Chapter 1.

All animals were maintained at approximately 23°C in a reversed light dark cycle (L:D - 16:8 h). We monitored the individual containers daily and recorded the day when the crickets molted into adults. Males used in trials were within the age range of 7 - 15 days post final molt. Before testing, all crickets had their wing morph, muscle morph, age and mass recorded. We determined wing morph by comparing the length of the hindwings to the forewings (LW: hindwings longer than forewings; SW: hindwings shorter than forewings). We determined flight muscle morph by lifting a hind leg and recording the color of the muscle patch behind the thin membrane between the body and the leg (Chapter 1). On crickets with developed, pink flight muscles, the patches are pink in color. On crickets with undeveloped or histolyzed flight muscles, the patches are either not visible or are a bright white in color.

Experiment 1: Morph-specific differences in of singing activity

In order to examine variation among morphs in the singing activity, we monitored males for five hours and recorded their singing behavior (as in Mitra et al., 2011). To do this, we transferred males in their containers to an isolated testing room lit with dim red lights and maintained at 24 - 25°C. We tested 15 males at a time, arranged around the testing room at a distance of 30 cm from each other. These densities were not unrealistic, as we have observed males in high density field populations sing within short distances of each other (observations by CM and WEW). As density of calling males may have affected singing activity, on days when we did not have sufficient numbers of crickets to test, we maintained test group size at 15 by adding previously tested crickets. We did not record the singing activity of these dummy males.

We monitored test males for five hours, collecting singing data for each cricket for every 10 min segment within the five hours. If a cricket sang during one of these segments, it was scored as a one, and if it did not sing during a segment, it was scored as a zero. Therefore, at the end of monitoring period, any male could have a singing activity score between zero (never sang) and 30 (sang in every segment). Male crickets in this species can produce calling song (a long distance mate attraction signal), courtship song (a short distance mate attraction signal), and aggressive song (usually produced in response to physically encountering another male). In this experiment, we only scored males as singing if they produced calling song; while we heard some courtship song being produced during the trials, we never heard any aggressive song.

We tested a total of 112 males: 36 LW_P, 25 LW_W, 26 SW_P and 25 SW_W. We tested for morph-specific variation in nightly amount of singing using a linear mixed model, with wing morph and muscle morph as fixed factors, family as a random factor, and age as a covariate. All analyses were carried out using SPSS 19.0.0.

Experiment 2: Morph-specific differences in mass of testes and accessory glands

In order to examine variation among morphs in the masses of testes and accessory glands, we cold anesthetized and dissected 196 males (48 LW_P, 50 LW_W, 49 SW_P and 49 SW_W). After dissecting a male, we removed and weighed his testes and accessory glands to the nearest 0.1 mg.

We tested for morph-specific variation in testes and accessory glands using a linear mixed model, with wing morph and muscle morph as fixed factors, family as a

random factor, and age and body mass as covariates. All analyses were carried out using SPSS 19.0.0.

Experiment 3: Morph-specific differences in spermatophore size and speed of spermatophore replacement

In order to examine variation among morphs in spermatophore size, we ran mating trials. All trials were run under red light, with room temperature maintained at 23-24°C. During trials, we played a synthetic male calling song with a high chirp rate (4.2 chirps/second) using a SME-AFS portable field speaker attached to a CD player, at 75 dB SPL (re: 20 μ Pa at 30 cm from the speaker), to help stimulate experimental males to sing, and stimulate females to mate. For a detailed description of how the synthetic song was created see Chapter 1. Sound pressure level was calibrated prior to each trial, using a Casella CEL-254 Digital Impulse Sound Level Meter (impulse RMS).

To start the trial, four unrelated individuals (one previously unmated male, and three females) were randomly selected and placed in a 10 gallon glass aquarium (40 x 20 x 25 cm) lined with paper towels. As soon as the male mated with one of the females, we removed the female and detached the spermatophore. We used a 2mm slide micrometer and a dissecting microscope to measure the length (excluding the tail) and maximum width of the spermatophore. If the male did not mate within the first 30 minutes, he was removed from the experiment. Females were reused between trials.

A subset of the above males were monitored after they mated the first time, to record speed of spermatophore replacement. With these males, as soon as the male mated the first time, we started a stopwatch. We then closely monitored the male to note the

length of time it took for a second spermatophore to become visible in the male's aedeagus. We recorded this as the time required for spermatophore replacement.

Sixty-one of the tested males mated within the first 30 minutes of the trial (28 LW_P, 9 LW_W, 10 SW_P and 14 SW_W). Of these males, 38 (18 LW_P, 6 LW_W, 4 SW_P and 10 SW_W) were monitored to determine speed of spermatophore replacement. We tested for morph-specific variation in spermatophore width and length, and speed of spermatophore replacement using a linear mixed model, with wing morph and muscle morph as fixed factors, family as a random factor, and age as a covariate. We log transformed the data for speed of spermatophore replacement as it was not normally distributed. We also examined whether spermatophore size affected speed of spermatophore replacement, using a linear mixed model, with family as a random factor. All analyses were carried out using SPSS 19.0.0.

RESULTS

Experiment 1: Morph-specific differences in of singing activity

Males with white flight muscles sang significantly more frequently than did males with pink flight muscles ($F = 14.41$, $P < 0.001$; Figure 3.1). There was no effect on singing activity of either wing morph (LW vs. SW; $F = 0.07$, $P = 0.787$), or the interaction between wing morph and muscle morph ($F = 2.52$, $P = 0.116$). Neither family ($F = 1.33$, $P = 0.154$), nor age ($F = 0.07$, $P = 0.789$), significantly affected singing activity.

Experiment 2: Morph-specific differences in mass of testes and accessory glands

Males with white flight muscles had greater absolute accessory gland mass than did males with pink flight muscles ($F = 8.71$, $P = 0.004$; Figure 3.2a). There was no effect on absolute accessory gland mass of either wing morph (LW vs. SW; $F = 0.58$, $P = 0.449$), or the interaction between wing morph and muscle morph ($F = 0.31$, $P = 0.578$). Both family ($F = 2.03$, $P = 0.001$) and age ($F = 12.07$, $P = 0.001$) significantly affected absolute accessory gland mass, with older males having heavier accessory glands.

Relative accessory gland mass (using body mass as a covariate) showed similar patterns: males with white flight muscles had larger relative accessory gland mass than males with pink flight muscles ($F = 16.448$, $P = 0.004$; Figure 3.2b), while there was no effect on accessory gland mass of either wing morph ($F = 1.44$, $P = 0.233$) or the interaction between wing morph and muscle morph ($F = 0.82$, $P = 0.366$). Family ($F = 2.02$, $P = 0.001$), age ($F = 15.41$, $P = 0.001$) and body mass ($F = 52.04$, $P < 0.001$) significantly affected relative accessory gland mass, with older and heavier males having heavier accessory glands.

There was no effect of flight muscle morph ($F = 2.18$, $P = 0.142$), wing morph ($F = 3.23$, $P = 0.075$), or the interaction between wing and flight muscle morph ($F = 1.74$, $P = 0.189$) on absolute testes mass (Figure 3.3a). Both family ($F = 2.55$, $P < 0.001$) and age ($F = 9.27$, $P = 0.003$) significantly affected absolute testes mass, with younger males having heavier testes.

However, relative testes mass (using body mass as a covariate) showed patterns similar to those of accessory gland mass. Males with white muscles had greater relative testes mass than males with pink flight muscles ($F = 6.71$, $P = 0.011$; Figure 3.3b). There

was no effect of wing morph ($F = 0.13$, $P = 0.717$), and a trend for an interaction between wing morph and muscle morph ($F = 3.82$, $P = 0.053$), with the difference in relative testes mass between individuals with developed and undeveloped flight muscles being larger for long-winged individuals than for short-winged individuals. Family ($F = 2.87$, $P < 0.001$), age ($F = 15.69$, $P = 0.001$) and body mass ($F = 77.98$, $P < 0.001$) significantly affected relative testes mass, with younger and heavier males having heavier testes.

Experiment 3: Morph-specific differences in spermatophore size and speed of spermatophore replacement

Males with white flight muscles had wider spermatophores than did males with pink flight muscles ($F = 7.50$, $P = 0.010$), and long-winged males had wider spermatophores than did short-winged males ($F = 18.14$, $P < 0.001$; Figure 3.4a). However, there was no effect of the interaction between wing and muscle morph ($F = 1.55$, $P = 0.223$) on spermatophore width. Both family ($F = 2.85$, $P = 0.003$) and age ($F = 19.38$, $P < 0.001$) significantly affected absolute spermatophore width, with older males having wider spermatophores. Spermatophore length, however, was not affected by muscle morph ($F = 1.25$, $P = 0.272$), wing morph ($F = 0.99$, $P = 0.328$), or the interaction between wing and muscle morph ($F = 0.11$, $P = 0.744$; Figure 3.4b). In addition, neither family ($F = 0.74$, $P = 0.782$) nor age ($F = 3.21$, $P = 0.083$) significantly affected absolute spermatophore length. We did not use body mass as a covariate in the above analyses, as we were interested in morph-specific differences in absolute spermatophore size (because absolute, not relative, spermatophore size should affect a male's fitness).

Speed of spermatophore replacement was significantly affected by the interaction between wing morph and muscle morph ($F = 8.52$, $P = 0.009$; Figure 3.5): SW_W males remated the fastest, and LW_W males remated the slowest, with the two pink muscled morphs having intermediate speeds of spermatophore replacement. Neither family ($F = 1.77$, $P = 0.123$) nor age ($F = 0.06$, $P = 0.806$) significantly affected speed of spermatophore replacement.

In addition, spermatophore width had a significant effect on speed of spermatophore replacement ($F = 2.67$, $P = 0.023$), with males who produced wider spermatophores having slower speeds of spermatophore replacement.

DISCUSSION

With this series of experiments, we tested the hypothesis that flight capable and flightless males express conditional alternate mating tactics. We suggested that males in lower density populations should emerge as flight capable, so that they can disperse to areas with more males to display with and more potential mates. As these males allocate limited available resources to flight, they should decrease their investment in reproduction. In contrast, males in high density populations should emerge as flightless and invest heavily in current reproduction. Our results partially supported our hypothesis.

Resource allocation to mate attraction signals

The amount of time a male spends producing mate attraction signals has been shown to strongly affect male mating success in many species (e.g., Bertram, 2000; Greer and Wells, 1980; Wagner and Sullivan, 1995). We found that males with undeveloped

flight muscles sing more than males with developed flight muscles. This result supports our hypothesis that flightless individuals invest more in reproduction than flight capable individuals. As singing behavior is known to be energetically expensive (Robertson, 1986; Simmons et al., 1992), flight capable individuals, who have high allocation of limited energy resources to flight capability, may have decreased energy to allocate towards producing song. These results are consistent with previous work on morph-specific singing activity that found that SW_W and LW_W sing significantly more than LW_P (Crnokrak and Roff, 1998; Mitra et al., 2011). Our study additionally shows that SW_P individuals behave similarly to LW_P individuals, and appear to pay a reproductive cost in terms of singing activity for flight muscles they cannot use to fly.

Resource allocation to reproductive tissues

Increasing investment in reproductive tissues, such as testes and accessory glands, has been shown to positively affect male reproductive success in a number of species (Bangham et al., 2002; Demary and Lewis, 2007; Leopold, 1976; Schulte-Hostedde and Millar, 2004; Wolfner, 1997). We found that individuals with undeveloped flight muscles have larger accessory glands, both absolute, and relative to their body mass, than individuals with developed flight muscles. This result supports our hypothesis that flightless individuals invest more in reproduction than flight capable individuals. This is in contrast to Zhao et al. (2010), who found no differences between morphs in accessory gland mass in another wing dimorphic field cricket, *Velarifictorus ornatus*. In addition, previous studies in insects have found that seminal products transferred to the female during mating, which are mostly produced by the accessory glands, can affect sperm

competition, female fecundity and lifespan, and maternal allocation to offspring (Chapman et al., 2000; Simmons, 2011; Wagner and Harper, 2003; Wolfner, 1997). Therefore, having larger accessory glands may increase mating and/or reproductive success of males with undeveloped flight muscles. This study also shows that SW_P individuals are similar to LW_P individuals in that they appear to pay a reproductive cost in terms of accessory gland mass for flight muscles they cannot use to fly.

Interestingly, while we found no differences in absolute testes mass between morphs, individuals with undeveloped flight muscles have larger testes relative to their body mass, than do individuals with developed flight muscles. As all past studies we have found examined the effects of absolute testes size on male reproductive success, we are unsure whether having larger relative testes size would have fitness consequences for males with white flight muscles. This result, therefore, only partially supports our hypothesis that flightless individuals are investing more in reproduction than flight capable individuals. In addition, there is a near significant trend that the difference in relative testes mass is larger within LW males than within SW males. Therefore, LW_W males are investing in larger testes relative to their body mass, in comparison to LW_P males. As almost all LW individuals emerge with developed flight muscles (CM, unpublished data), these results suggest that after flight muscle histolysis long-winged individuals reallocate resources to increasing relative testes mass. We do not know whether SW_P individuals undergo a similar reallocation of resources post flight muscle histolysis, as we did not separate SW_W individuals into those who emerged with undeveloped flight muscles, and those who histolyzed their flight muscles as adults. Lastly, this study shows that SW_P individuals are similar to LW_P individuals in that they

appear to pay a reproductive cost in terms of relative testes mass for flight muscles they cannot use to fly.

Resource allocation to spermatophore production

Previous studies in insects have found that males on low nutrition diets, presumably with less available energy to allocate to mating, may decrease the size of spermatophore they produce, may increase the time they take to produce a spermatophore, or may do both simultaneously (Jia et al., 2000; Wagner, 2005). We found that individuals with undeveloped flight muscles have wider spermatophores than individuals with developed flight muscles, and that individuals with long wings have wider spermatophores than individuals with short wings. Therefore LW_w males produced the largest spermatophores. As a male's reproductive success has been shown to increase with increasing spermatophore size in a number of species (McNamara et al., 2009; Oberhauser, 1989; South et al., 2011), having wider spermatophores may increase reproductive success of LW_w males. Comparing Figure 3.3a, 3.3b and 3.4a, we see very similar patterns: LW_w males have larger testes relative to their body size, and produce the largest spermatophores, suggesting that flight muscle histolysis in this wing morph may be followed by a large reallocation of energy to reproduction.

We see very different patterns for morph-specific differences in speed of spermatophore replacement. There is an interaction between wing and muscle morph, with LW_w individuals being the slowest, and SW_w individuals being the fastest, at replacing their spermatophores (Figure 3.5). Wedell (1993) suggested that males who produce costlier, better quality, spermatophores should take longer to produce new ones.

Given that we found that LW_w males produce the largest spermatophores and take the longest to replace them, LW_w males may trade off size of spermatophore and speed of replacement. This hypothesis was supported by our results, as we found that increasing spermatophore width significantly decreased speed of spermatophore replacement. SW_w males, in contrast, may be increasing mating success by quickly replacing spermatophores and decreasing latency to remate, but producing smaller spermatophores than LW_w males.

Therefore, these results partially support our hypothesis that flightless individuals invest more in reproduction than flight incapable individuals: LW_w males produce larger spermatophores but take longer to replace them, while SW_w males produce intermediate sized spermatophores, but replace them quickly.

Alternative reproductive tactics: tactics within tactics?

We had hypothesized that flightless and flight capable males would express alternate mating tactics, with flightless males investing more in current reproduction. This hypothesis was partly supported by our results. We found that males with non-functional, white flight muscles sing more, and have larger male accessory glands than males with developed flight muscles. As developed flight muscles are more energetically expensive, with more and larger fibers, higher respiration rates, and higher in vitro enzyme activity (Zera et al., 1997), individuals with developed flight muscles may have less resources available to invest in song production and accessory gland mass.

However, males with non-functional, white flight muscles of different wing morphs may also differ in their tactics. A male can increase his fitness by signaling more,

investing more in his reproductive tissues and investing more in spermatophore production. While both LW and SW males with undeveloped flight muscles sing more and have large accessory glands, our results suggest that after flight muscle histolysis, LW males reallocate resources to increasing relative testes mass, and producing larger spermatophores which take longer to replace. SW males, in contrast, trend towards having intermediate relative testes mass, and produce intermediate sized spermatophores, but replace spermatophores quickly. Therefore, while LW_W males may invest more in each mating, SW_W males may prioritize remating more quickly. Whether these tactics result in differential fitness is an open question.

What about the SW_P males? Like SW_P females, they appear to pay a reproductive cost in terms of signaling time, accessory gland mass and spermatophore width, for flight muscles they cannot use to fly. This adds support to our hypothesis that this morph may result from developmental constraints, and may be a possible cost of phenotype-environment mismatching (Chapter 1).

In spite of considerable work on conditional alternative reproductive tactics (Brockmann, 2001; Gross, 1996), the effects of external environmental conditions, such as social group, population density, etc., have been largely ignored (but see Formica et al., 2004; Formica and Tuttle, 2009; Ribeiro et al., 2010). The study of such external environmental factors are important, because such factors would not restrict the evolution of alternative reproductive tactics only to systems with intense competition of mates (Shuster and Wade, 2003). Here, alternative reproductive tactics would evolve because of temporal or spatial variation in habitat quality. If individuals in poor habitats can increase their fitness by delaying reproduction and dispersing, they should express this strategy.

And, if individuals in good habitats can increase their fitness by early investment in reproduction, they should express that strategy. As individuals of many species, both male and female, are faced with a tradeoff between early reproduction and dispersal, such tactics may not be restricted to non-polymorphic/polyphenic animals. Such conditional alternative reproductive tactics, based on external environmental factors, may be common in both sexes of many species.

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FIGURE LEGENDS**Figure 3.1**

Differences in average number of time periods during which a male sang, controlling for age, between wing and muscle morphs. While there was no effect of wing morph (LW vs. SW), individuals with undeveloped, white flight muscles sang significantly more than individuals with developed, pink flight muscles. Different letters designate statistically significant differences ($P \leq 0.05$).

Figure 3.2

(a) Differences in absolute accessory gland mass, controlling for age, between wing and muscle morphs. While there was no effect of wing morph (LW vs. SW), individuals with undeveloped, white flight muscles had significantly larger absolute accessory gland mass than individuals with developed, pink flight muscles. (b) Differences in relative accessory gland mass, controlling for age and body mass, between wing and muscle morphs. While there was no effect of wing morph (LW vs. SW), individuals with undeveloped, white flight muscles had significantly larger relative accessory gland mass than individuals with developed, pink flight muscles. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 3.3

(a) Differences in absolute testes mass, controlling for age, between wing and muscle morphs. There was no effect either wing morph (LW vs. SW) or muscle morph (P vs. W). (b) Differences in relative testes mass, controlling for age and body mass, between wing

and muscle morphs. While there was no effect of wing morph (LW vs. SW), individuals with undeveloped, white flight muscles had significantly larger relative testes mass than individuals with developed, pink flight muscles. In addition, there was a near significant interaction between wing and muscle morph. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 3.4

(a) Differences in spermatophore width, controlling for age, between wing and muscle morphs. Individuals with undeveloped, white flight muscles had significantly wider spermatophores than individuals with developed, pink flight muscles, and Individuals with long wings had significantly wider spermatophores than individuals with short wings. (b) Differences in spermatophore length, controlling for age, between wing and muscle morphs. There was no effect either wing morph (LW vs. SW) or muscle morph (P vs. W). Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 3.5

Differences in time to replace a spermatophore, controlling for age, between wing and muscle morphs. There was a significant interaction between wing and flight muscle morph. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 3.1

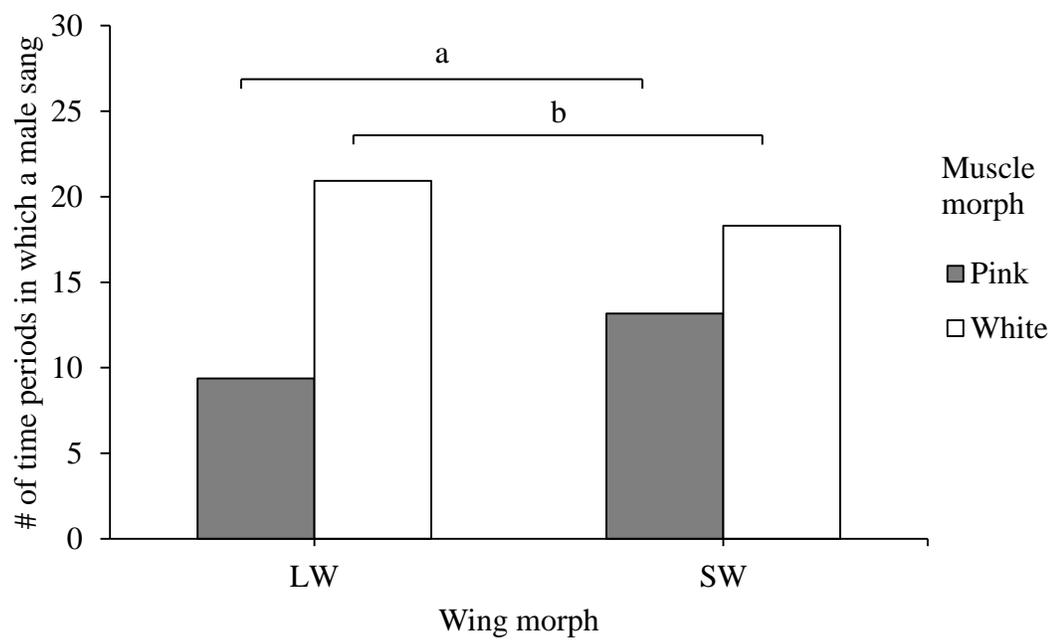


Figure 3.2

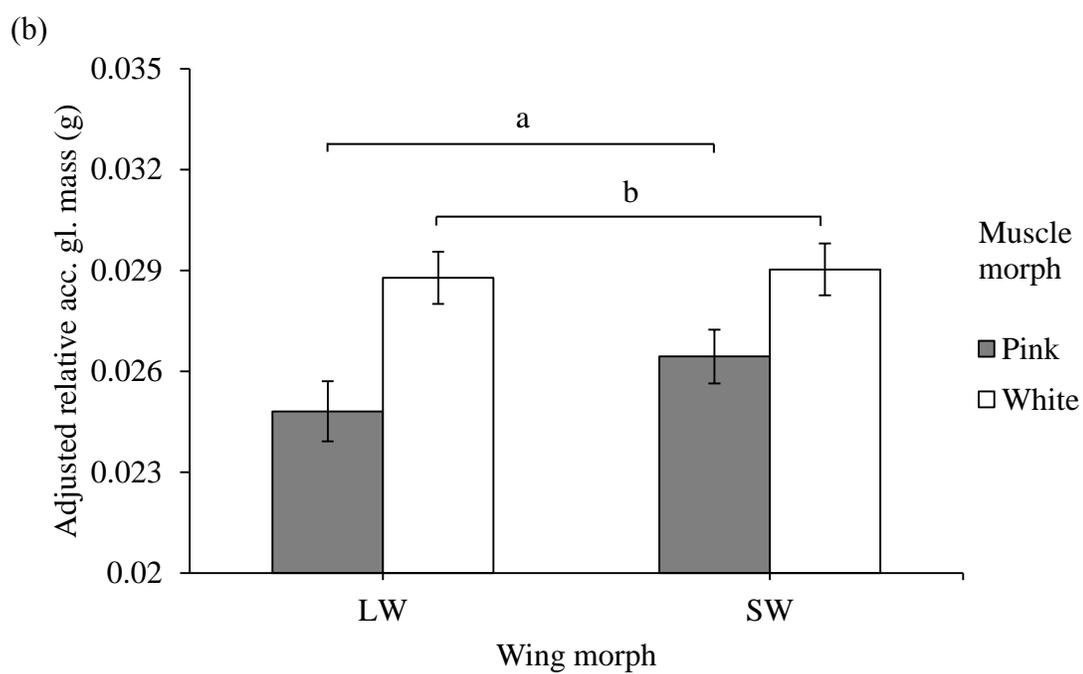
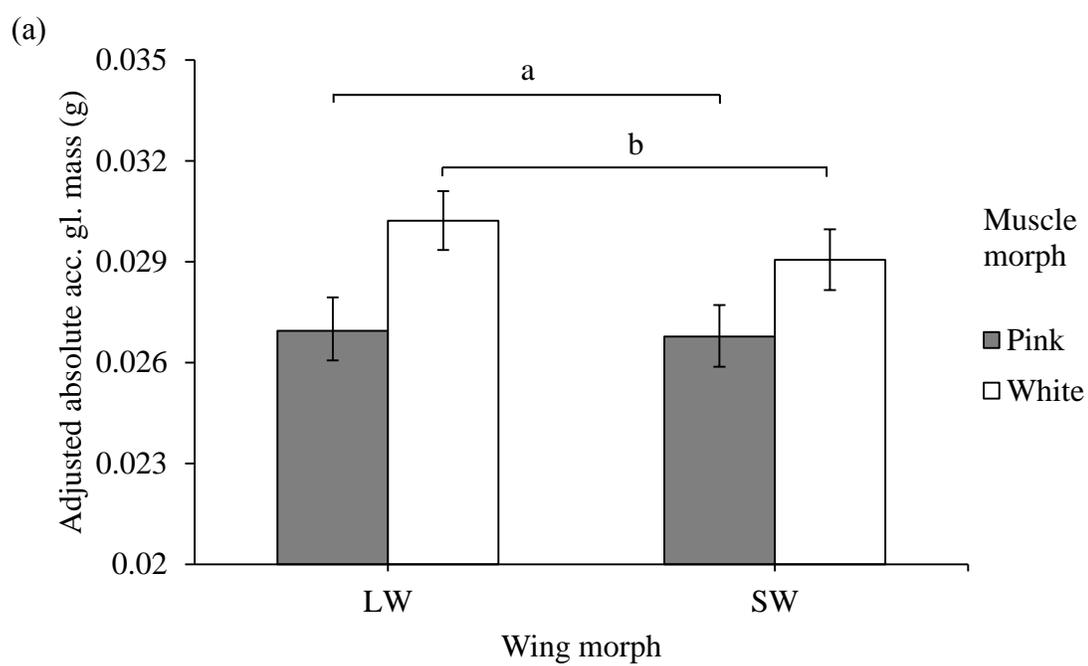


Figure 3.3

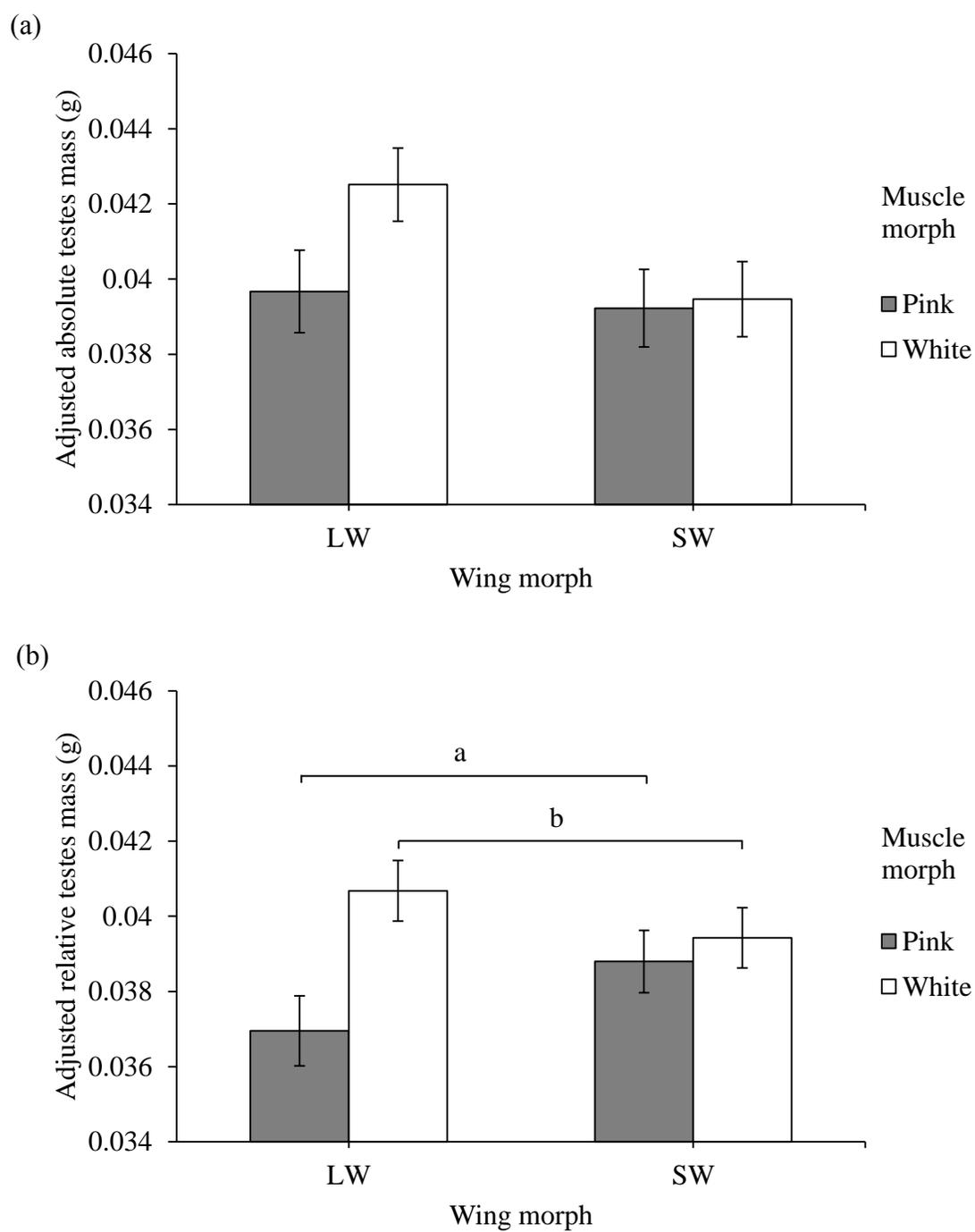


Figure 3.4

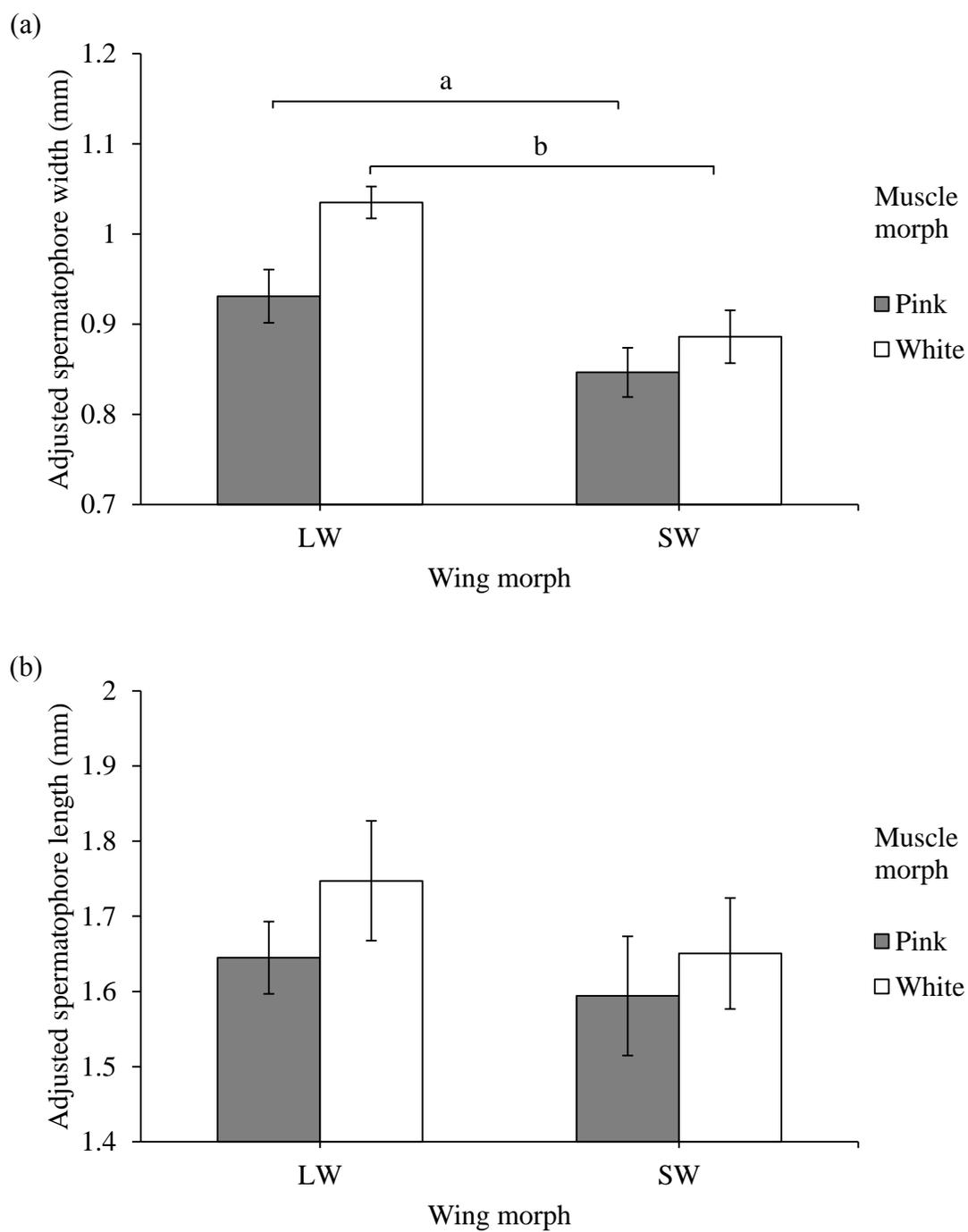
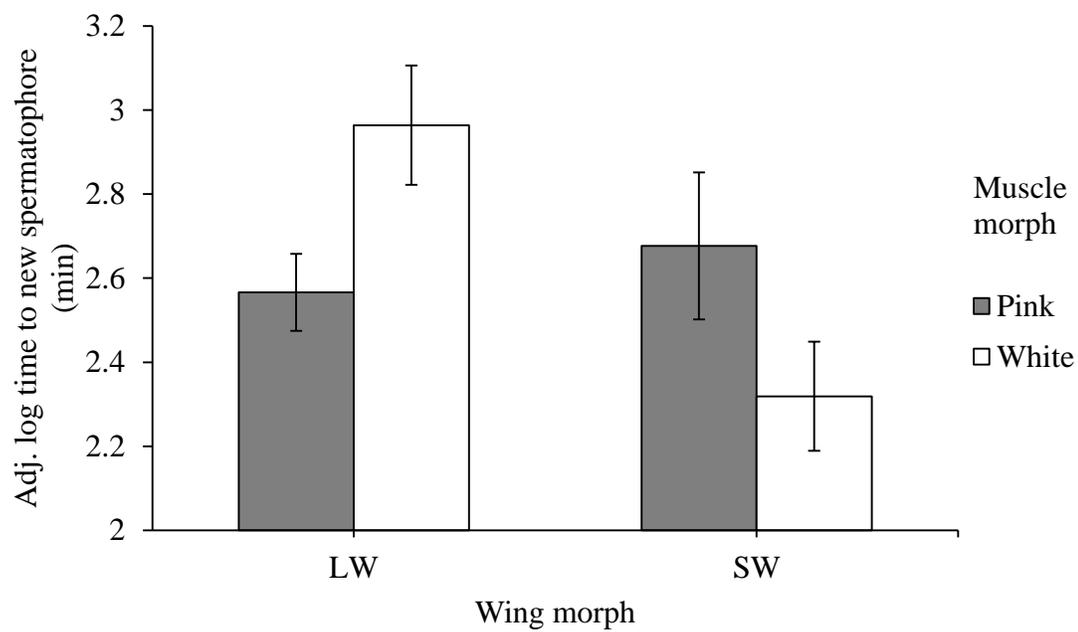


Figure 3.5



CHAPTER 4: Life history tradeoffs in a flight polyphenic field cricket: examining morph specific variation in a field population

ABSTRACT

The physiological mechanisms underlying life history tradeoffs have been extensively studied under laboratory conditions. However, examination of these mechanisms are rare in the field. Comparisons of lab and field results are still rarer. We examined the physiology underlying the flight capability vs. reproduction tradeoff in a species of flight polyphenic field cricket, *Gryllus lineaticeps*, in field caught individuals. In this species, flight phenotype is determined by a combination of wing length (short or long), and flight muscle morphology (functional pink or non-functional white). We examined morph specific differences in (1) investment in reproductive tissues (females: ovaries; males: testes and accessory glands), and (2) investment in lipids (triglycerides and phospholipids). We found that field caught flightless individuals invest more in ovaries and male accessory glands than flight capable individuals. However, flight capable males invest more in testes mass than do flightless individuals. Additionally, we found that flightless and flight capable males and females invest differentially in different lipids. Long winged females have high triglyceride mass in their somatic tissues, and long winged females with histolyzed flight muscles have high triglyceride mass in their ovarian tissues. In contrast, males of the different morphs did not differ in triglyceride mass. Flight capable males and females had higher phospholipid mass in their somatic tissues than did flightless males and females. However, flightless females had higher phospholipid mass in their ovarian tissues. These results suggest that the tradeoff between

flight capability and reproduction is similar in the lab and field for some traits, but not others, perhaps due to field individuals being exposed to more heterogeneous environments, more selective pressures, and more extreme resource limitations.

Key words. Life history tradeoffs, wing polymorphism, phenotypic plasticity, lipids, reproductive tissues, stoichiometry.

INTRODUCTION

Life history traits affect either the survival or reproduction of an individual (Ricklefs and Wikelski, 2002). While increasing investment in these traits should be advantageous for individuals, they can often be expensive in terms of internal resources. As most organisms are resource limited, life history traits are often negatively correlated with each other (Partridge and Harvey, 1988; Stearns, 1976; 1989; Zera and Harshman, 2001). The physiological causes of, and the mechanisms underlying, life history tradeoffs have been extensively studied over several decades (reviewed in Zera and Harshman, 2001). However, most studies examining the physiological causes and mechanisms of life history tradeoffs have been confined to lab reared animals (but see Agosta, 2008; Zera et al., 2007). Tradeoffs between traits under lab and field conditions may be vastly different, because tradeoffs may be context specific (Reznick and Ghalambor, 2005). In the lab, organisms may be shielded from environmental factors, such as predation, disease, etc., that would have a large effect in nature. In the field, organisms are exposed to heterogeneous environments, many more selective pressures, and often, more extreme resource limitations (Reznick and Ghalambor, 2005). Consequently, the lack of studies

examining the physiology underlying life history tradeoffs in natural populations presents a large gap in our current knowledge of life history evolution.

Flight polyphenism in insects, in which individuals tradeoff investment in flight and investment in reproduction, is a life history tradeoff which has been extensively studied (reviewed in Zera and Denno, 1997). In these animals, individuals occur as discrete morphs differing in flight capability, determined by variation in wing morphology, flight muscle morphology, or both (Zera and Denno, 1997). Such polyphenisms, in which an individual's phenotype is determined by both environmental factors and genes, are thought to arise and be maintained because different morphs have higher fitness in different environments (Denno et al., 1996; Denno, 1994; Roff, 1990). The physiological underpinnings of this flight-reproduction tradeoff have been most thoroughly examined in field crickets (reviewed in Zera, 2009). In these species, individuals may differ in wing length, having either long wings (LW) or short wings (SW). They may also differ in flight muscle development, maturing with either developed, pink flight muscles (P), or undeveloped, white flight muscles (W). In addition, pink flight muscles can degenerate or be histolyzed, with age and become flightless. Previous studies have shown that maintaining flight capability is energetically expensive: developed, pink flight muscle has more fibers that are larger in size, and exhibit higher respiration rates and higher in vitro enzyme activity than white flight muscle (Zera, 2009; Zera et al., 1997). In addition, flight capable females produce and maintain extensive lipid, mostly triglyceride, fuel stores (Zera et al., 1994). Due to this high allocation of available resources to producing and maintaining necessary muscles

and flight fuels, flight capable individuals invest less in current reproduction (Crnokrak and Roff, 1998; Mole and Zera, 1993; Roff and Fairbairn, 1991; Chapter 1 and 3).

In our study species *Gryllus lineaticeps*, adults occur as four flight morphs varying in wing and/or flight muscle morph. Individuals can have long wings with pink flight muscles (LW_P), long wings with white flight muscles (LW_W), short wings with white flight muscles (SW_W), and, paradoxically, short wings with pink flight muscles (SW_P ; Chapter 1). We have previously shown that lab reared females (Chapter 1) and males (Chapter 3) of both wing morphs that have developed, pink flight muscles pay a reproductive cost for these muscles, but only LW_P crickets are flight capable (Chapter 2).

In this study we examined morph specific differences in, (1) investment in reproductive tissues (females: ovaries; males: testes and accessory glands), and (2) investment in lipids (triglycerides and phospholipids). We hypothesized that if individuals in the field, like lab reared individuals, trade off investment in reproduction with investment in flight, then flightless individuals (SW_W and LW_W) should invest more in reproductive tissues than flight capable individuals (LW_P). Secondly, previous work on lines selected to be almost pure breeding LW and SW in a different species of field cricket, *G. firmus*, found that LW_P females, relative to SW_W females, have (1) higher levels of whole-body and somatic triglycerides, but lower levels of ovarian triglycerides, and (2) have similar levels of somatic phospholipids, but lower levels of ovarian phospholipids (Zera, 2005). In insects, these two lipid classes make up more than 90% of total lipid, with triglycerides being the major energy storage material and phospholipids being the major component of biological membranes (Zera, 2005). The increased amount of triglycerides found in the somatic tissues of flight capable females may be explained

by these females requiring energy to fuel flight (Zera et al., 1999). Likewise, the increased amount of phospholipids in the ovaries of flightless females may be explained by phospholipids being an important component of vitellogenin in eggs (Beenakkers et al., 1985). Therefore, we hypothesized that flightless and flight capable field caught *G. lineaticeps* females should show similar patterns, with flight capable females having higher body triglyceride content, and flightless females having higher ovary phospholipid content. In addition, we examined lipid levels in field caught males, hypothesizing that as flight capable males, like flight capable females, require flight fuels, they should have higher amounts of triglycerides than flightless males. To our knowledge, this is first study to examine lipid types and amounts in males of any flight polyphenic insect.

While there has been much research on the physiological basis of the life history tradeoffs under laboratory conditions, specifically in flight polyphenic insects, there is a paucity of studies examining how such tradeoffs manifest in natural populations. The results of this study will bring us a long way in assessing how the physiology underlying the life history tradeoff between flight and reproduction is expressed in the wild.

METHODS

We collected 111 male and female field crickets at Sedgwick Reserve, Santa Ynez Valley, California over several nights between 1st and 18th July, 2008. Within two hours of the collection time, we recorded the wing and flight muscle morph of the individuals, and froze them in dry ice for transportation back to the University of Nebraska–Lincoln. The crickets were thereafter stored at -80°C. All measures reported here were from these field collected animals.

In the lab, we first recorded the mass of the collected individuals (56 females: 16 LW_P, 7 LW_W, and 33 SW_W; 55 males: 12 LW_P, 8 LW_W, 1 SW_P, and 34 SW_W) to 0.1 mg. As we only had collected 1 SW_P, we did not include this morph in further analyses.

In order to examine morph-specific variation in mass of reproductive tissues in females, we removed and weighed ovaries to the nearest 0.1 mg, and also noted flight muscle color and wing morph. The ovaries and the rest of the body were placed separately in 1.5ml microcentrifuge tubes. With males, we removed and separately weighed the testes and the accessory glands to the nearest 0.1mg, and noted flight muscle color and wing morph. We placed the whole male body in a labeled 1.5ml microcentrifuge tube (as testes and accessory glands are too small for individual analyses of lipids or C:N ratios). We used ANOVA to examine differences in testes and accessory gland mass; as the ovary mass was not normally distributed, we used a Kruskal-Wallis H test to examine differences among morphs, using Mann-Whitney U tests for posthoc comparisons. We controlled for multiple comparisons by using Holm-Bonferroni corrections. All analyses were carried out using SPSS 19.0.0.

The samples in these tubes were freeze-dried, reweighed, and homogenized in 2:1 chloroform/methanol (CHCl₃/MeOH) to extract total lipids (Christie, 1982; Zhao and Zera, 2001). In brief, we homogenized the sample and filtered it. The residue left on the filter paper was placed back in a tube, more 2:1 chloroform/methanol was added, and the sample was re-homogenized and re-filtered. This procedure was repeated one more time for three total homogenizations followed by filtrations. This left us with a liquid extract that contained lipids as well as some carbohydrates and proteins. The liquid extract was vortexed with 0.88% KCl in water and centrifuged. The aqueous supernatant, containing

carbohydrates and proteins, was then removed. This was repeated two more times. At the end of this procedure, we were left with total extracted lipids in 2:1 chloroform/methanol solution

Next, we separated the lipid solution into triglycerides and phospholipids using column chromatography (Zhao and Zera, 2002). We washed the columns with methanol and allowed them to dry. Next we added an aliquot of the extracted lipids to the column, followed by 8:2 hexane:diethyl ether to elute the triglycerides (and a small amount of other neutral lipids). Then we added 65:30:5 chloroform:methanol:water to elute the phospholipids. We verified the separation of triglycerides and phospholipids by thin-layer chromatography using triglyceride and phospholipid standards. Finally, we measured the amounts of triglycerides and phospholipids using the vanillin assay (Van Handel, 1985) in a subset of the collected animals. We assayed the bodies of a total of 47 individuals (23 females: 8 LW_P, 7 LW_W, and 8 SW_W; 24 males: 8 LW_P, 8 LW_W, and 8 SW_W), and the ovaries of 22 females (the lipid extracts of one assayed LW_P female's ovaries were misplaced). Triolein was used as a standard for this assay (Zera and Larsen, 2001). We used ANCOVA to assess morph-specific differences between morphs in triglyceride and phospholipid mass of bodies (male: whole body, female: whole body minus ovaries), and of ovaries, using dry total body mass as a covariate. We controlled for multiple comparisons by using Holm-Bonferroni corrections. All analyses were carried out using SPSS 19.0.0.

RESULTS

Differential resource allocation to reproductive tissues

Ovary mass in females varied significantly among morphs ($H_{2,57} = 23.28$, $P < 0.001$; Figure 4.1), with SW_W ($U = 508.5$, $P < 0.001$, critical $P = 0.017$) and LW_W ($U = 108.00$, $P < 0.001$, critical $P = 0.025$) females having significantly larger ovaries than LW_P females. However, there was no difference between the SW_W and LW_W females ($P = 0.073$, $U = 53.00$, critical $P = 0.05$).

Accessory gland mass in males varied significantly among morphs ($F_{2,51} = 9.66$, $P < 0.001$; Figure 4.2), with SW_W males having significantly larger accessory glands than LW_P males ($P < 0.001$, critical $P = 0.017$). There was a non-significant trend that SW_W and LW_W males have larger accessory glands than LW_P males ($P = 0.026$, critical $P = 0.025$). There was no difference between the SW_W and LW_W males ($P = 0.284$, critical $P = 0.05$).

Testes mass in males showed the reverse pattern. While it also varied significantly among morphs ($F_{2,51} = 23.68$, $P < 0.001$; Figure 4.3), LW_P males had significantly larger testes than both SW_W ($P < 0.001$, critical $P = 0.017$) and LW_W ($P = 0.001$, critical $P = 0.025$) males. As with previous measures, there was no difference between the SW_W and LW_W individuals ($P = 0.108$, critical $P = 0.05$).

Differential resource allocation to lipids: Triglycerides and phospholipids

The mass of triglycerides in the bodies of individual crickets was significantly affected by the interaction between sex and morph ($F_{2,40} = 5.92$, $P = 0.006$). Therefore, we analyzed the data for each sex separately. We found that in females, morph

significantly affected triglyceride mass in the body ($F_{2,19} = 26.92$, $P < 0.001$, Figure 4.4a), with SW_W females having significantly less triglycerides than both LW_P ($P < 0.001$, critical $P = 0.017$) and LW_W females ($P < 0.001$, critical $P = 0.025$). There was a non-significant trend in which LW_P females had more triglycerides than LW_W females ($P = 0.051$, critical $P = 0.05$). Dry total body mass was a significant covariate ($F_{1,19} = 10.64$, $P = 0.004$), with heavier crickets having more triglycerides. In males, we found no effect of morph ($F_{2,20} = 2.91$, $P = 0.078$; Figure 4.4b) or dry total body mass ($F_{1,20} = 2.79$, $P = 0.110$) on triglyceride mass.

There was a significant effect of morph ($F_{2,40} = 17.87$, $P < 0.001$; Figure 4.5) on the phospholipid mass in bodies of individual crickets, with LW_P individuals having significantly more phospholipids than both LW_W ($P = 0.002$, critical $P = 0.025$) and SW_W ($P < 0.001$, critical $P = 0.017$) individuals. SW_W and LW_W individuals did not differ in phospholipid mass ($P = 0.092$, critical $P = 0.05$). There was also a significant effect of sex on phospholipid mass in bodies of individual crickets ($F_{1,40} = 6.76$, $P = 0.013$), with males having higher masses than females. There was no effect of the interaction between sex and morph ($F_{2,40} = 1.33$, $P = 0.276$). Dry total body mass was a significant covariate ($F_{1,40} = 4.41$, $P = 0.042$), with heavier crickets having less phospholipids.

Our analyses of lipid content of ovaries yielded very different patterns. While we found that morph significantly affected triglyceride mass in the ovaries ($F_{2,18} = 12.47$, $P < 0.001$, Figure 4.6), LW_W females had significantly more triglycerides in their ovaries than either LW_P ($P < 0.001$, critical $P = 0.017$) or SW_W (SW_W : $P = 0.004$, critical $P = 0.025$) females. LW_P and SW_W females did not differ in triglyceride levels ($P = 0.729$,

critical $P = 0.05$). Dry total body mass was not a significant covariate ($F_{1,18} = 4.10$, $P = 0.058$).

Lastly, we found that morph significantly affected phospholipid mass in the ovaries ($F_{2,17} = 5.89$, $P = 0.011$, Figure 4.7), with SW_W ($P = 0.004$, critical $P = 0.017$) and LW_W ($P = 0.022$, critical $P = 0.025$) females having significantly more phospholipid in their ovaries than LW_P females. SW_W and LW_W females did not differ in ovary phospholipid mass ($P = 0.351$, critical $P = 0.05$). Dry total body mass was not a significant covariate ($F_{1,17} = 1.67$, $P = 0.214$).

DISCUSSION

Life history traits are often negatively correlated within resource limited individuals, because increasing resources to one trait results in decreasing resources to others (Van Noordwijk and De Jong, 1986). Such tradeoffs, and the physiology underlying them, have been extensively studied in many species, especially under lab conditions (reviewed in Zera and Harshman, 2001). However, in order to explore how such tradeoffs affect life history evolution in the wild, we need to assess trait variation in both the lab and the field. We need such multiple, complementary approaches because both lab and field studies have certain advantages and disadvantages. Under lab conditions, we can precisely control environmental factors, such as resource availability, and manipulate factors of interest separately. However, from a different perspective, this is also a disadvantage because in nature, the life history traits of organisms evolve in heterogeneous environments, shaped by multiple, temporally and spatially changing selective forces (Reznick and Ghalambor, 2005). Therefore, in order to assess how the

tradeoffs we find in the lab are expressed under natural conditions, we need to check our lab results via field experiments. Here, we assessed the tradeoff between flight and reproduction using field caught individuals of a flight polyphenic field cricket.

Differential resource allocation to reproductive tissues

We found that field caught females with developed flight muscles have significantly smaller ovaries than females with undeveloped or histolyzed flight muscles. This finding is consistent with our results from lab-reared females (Chapter 1). Ovary mass is a good measure of female fecundity, as ovary mass is highly correlated to egg number (Roff, 1994). Therefore, this finding supports the hypothesis that flightless females invest more in reproduction than flight capable females.

The data for field caught males partially matches what we found with lab-reared animals. We found that field caught males with developed muscles have significantly smaller accessory glands, which produce seminal fluids, than males with undeveloped or histolyzed flight muscles. This finding is consistent with our results from lab-reared males (Chapter 3). As accessory gland size has been shown to affect mating and/or reproductive success in some insects (Bangham et al., 2002; Demary and Lewis, 2007; Leopold, 1976; Wolfner, 1997), this finding is consistent with the hypothesis that flightless males invest more in reproduction than flight capable males.

In contrast, we found that field caught males with developed muscles have significantly larger testes than males with undeveloped or histolyzed flight muscles. As increasing testes size has been shown to increase male reproductive success, often by increasing success in sperm competition, in a number of species (e.g., Bangham et al.,

2002; Demary and Lewis, 2007; Schulte-Hostedde and Millar, 2004), these results suggest that in the field, flight capable males are investing more in this component of reproduction than flightless males. Work on other field crickets has shown that egg fertilization is mainly determined by lottery (e.g., Sakaluk, 1986; Simmons, 1987). Therefore, if having larger testes affects the amount of sperm transferred per mating, flight capable males may be trading off their investment in different reproductive tissues, and investing in testes at the cost of accessory glands. However, we found no effect of morph on testes size in lab-reared males (Chapter 3). A possible explanation for these divergent results in lab-reared and field caught animals is that resources are more limiting in the field than in the lab. Therefore, while lab-reared males can afford to invest in both testes and accessory glands, morphs have to prioritize investing in one over another in the field. This hypothesis can be tested by varying the diet of lab-reared males, and assessing whether testes and accessory glands are negatively correlated under low diet conditions.

Differential resource allocation to lipids: Triglycerides

Triglycerides are the most common lipid in insects, and are used as an energy storage molecule (Zera, 2005). Previous work with selected lines of nearly pure breeding LW and SW *G. firmus* individuals found that LW_P females have significantly more triglycerides in their somatic tissues than do SW_W females (Zera, 2005; Zera and Larsen, 2001). We found similar results in the somatic tissues of our field caught females: LW_P had significantly more triglycerides than SW_W females. LW_W females also had significantly more triglycerides than SW_W females in their somatic tissues, and although there was a strong trend for LW_P to have more triglycerides than LW_W females, this was

not statistically significant. In contrast, morph-specific triglyceride amounts in the ovaries showed very different patterns. We found no difference between LW_P and SW_W females. However, LW_W females had significantly higher triglyceride amounts in their ovaries when compared to the other two morphs. The results of these morph-specific differences in somatic and ovarian triglyceride amount tell a clear story: LW_P females invest highly in triglycerides in their somatic tissue at least partially for use as a flight fuel (Zera, 2005). After flight muscle histolysis, LW_W females shift allocation of somatic triglycerides to their ovaries, and increase investment in reproduction. SW_W females, who have lower energetic requirements since they do not disperse, have the lowest amounts of triglycerides in all tissues.

Interestingly, there were no morph-specific differences in triglyceride mass in males. One explanation for these results may be that flightless and flight capable males both require triglycerides, potentially for different purposes. We previously found that flightless males spend significantly more time producing mate attraction signals than do flight capable males (Chapter 3). Males rub their forewings together to produce long distance calling songs to attract females (Wagner, 1996), and producing song has been shown to be energetically expensive in many species of crickets (Hoback and Wagner, 1997; Kolluru et al., 2004; White et al., 2008). In addition, in a different species of field cricket, increasing lipid weight has been shown to be correlated with increasing call duration (Crnokrak and Roff, 2000). To our knowledge, no one has assessed whether triglycerides affect singing activity in insects. Therefore, while flightless and flight capable males may be trading off investment in reproduction and flight, both investment

in reproduction and flight may require high levels of energy storage molecules, here triglycerides.

Differential resource allocation to lipids: phospholipids

Phospholipids are the second most abundant type of lipid in insects, found in all biological membranes and an important component of the yolk protein vitellogenin in eggs (Zera, 2005). Previous work has found that in selected lines of *G. firmus*, the short wing and long wing lines differ in how they allocate phospholipids. Females of SW_w lines have more total phospholipids than females of LW_p lines (Zera and Larsen, 2001). However, females of LW_p lines allocate more phospholipids to their somatic tissues, while females of SW_w lines allocate more to their ovarian tissues (Zera, 2005).

We found similar results in the ovarian tissues of our field caught females: the two flightless morphs (SW_w and LW_w) had significantly more ovarian phospholipids than the flight capable morph (LW_p). This is not surprising given that we found that the flightless females have larger ovaries, and therefore likely produce more eggs, than flight capable females. In contrast, flight capable individuals had more phospholipids in their somatic tissues than flightless individuals, and males had more phospholipids in their somatic tissue than females. As pink flight muscles have been shown to contain more phospholipids than white muscles in a species of locust (Nováková et al., 1976), the large, pink flight muscles of flight capable individuals may contain more phospholipids than reduced, white flight muscles of flightless individuals. Secondly, this may also explain why males, who need developed muscles for producing calling song, have more phospholipids than females.

Life history tradeoffs in the lab and in the field

The physiological basis of the life history tradeoff between reproduction and flight capability has been extensively examined in lab-reared flight polyphenic field crickets (Zera, 2009). We investigated this same tradeoff in the field in order to examine whether we see similar patterns under lab conditions and in the field. Our results suggest that this tradeoff is similarly manifested in the wild and in the lab for some traits: flightless and flight capable individuals tradeoff investment in some reproductive tissues (ovaries and male accessory glands), and lipids (triglycerides and phospholipids). However, other traits such as testes size show very different patterns, suggesting that some traits may be more strongly affected by field conditions. This study highlights the importance of such multiple, complementary approaches to exploring life history evolution.

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FIGURE LEGENDS

Figure 4.1

Boxplot of variation between females of different flight morphs in ovary mass: flightless females (SW_W and LW_W) have significantly larger ovaries than flight capable females (LW_P).

Figure 4.2

Variation between males of different flight morphs in accessory gland mass: SW_W males have significantly larger accessory glands than LW_P males, while LW_W males do not differ significantly from either. Cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 4.3

Variation between males of different flight morphs in testes mass: flight capable males (LW_P) have significantly larger testes than flightless males (SW_W and LW_W). Cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 4.4

Variation between (a) females and (b) males of different flight morphs in the amount of triglycerides in the body, controlling for dry total body mass. (a) Long winged female bodies (LW_P and LW_W) have significantly more triglycerides than SW_W female bodies. (b) Male morphs do not differ in the amount of triglycerides in their bodies. Adjusted cell

means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 4.5

Variation between individuals of different sexes and flight morphs in the amount of phospholipids in the body, controlling for dry total body mass. Flight capable individuals (LW_P) have significantly more phospholipids in their bodies than flightless individuals (SW_W and LW_W), and males have more phospholipids in their bodies than females. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 4.6

Variation between females of different flight morphs in the amount of triglycerides in ovaries, controlling for dry total body mass. LW_W females had significantly more triglycerides in their ovaries than either SW_W or LW_P females. Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 4.7

Variation between females of different flight morphs in the amount of phospholipids in ovaries, controlling for dry total body mass. Flightless females (SW_W and LW_W) had significantly more phospholipids in their ovaries than flight capable females (LW_P).

Adjusted cell means and SE are plotted, and the different letters designate statistically significant differences ($P \leq 0.05$).

Figure 4.1

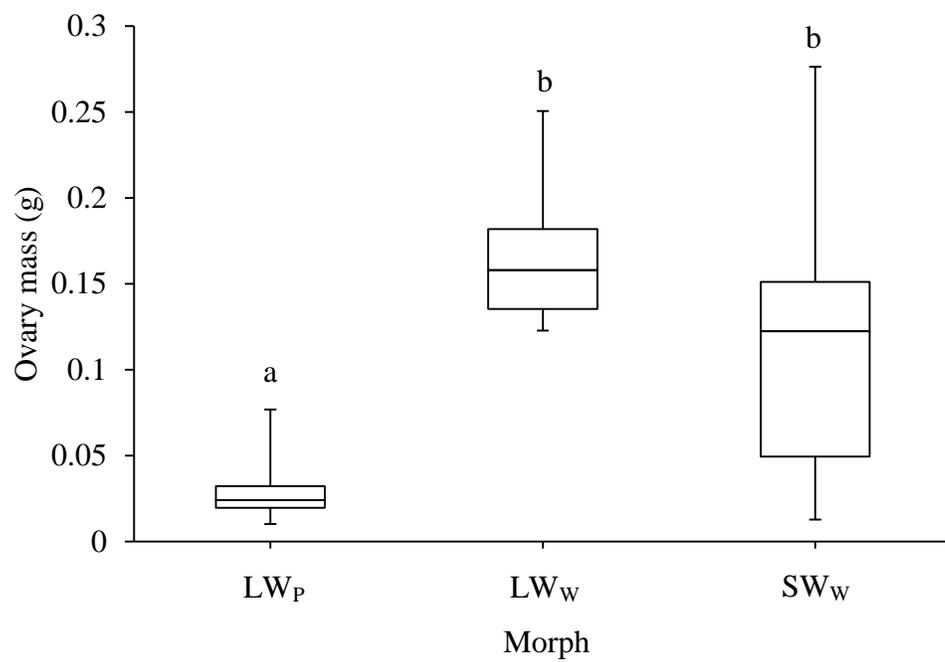


Figure 4.2

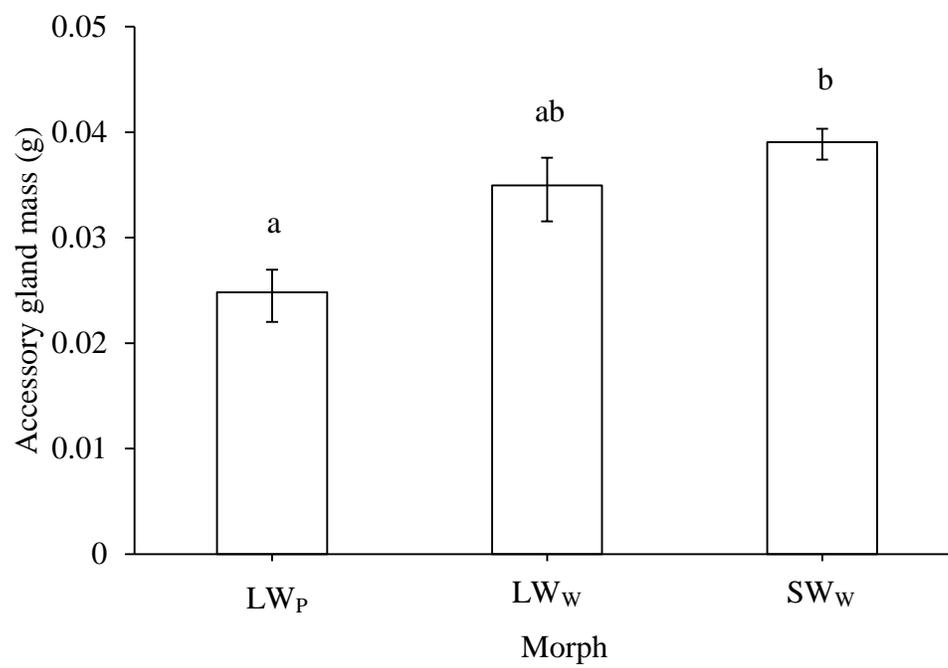


Figure 4.3

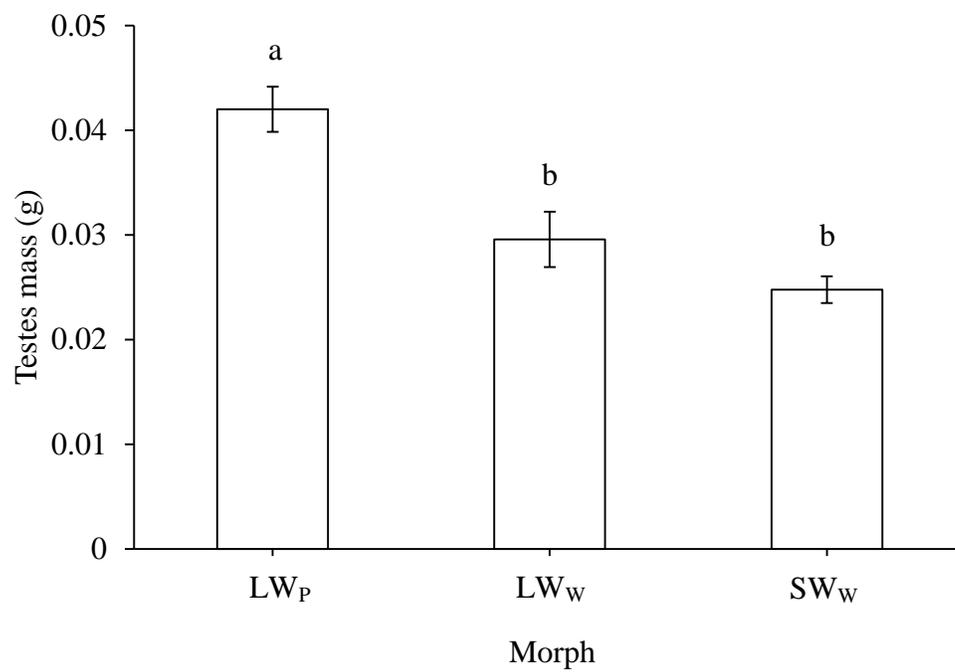


Figure 4.4

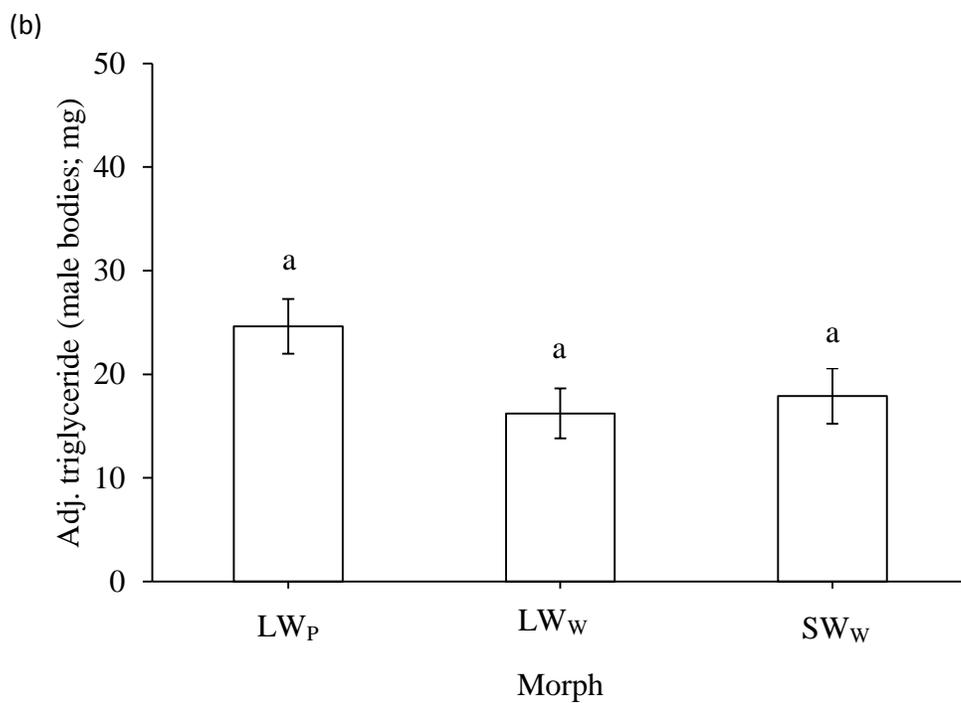
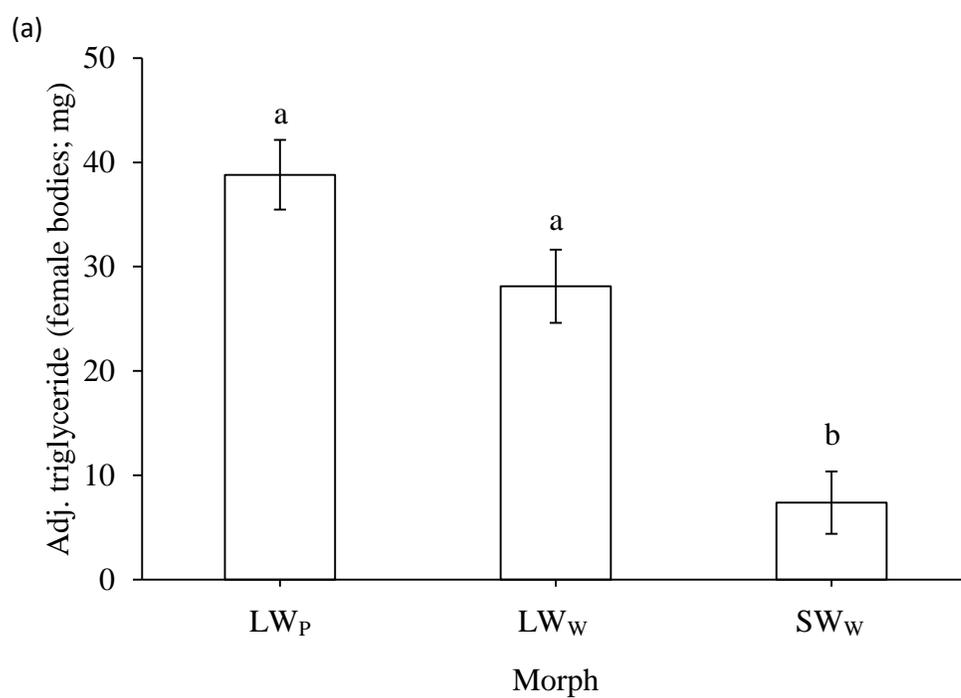


Figure 4.5

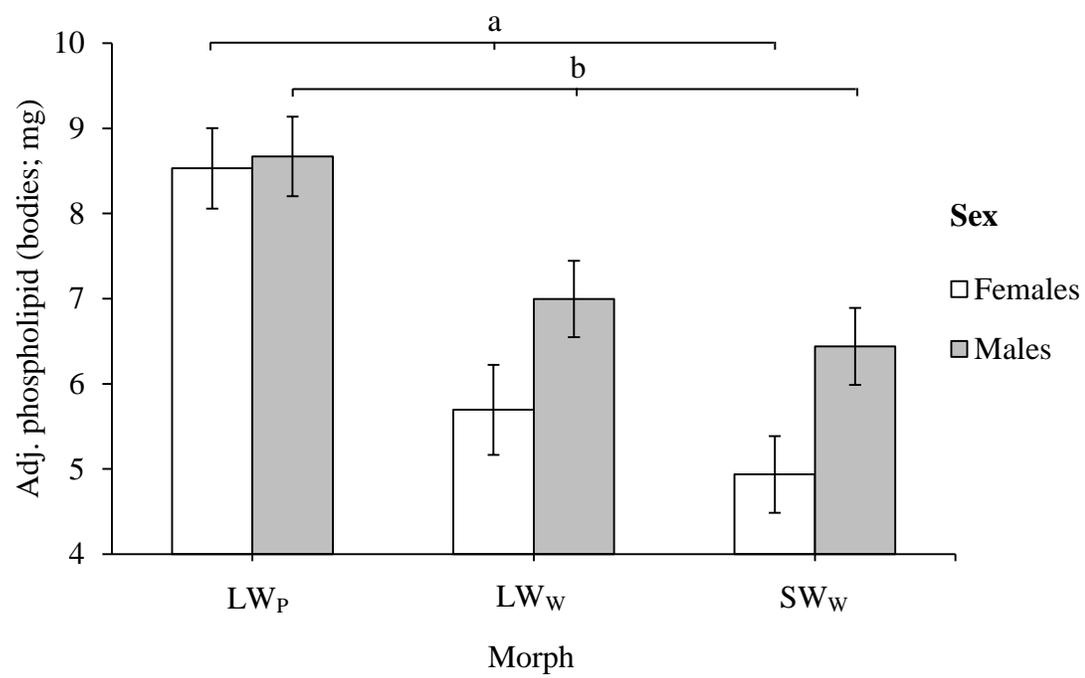


Figure 4.6

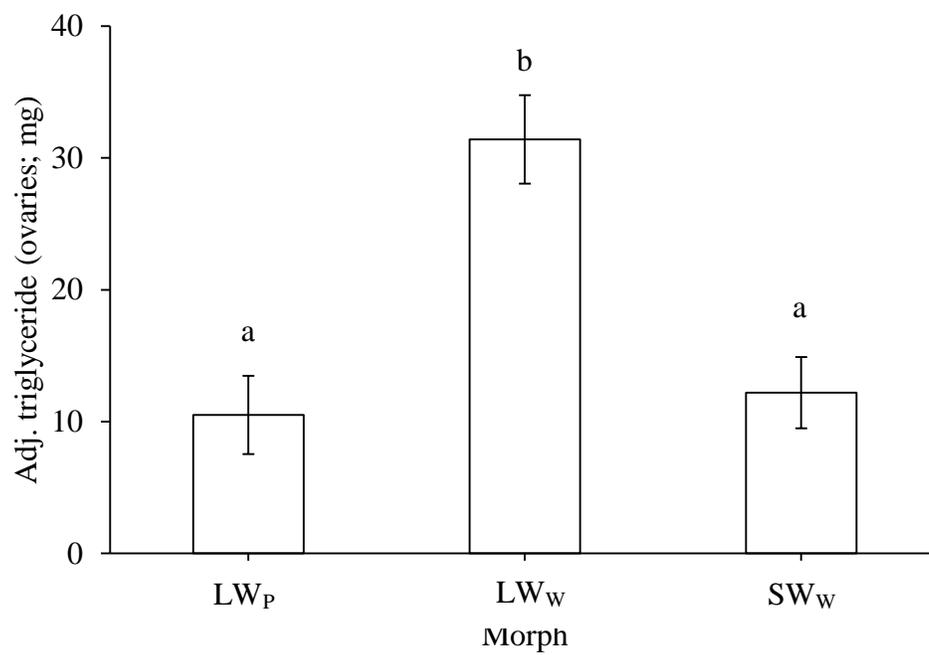


Figure 4.7

