PROXIMATE CAUSES OF VARIATION IN SIGNALING AMONG THREE SISTER SPECIES OF *GRYLLUS* CRICKETS

A thesis submitted in partial fulfillment of the requirements
For the degree of Masters of Science
in Biology

By
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ABSTRACT

PROXIMATE CAUSES OF VARIATION IN SIGNALLING AMONG THREE SISTER SPECIES OF *GRYLLUS* CRICKETS

By

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Master of Science in Biology

Mating signals play an important role in both mate selection and species recognition. Acoustic mating signals have been shaped under various evolutionary constraints with remarkable malleability in response to variable conditions. These same traits have been shown to serve as indicators of species identity and mate quality. But how will sexually selected signal traits be expressed as environmental conditions change and as species diverge? Using three closely related *Gryllus* crickets (*Gryllus lineaticeps*, *G. personatus*, and *G. sp15/“G. staccato”*), I assessed how components of male song (chirp rate, pulse rate, frequency, and pulses per chirp) and morphology responded to varying environmental dietary conditions within and among genetically structured family groups (sibships). Experimental diets had some effects on morphology with differences in mass and pronotum width in some species, but the diets had no effects on body condition or components of calling song, save one trait in one species. In contrast, sibship consistently affected chirp rate and frequency but did not affect pulse rate and pulses per chirp either within or among species. Interestingly, the dynamic song traits, chirp rate and
pulses per chirp, exhibited broad variation in all species, as expected, but responded differently from each other to sibship. Likewise, the static song traits, frequency and pulse rate, exhibited limited within-species variation, as expected, but had different contrasting effects of sibship. Overall genetic variation in song traits was most strongly associated with species divergence, and then with dam but not sire effects within species. Overall, these results suggest conservation across species of the major sources of within-species song variation; that is, divergence among species appears to be associated with changes in the average values of song traits, especially static ones, but not consistent divergence in the sources of within-species variation.
INTRODUCTION

Acoustic mating signals provide opportunities to study sexually selected trait evolution and how such traits diverge or are conserved among closely related species. Acoustic signals form the basis of mating systems and some of the most well-studied acoustic mating systems are found in the Orthoptera (crickets, grasshoppers, katydids) (Gerhardt 1991, Gerhardt & Huber 2002). These insects produce acoustic signals through energetically costly stridulatory motion of their anatomy, playing a part both in male–male competition and female choice. These signals are typically species specific and can also be subject to female mate preferences. Often, some signals show low within-species variation, and are termed ‘static’ – such signals are usually subject to stabilizing or weakly directional female preferences. Other signals traits may show higher levels of within-species and/or within-individual variation, and are termed ‘dynamic’ signals, and are often subject to directional female preferences (Gerhardt & Huber 2002).

Among orthopterans, members of the subfamily Gryllinae, which includes \textit{Gryllus} (field crickets), are prime examples for investigating the role of signal divergence in speciation (Weissman 1980, Lande 1981, West-Eberhard 1983, 1984). Male field crickets rub their forewings together to producing a calling song to attract female crickets for mating; female crickets respond to male song by walking or flying toward a male whose song they find attractive (Zuk & Simmons 1997). Historically, North American \textit{Gryllus} were considered to be one widespread but highly variable species until recording and analysis of cricket song revealed that cricket species could be easily distinguished by examining differences in male calling song in tandem with morphology, life cycles, and geographic variation (Alexander 1957). Calling song can vary in several characteristic
components including chirp rate, chirp duration, inter-chirp period, pulse rate, pulse duration and inter-pulse duration (Walker 1957, Hedrick 1986, Otte 1974). Some differences in song are even the primary means by which crickets distinguish conspecifics as evidenced by lack of female response when presented with the songs of heterospecific males (Gray 1997, Gray & Cade 2000, Izzo & Gray 2004 Gray et al. 2015).

The songs of *Gryllus* males demonstrate a wide range of potential acoustic variability. Some songs consist of simple chirps, a series of chirps, or a relatively continuous series of pulses (20 or more) referred to as a trill, while others present a variable mix of two or more components. These components are important to informing female choice, which may reflect aspects of male quality, such as body size, diet, and even the immune system, via specific song traits, such as chirp rate and chirp duration (Gray 1997, Wagner & Hoback 1999, Ryder & Siva-Jothy 2000). In some cases, females prefer ‘dynamic’ highly exaggerated and energetically costly male signals as opposed to values that are near the average for the population (Hoback & Wagner 1997). This form of mate discrimination is important especially among very closely related species, as it can be the mechanism by which rapid divergence occurs. Several species within *Gryllus* show evidence of a recent divergence according to molecular phylogenetic analyses (Gray et al. 2008). In the Southwestern U.S., one such sister-species complex is *Gryllus lineaticeps*, *G. personatus*, and *G. sp15* (Gray et al. 2015). *G. sp15* is currently being described and will be formally named “*Gryllus staccato*”, so throughout this thesis I will use “*G. staccato*” to provide continuity in the literature.
*Gryllus* crickets have served as a model for investigating evolution and sexually selected trait expression (Fitzpatrick & Gray 2001, Scheuber et al. 2003, Holzer et al. 2003, Ritz & Kohler 2010, Tolle & Wagner 2011), especially into components of male calling song such as chirp rate and chirp duration. However, to date, other song characteristics, such as pulse duration and interpulse duration have yet to be assessed for their part in reflecting male mate quality. Coefficients of variation associated with such a trait could potentially indicate the form of selection (i.e. directional or stabilizing) (Gerhardt 1991, Murphy & Gerhardt 2000). The sources of variation that affect these traits are generally unknown, but could include both environmental and genetic effects.

My study seeks to explore how components of male cricket calling song exhibit divergence in response to varying environmental condition within this sister-species complex of crickets by using dietary manipulation as a means to investigate condition-dependent trait expression and structured breeding lineages to compare genetic effects within and among species. The qualitatively different diets I used were meant to provide a means of exploring potential genotype-by-environment interactions (GEI) (Ingleby et al. 2010). Dietary manipulation in crickets is in principle simple (Gray & Eckhardt 2001), and several studies, including one with *G. lineaticeps*, have shown that diet alters male song (Wagner & Hoback 1999). Studies investigating the role of diet in cricket development and signaling have shown that varying the amounts of single dietary components can have an important impact on morphological development and signaling effort; however, timing of delivery is also important (Bertram et al. 2011). Qualitatively variable diets given during adulthood provide individuals with energy that can be allocated to signaling effort, but the same diets given during the nymph stages allow
allocation of energy to development that can have a direct impact on signaling effort as a result of increased body size and structures relevant to signaling effort (Hunt et al. 2004). Hunt et al. showed that because males reared under different dietary conditions did not show significant differences in mass, males were allocating the excess energy toward increasing calling effort to increase their attractiveness to females. Hunt et al.’s results deserve to be replicated to assess whether and how additional song components are responding, clarification of which could change our outlook on these formative processes.

The objective of my study is to observe how diet and genotype affect morphology (body size and overall condition) and male calling song traits in these three sister species to elucidate potential patterns of song trait expression within and among species. For example, signals of mate quality are more likely to remain constant across speciation events, whereas arbitrary signals unrelated to mate quality are more likely to change (Via & Lande 1985). Thus, signals conveying information about mate quality should remain fixed under stabilizing selection with conservation of quality indicated by similarity among species and that signals unrelated to mate quality should be highly variable with divergence indicated by differences among species in the traits assessed.

Prior research and preliminary dietary manipulations resulted in qualitative differences due to diet treatment. Controlling for diet has a discernible effect on male calling song (Wagner & Hoback 1999, Scheuber et al. 2003, Tolle & Wagner 2011). It also accounts for differences in heritable traits attributable to sire or maternal effects attributable to the dam (Mousseau & Roff 1989, Hedrick 2005, Bertram et al. 2007). For each of the three species, offspring from wild-caught females were reared in the lab and
randomly assigned into breeding groups consisting of a single sire with three dams, producing full-sibling offspring within each sire–dam pairing and half-sibling offspring among each of the three dams for each species to assess the potential genetic effect of sires and dams on their offspring. The full-sibling offspring of each sire–dam pairing were reared on two qualitatively different diets—one of low/poor nutritive quality and one of high/good nutritive quality—to assess the potential effect of diet on trait expression among species.

By comparing species, differences in the male calling songs for the offspring of all three species can be assessed for a suite of signal traits and the factors affecting them. Divergence in trait expression is indicated by differences among species in arbitrary male song traits, while conservation of quality-indicating traits is indicated by similarity among species, meaning that those traits may indicate male quality regardless of species. My study will allow for better characterization of trait evolution in this closely related group of crickets. To date, the role of genetic effects by way of inherited traits and environmental effects by way of diet on the variation in trait expression and potential patterns in expression have yet to be investigated through a comparative approach for crickets with a closely related sister-species complex of *Gryllus* crickets.

Given these interests and the progress I was able to make, my thesis will be structured around three topics. (1) Did differences in the quality of diets represent major environmental effects? (2) Is there quantitative genetic variation among sibships in song traits and aspects of morphology? (3) How does this variation within species relate to divergence among species in those same traits?
MATERIALS AND METHODS

To explore the variation of traits expressed in male calling song, I collected female crickets to initiate laboratory colonies, conducted a dietary manipulation experiment, and analyzed the songs of adult male half-siblings from high-quality and low-quality diet treatments.

Field Collection and Identification

Wild-caught female field crickets were collected in summer 2010 and summer/fall 2011 from three field sites: *Gryllus lineaticeps*, Malibu Creek, Los Angeles Co., CA (34.0986840°N, -118.7167490°W, 171 m); *G. personatus*, Winslow, Yavapai Co., AZ (35.0288670°N, -110.6850460°W, 1476 m); and “*G. staccato*”, Agua Fria National Monument, Navajo Co., AZ (34.2391600°N, -112.0266220°W, 1137 m). In summer 2010, 28 *G. lineaticeps* females, 11 *G. personatus* females, and 14 “*G. staccato*” females were collected. In summer/fall 2011, 17 *G. lineaticeps* females, 28 *G. personatus* females, nine *G. personatus* males, 15 “*G. staccato*” females, and 17 “*G. staccato*” males were collected. Crickets were collected during the day by inspecting potential refuges and at night by broadcast playback of male calling song. Females perceive playbacks as conspecific potential mates.
Laboratory Rearing

The wild-caught females were used to initiate laboratory colonies. Female crickets exhibit polyandrous mating (Tregenza & Wedell 2002, Ivy et al. 2005) with typically multiple sires per clutch (Bretman & Tregenza 2005); thus, the offspring of multiple females are typically broadly representative of the genetic diversity of wild populations. Wild-caught female crickets were housed in species-specific 167-L tubs at 27 ± 1°C with a photoperiod of 12:12 and provisioned with dry cat food (Purina Cat Chow, St. Louis, MO), water in cotton-plugged vials available ad libitum, and cardboard egg cartons for shelter. Dishes (deli containers, 16 oz, 453 g) with a moist mixture of peat moss and vermiculite were provided for 1 wk to allow for egg deposition, and subsequently replaced with a new dish to allow for continued oviposition. Each egg dish was housed in a rearing tub at 27 ± 1°C and provisioned with food and water for nymph development (Gray & Cade 2000).

Breeding Design

Offspring of wild-caught females were used for my experimental manipulations. The sample size for experimental manipulation called for sires from each of the three study species (30 sires per species, 90 sires total) with three dams per sire (90 dams per species, 270 dams total), and no fewer than six male offspring per dam family (540 sons per species, 1620 sons total) with one half assigned to each of two dietary treatments. Offspring were at least one generation removed from wild-caught breeding stock to alleviate maternal effects. Rearing containers were checked twice weekly for food, water, and last-instar juveniles. Penultimate-instar juveniles were removed and reared in
separate-sex containers until post-imaginal molt. Virgin first-generation offspring were then randomly paired into unique family groups (three dams per sire) approximately 6 d post-imaginal molt in 3.1-L containers. Sires and dams were housed together for 1 wk to allow for insemination. Each dam was then housed with a soil egg dish for 1 wk to allow for oviposition. This breeding design establishes full-sibling dam families within half-sibling sire families, which allowed me to investigate genetic effects following diet manipulations (Gray & Cade 1999).

*Offspring Diet Manipulation*

Second-generation offspring of wild-caught females were reared in full-sibling groups at 27 ± 1°C with food and water provided *ad libitum*. Two to three instars prior to adult eclosion, full-siblings within each dam family were randomly assigned to high- and low-quality dietary treatments with 13 individuals in each treatment. Juvenile females were selectively removed from family groups as they matured to mitigate the effects of density such as increased development time or reduced body size or condition (Niemela 2011). Dietary treatments were qualitatively different based on relative amounts of essential nutrients and performance previously investigated for *Acheta domesticus* in Patton 1967 (Table 1). Full-sibs were maintained on their respective diets for the remainder of nymph development and throughout adulthood.
Table 1. Components and nutrient composition of experimental diets.

<table>
<thead>
<tr>
<th>Components (g/100)</th>
<th>High Quality</th>
<th>Low Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewer’s yeast ¹</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Cornmeal ²</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Liver powder ³</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Milk, dried skim ⁴</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Soybean meal ⁵</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Wheat middlings ⁶</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td><strong>Composition (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>20.4</td>
<td>23.8</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>47.0</td>
<td>36.2</td>
</tr>
<tr>
<td>Fat</td>
<td>3.2</td>
<td>4.9</td>
</tr>
</tbody>
</table>

¹ Solgar Brewer’s Yeast; ² Bob’s Red Mill Cornmeal, Medium Grind; ³ NOW foods Argentine Beef Liver Powder; ⁴ Albertsons Instant Nonfat Dry Milk; ⁵ Bob’s Red Mill Soy flour; ⁶ Miller Milling Company (Commerce, CA)
Song Recording and Analysis

Calling songs of second-generation full-sib laboratory-reared males of each species were recorded several days post-imaginal molt (Figure 1). Calling males were recorded to a computer (16 bit, 44.1 kHz; Dell, Inspiron, Round Rock, TX) using a Sony PC-62 stereo microphone (Japan) with The Sound Professionals dual channel microphone preamplifier (Phoeniz, AZ). After recording, morphological measurements (mass [g] and pronotum width [mm]) were taken using a digital scale and digital calipers (± 0.01 mm). Male age at the time of audio recording was calculated as the number of days post imaginal molt and is accurate to within 3 d. Songs were analyzed with the acoustics software program CoolEdit2000® (Syntrillium Software Corporation, Phoenix, AZ). Each song was analyzed to measure key song traits including pulse duration (ms), interpulse interval (ms), frequency (Hz), the number of pulses per chirp, and the interchirp interval (ms). Temperature at the time of recording (°C) was also noted with additional song traits (chirp rate [chirps/s] and pulse rate [pulses/s]) derived from the initial measurements.

Figure 1. Songs of the three cricket study species. Shown (top to bottom) is 1.5 s of song for *Gryllus personatus* (22.0°C), *Gryllus lineaticeps* (22.1°C), and “*Gryllus* staccato” (21.3°C).
Data Analysis

Analyses were conducted using SYSTAT 13 (13.1, San Jose, CA) and StatPlus:mac LE (Build 6.1.7.0/Core v6.1.60, Walnut, CA). For each species separately, I used the GLM module calculating univariate analysis of variance to determine the effect of diet on mass, pronotum width, and body condition. Type III sum of squares were used. General linear models were used to determine the effect of sire, dam within sire, diet, and diet by sire on mass, pronotum width, and body condition with estimated marginal means for the interaction of sire by diet on body condition to develop GEI reaction norms. Effects of sire and dam within sire are random variables, effect of diet is a fixed factor, and all effects were normal and independent with expectations equal to zero. Variance components were calculated in SYSTAT using the Mixed Model module set for restricted maximum likelihood.

Body Condition

For males of each species, body condition was estimated as residual mass, based on a linear regression of mass on pronotum width (Wagner & Hoback 1999, Ritz & Kohler 2010) (Figure 2). In Gray & Eckhardt (2001), this measure has been shown to reflect energetic fat reserves in crickets, particularly those on low quality diets.
Figure 2. An illustration of how body condition was calculated. Shown is the relationship between mass (g) and pronotum width (mm) for male *G. lineaticeps*. Individual body condition was estimated as the residuals from the linear regression line.
Temperature Correction of Song Traits

All songs were corrected for differences in recording temperature using the slopes from linear regressions of the song characters on temperature. Song characters were corrected to 22.5°C (Fitzpatrick & Gray 2001):

corrected song character = song character + slope * (recording temperature − 22.5)

<table>
<thead>
<tr>
<th></th>
<th>Chirp Rate¹</th>
<th>Pulse Rate²</th>
<th>Frequency³</th>
<th>Pulses per Chirp⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gryllus lineaticeps</em></td>
<td>0.02</td>
<td>1.9</td>
<td>-30.0</td>
<td>-0.249</td>
</tr>
<tr>
<td><em>Gryllus personatus</em></td>
<td>0.05</td>
<td>2.1</td>
<td>-38.1</td>
<td>-0.400</td>
</tr>
<tr>
<td><em>“Gryllus staccato”</em></td>
<td>1.2</td>
<td>0.16</td>
<td>16.9</td>
<td>-0.217</td>
</tr>
</tbody>
</table>

¹ Chirp rate (chirps/s); ² Pulse rate (pulses/s); ³ Frequency (Hz); ⁴ Pulses per chirp.
RESULTS

Song

Results are presented based on recordings of 447 males recorded in three species for two diets:

- 93 *G. lineaticeps* from 10 sires and 15 dams, 47 on diet A, 46 on diet B
- 186 “*G. staccato*” from 10 sires and 22 dams, 97 on diet A, 89 on diet B
- 164 *G. personatus* from 16 sires and 22 dams, 81 on diet A, 83 on diet B

Number of analyzed recordings varied among species and diet treatments due to asynchrony in sibship maturation, lack of calling effort, and mortality before a song could be recorded. Song data are presented in Tables 3–6 with details of means and standard errors for random variables in Appendices A–D.

*Were the diet treatments really different?*

Diet affected adult mass in “*G. staccato*” and in *G. personatus* but not in *G. lineaticeps* (Table 3). Diet did not affect pronotum width in any of the species. Standardizing biomass by pronotum width, adult condition was only affected in *G. personatus* but not in *G. lineaticeps* or “*G. staccato*”. When a difference was significant, diet A resulted in larger crickets than diet B.
Table 3. One-way ANOVAs for effect of diet on morphological measurements of males in three species (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Mass (g)</th>
<th>Pronotum width (mm)</th>
<th>Body Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G. lineaticeps</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet A</td>
<td>0.491 ± 0.012</td>
<td>5.105 ± 0.062</td>
<td>0.00732 ± 0.00749</td>
</tr>
<tr>
<td>Diet B</td>
<td>0.479 ± 0.013</td>
<td>5.120 ± 0.062</td>
<td>-0.00748 ± 0.00783</td>
</tr>
<tr>
<td>$F_{1,91} = 0.51$</td>
<td>$F_{1,91} = 0.03$</td>
<td>$F_{1,91} = 1.87$</td>
<td></td>
</tr>
<tr>
<td>$P = 0.477$</td>
<td>$P = 0.867$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G. personatus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet A</td>
<td>0.448 ± 0.008</td>
<td>5.011 ± 0.044</td>
<td>0.00698 ± 0.00479</td>
</tr>
<tr>
<td>Diet B</td>
<td>0.425 ± 0.007</td>
<td>4.943 ± 0.040</td>
<td>-0.00682 ± 0.00506</td>
</tr>
<tr>
<td>$F_{1,162} = 4.73$</td>
<td>$F_{1,162} = 1.31$</td>
<td>$F_{1,162} = 3.92$</td>
<td></td>
</tr>
<tr>
<td>$P = 0.031^*$</td>
<td>$P = 0.255$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>“G. staccato”</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet A</td>
<td>0.567 ± 0.009</td>
<td>5.685 ± 0.035</td>
<td>0.00680 ± 0.00609</td>
</tr>
<tr>
<td>Diet B</td>
<td>0.540 ± 0.009</td>
<td>5.608 ± 0.039</td>
<td>-0.00668 ± 0.00618</td>
</tr>
<tr>
<td>$F_{1,184} = 4.57$</td>
<td>$F_{1,184} = 2.16$</td>
<td>$F_{1,184} = 2.41$</td>
<td></td>
</tr>
<tr>
<td>$P = 0.034^*$</td>
<td>$P = 0.143$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P$ value with significant effect.
Song traits seemed rarely affected by diet (Table 4). Diet did not affect chirp rate, pulse rate, or frequency in “G. staccato”, G. lineaticeps, or G. personatus. The only effect of diet on any song trait was that G. personatus had more pulses per chirp on diet A than on diet B. There was no effect in the other two species.

**Table 4.** One-way mixed model ANOVAs of effect of diet on characteristics of temperature corrected male calling songs of all three species (mean ± SE).

<table>
<thead>
<tr>
<th>Species</th>
<th>Chirp rate (c/s)</th>
<th>Pulse rate (p/s)</th>
<th>Frequency (Hz)</th>
<th>Pulses/chirp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G. lineaticeps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet A</td>
<td>2.16 ± 0.07</td>
<td>68.64 ± 0.71</td>
<td>5256 ± 42</td>
<td>7.66 ± 0.12</td>
</tr>
<tr>
<td>Diet B</td>
<td>2.34 ± 0.08</td>
<td>68.11 ± 0.77</td>
<td>5200 ± 28</td>
<td>7.57 ± 0.12</td>
</tr>
<tr>
<td>(F_{1,91} = 2.96)</td>
<td>(F_{1,91} = 0.27)</td>
<td>(F_{1,91} = 1.26)</td>
<td>(F_{1,91} = 0.31)</td>
<td></td>
</tr>
<tr>
<td>(P = 0.089)</td>
<td>(P = 0.607)</td>
<td>(P = 0.265)</td>
<td>(P = 0.578)</td>
<td></td>
</tr>
<tr>
<td><strong>G. personatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet A</td>
<td>1.69 ± 0.05</td>
<td>62.76 ± 0.38</td>
<td>3998 ± 21</td>
<td>7.47 ± 0.07</td>
</tr>
<tr>
<td>Diet B</td>
<td>1.61 ± 0.05</td>
<td>62.04 ± 0.44</td>
<td>4042 ± 23</td>
<td>7.53 ± 0.09</td>
</tr>
<tr>
<td>(F_{1,162} = 1.54)</td>
<td>(F_{1,162} = 1.54)</td>
<td>(F_{1,162} = 2.04)</td>
<td>(F_{1,162} = 13.05)</td>
<td>(P &lt; 0.001^*)</td>
</tr>
<tr>
<td>(P = 0.216)</td>
<td>(P = 0.216)</td>
<td>(P = 0.155)</td>
<td>(P = 0.578)</td>
<td></td>
</tr>
<tr>
<td><strong>“G. staccato”</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet A</td>
<td>2.31 ± 0.78</td>
<td>80.62 ± 0.62</td>
<td>5342 ± 23</td>
<td>8.27 ± 0.11</td>
</tr>
<tr>
<td>Diet B</td>
<td>2.28 ± 0.06</td>
<td>80.42 ± 0.55</td>
<td>5304 ± 28</td>
<td>8.33 ± 0.10</td>
</tr>
<tr>
<td>(F_{1,184} = 0.09)</td>
<td>(F_{1,184} = 0.06)</td>
<td>(F_{1,184} = 1.12)</td>
<td>(F_{1,184} = 0.14)</td>
<td></td>
</tr>
<tr>
<td>(P = 0.764)</td>
<td>(P = 0.814)</td>
<td>(P = 0.291)</td>
<td>(P = 0.712)</td>
<td></td>
</tr>
</tbody>
</table>

*\(P\) value with significant effect.
I was interested in the interaction between diet and sibship (Table 5). I performed statistical analyses using two-way mixed model ANOVAs with sibship as a random variable and diet as a fixed factor. \( F \)-values for the fixed factor were calculated as the mean square of diet divided by the mean square of the interaction term. For none of the three species was the interaction between diet and sibship even close to significant as evaluated for body condition \((P > 0.25)\). For the song traits, in general, the significance or non-significance of the interaction was not consistent among the three species. The only two significant interactions were for frequency in *G. lineaticeps* and for chirp rate in “*G. staccato*”. Comparing the two-way ANOVAs to the one-way ANOVAs, diet still affected pulses per chirp in *G. personatus* but became non-significant for body condition. It would seem that I failed to instigate a consistent and major effect of diet.
**Table 5.** Two-way mixed model ANOVAs for effect of sibship, diet, and their interaction.

<table>
<thead>
<tr>
<th></th>
<th>Chirp Rate (c/s)</th>
<th>Pulse Rate (p/s)</th>
<th>Frequency (Hz)</th>
<th>Pulses/Chirp</th>
<th>Body Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G. lineaticeps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship</td>
<td>( F_{14,63} = 2.02 )</td>
<td>( F_{14,63} = 1.04 )</td>
<td>( F_{14,63} = 3.30 )</td>
<td>( F_{14,63} = 1.61 )</td>
<td>( F_{14,63} = 1.27 )</td>
</tr>
<tr>
<td>Diet</td>
<td>( P = 0.030^* )</td>
<td>( P = 0.431 )</td>
<td>( P &lt; 0.001^* )</td>
<td>( P = 0.101 )</td>
<td>( P = 0.251 )</td>
</tr>
<tr>
<td>Diet x Sibship</td>
<td>( F_{14,63} = 1.00 )</td>
<td>( F_{14,63} = 0.58 )</td>
<td>( F_{14,63} = 2.17 )</td>
<td>( F_{14,63} = 0.97 )</td>
<td>( F_{14,63} = 1.25 )</td>
</tr>
<tr>
<td></td>
<td>( P = 0.466 )</td>
<td>( P = 0.868 )</td>
<td>( P = 0.019^* )</td>
<td>( P = 0.493 )</td>
<td>( P = 0.261 )</td>
</tr>
<tr>
<td><strong>G. personatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship</td>
<td>( F_{21,120} = 2.20 )</td>
<td>( F_{21,120} = 1.06 )</td>
<td>( F_{21,120} = 1.98, )</td>
<td>( F_{21,120} = 2.59 )</td>
<td>( F_{21,120} = 0.85 )</td>
</tr>
<tr>
<td>Diet</td>
<td>( P = 0.004^* )</td>
<td>( P = 0.404 )</td>
<td>( P = 0.011^* )</td>
<td>( P &lt; 0.001^* )</td>
<td>( P = 0.648 )</td>
</tr>
<tr>
<td>Diet x Sibship</td>
<td>( F_{21,120} = 0.69 )</td>
<td>( F_{21,120} = 2.55 )</td>
<td>( F_{1,120} = 1.51 )</td>
<td>( F_{1,120} = 14.58, )</td>
<td>( P = 0.140 )</td>
</tr>
<tr>
<td></td>
<td>( P = 0.407 )</td>
<td>( P = 0.113 )</td>
<td>( P = 0.221 )</td>
<td>( P &lt; 0.001^* )</td>
<td>( P = 1.02 )</td>
</tr>
<tr>
<td></td>
<td>( F_{21,120} = 1.06 )</td>
<td>( F_{21,120} = 1.00 )</td>
<td>( F_{21,120} = 0.87 )</td>
<td>( F_{21,120} = 0.55 )</td>
<td>( F_{21,120} = 0.451 )</td>
</tr>
<tr>
<td></td>
<td>( P = 0.398 )</td>
<td>( P = 0.463 )</td>
<td>( P = 0.631 )</td>
<td>( P = 0.942 )</td>
<td>( P = 0.451 )</td>
</tr>
<tr>
<td><strong>“G. staccato”</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship</td>
<td>( F_{21,142} = 1.87 )</td>
<td>( F_{21,163} = 1.10 )</td>
<td>( F_{21,142} = 1.95 )</td>
<td>( F_{21,142} = 2.12 )</td>
<td>( F_{21,142} = 0.98 )</td>
</tr>
<tr>
<td>Diet</td>
<td>( P = 0.017^* )</td>
<td>( P = 0.354 )</td>
<td>( P = 0.012^* )</td>
<td>( P = 0.005^* )</td>
<td>( P = 0.488 )</td>
</tr>
<tr>
<td>Diet x Sibship</td>
<td>( F_{1,142} = 1.14 )</td>
<td>( F_{1,142} = 0.06 )</td>
<td>( F_{1,142} = 1.68 )</td>
<td>( F_{1,142} = 0.25 )</td>
<td>( F_{1,142} = 3.44 )</td>
</tr>
<tr>
<td></td>
<td>( P = 0.287 )</td>
<td>( P = 0.804 )</td>
<td>( P = 0.197 )</td>
<td>( P = 0.616 )</td>
<td>( P = 0.066 )</td>
</tr>
<tr>
<td></td>
<td>( F_{21,142} = 1.79 )</td>
<td>( F_{21,142} = 1.16 )</td>
<td>( F_{21,142} = 0.71 )</td>
<td>( F_{21,142} = 1.29 )</td>
<td>( F_{21,142} = 1.13 )</td>
</tr>
<tr>
<td></td>
<td>( P = 0.025^* )</td>
<td>( P = 0.295 )</td>
<td>( P = 0.816 )</td>
<td>( P = 0.191 )</td>
<td>( P = 0.327 )</td>
</tr>
</tbody>
</table>

* \( P \) value with significant effect.
**Table 6.** ANOVAs for effect of sibship by itself without effect of diet and diet x sibship interaction.

<table>
<thead>
<tr>
<th></th>
<th>Chirp Rate (c/s)</th>
<th>Pulse Rate (p/s)</th>
<th>Frequency (Hz)</th>
<th>Pulses/Chirp</th>
<th>Body Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G. lineaticeps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship</td>
<td><em>F</em>{sub:14,78} = 2.02</td>
<td><em>F</em>{sub:14,78} = 3.19</td>
<td><em>F</em>{sub:14,78} = 1.73</td>
<td><em>F</em>{sub:14,78} = 1.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.026*</td>
<td><em>P</em> &lt; 0.001*</td>
<td><em>P</em> = 0.067</td>
<td><em>P</em> = 0.355</td>
<td></td>
</tr>
<tr>
<td><strong>G. personatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship</td>
<td><em>F</em>{sub:21,142} = 2.11</td>
<td><em>F</em>{sub:21,142} = 1.97</td>
<td><em>F</em>{sub:21,142} = 2.61</td>
<td><em>F</em>{sub:21,142} = 1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.005*</td>
<td><em>P</em> = 0.011*</td>
<td><em>P</em> &lt; 0.001*</td>
<td><em>P</em> = 0.471</td>
<td></td>
</tr>
<tr>
<td>“G. staccato”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship</td>
<td><em>F</em>{sub:21,164} = 1.75</td>
<td><em>F</em>{sub:21,164} = 2.01</td>
<td><em>F</em>{sub:21,164} = 2.16</td>
<td><em>F</em>{sub:21,164} = 0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.028*</td>
<td><em>P</em> = 0.008*</td>
<td><em>P</em> = 0.004*</td>
<td><em>P</em> = 0.540</td>
<td></td>
</tr>
</tbody>
</table>

* *P* value with significant effect.
Quantitative Genetics within Species

Given the weak effect of diet, I focused on variation among sibships as a random genetic variable. Table 5 evaluates the significance of sibship in the two-way mixed-model ANOVA. However, given the weak effects of diet, I evaluated the effect of sibship with the effects of diet and the interaction included in the error term. Table 6 gives the single classification model II ANOVAs. Whether sibship was significant or not was the same in both analyses for all variables. For all three species, chirp rate and frequency were significant. For all three species, pulse rate and condition were not significant. Pulses per chirp was significant in *G. personatus* and “*G. staccato*” and marginally non-significant in *G. lineaticeps*.

Given the consistent effect of sibship on certain morphological and song traits, nested analyses were done in an attempt to separate the effects of dams when the sire was the same from the effect of different sires. This was done for each species for each of the four song traits, body condition, and pronotum width. In these model II nested ANOVAs, $F$ of sires was tested as the $\text{MS}_{\text{sire}}$ over the $\text{MS}_{\text{dam(sire)}}$ (Table 7). These analyses have fewer degrees of freedom than previous analyses due to limited numbers of dams within sires. Sires with only one dam were removed from analysis. I found several variables in which half-siblings were affected by their dam even when they had the same sire. As examples, chirp rate varied significantly among dams within sires for *G. personatus* and for “*G. staccato*”, although it did not for *G. lineaticeps*. There was only one case of a significant effect of sire beyond any effect of dam within sire; sire affected condition in *G. lineaticeps*. Other results of the nested analysis will be discussed below for each trait.
Table 7. Results of nested analyses for sires and dams within sires.

<table>
<thead>
<tr>
<th></th>
<th>G. lineaticeps</th>
<th></th>
<th></th>
<th>G. personatus</th>
<th></th>
<th></th>
<th>“G. staccato”</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS&lt;sub&gt;error&lt;/sub&gt;</td>
<td>MS&lt;sub&gt;dam(sire)&lt;/sub&gt;</td>
<td>P&lt;sub&gt;dam(sire)&lt;/sub&gt;</td>
<td>MS&lt;sub&gt;sire&lt;/sub&gt;</td>
<td>P&lt;sub&gt;sire&lt;/sub&gt;</td>
<td>MS&lt;sub&gt;error&lt;/sub&gt;</td>
<td>MS&lt;sub&gt;dam(sire)&lt;/sub&gt;</td>
<td>P&lt;sub&gt;dam(sire)&lt;/sub&gt;</td>
<td>MS&lt;sub&gt;sire&lt;/sub&gt;</td>
</tr>
<tr>
<td>Chirp Rate</td>
<td>0.23 [53]</td>
<td>0.31 [5]</td>
<td>0.260</td>
<td>0.32 [3]</td>
<td>0.278</td>
<td>0.19 [75]</td>
<td>0.66 [6]</td>
<td>0.005*</td>
<td>0.30 [3]</td>
</tr>
<tr>
<td>Pulses per Chirp</td>
<td>0.69 [53]</td>
<td>0.65 [5]</td>
<td>0.465</td>
<td>1.79 [3]</td>
<td>0.149</td>
<td>0.56 [75]</td>
<td>0.70 [6]</td>
<td>0.317</td>
<td>1.85 [3]</td>
</tr>
<tr>
<td>Body Condition</td>
<td>0.00301 [53]</td>
<td>0.00115 [5]</td>
<td>0.860</td>
<td>0.01118 [3]</td>
<td>0.016*</td>
<td>0.00211 [75]</td>
<td>0.00203 [6]</td>
<td>0.458</td>
<td>0.00117 [3]</td>
</tr>
<tr>
<td>Pronotum</td>
<td>0.14 [53]</td>
<td>0.14 [5]</td>
<td>0.404</td>
<td>0.41 [3]</td>
<td>0.143</td>
<td>0.10 [75]</td>
<td>0.29 [6]</td>
<td>0.014*</td>
<td>0.15 [3]</td>
</tr>
</tbody>
</table>

* P value with significant effect.
In order to compare species, one more level was added to the top of the nested ANOVAs. Pure model II nested ANOVAs were calculated to see how species varied beyond the effect of sires, beyond the effect of sires within dams, and beyond the residual variance, which included any variation due to measurement error, individual development, or to diet (Table 8). \( F \)-ratios of species were tested as the MS\textsubscript{species} over the MS\textsubscript{sire}. Variance components were converted to percentages (Table 9). Chirp rate and pulses per chirp appeared to be one kind of variable with the effect of species being modest and the variance component due to dams within sire within species being significant. In contrast, pulse rate and frequency appear to be to be another kind of variable with an overwhelming variance component due to species. Condition and pronotum were included because size of a cricket may affect some of its song characteristics. For example, a larger pronotum, indicative of larger body size, seems to result in a lower frequency of song.
Table 8. Nested analyses among three species, sires nested within species, dams nested within sires within species.

<table>
<thead>
<tr>
<th></th>
<th>$\text{MS}_{\text{error}}$</th>
<th>$\text{MS}_{\text{dam(sire(species))}}$</th>
<th>$P_{\text{dam(sire(species))}}$</th>
<th>$\text{MS}_{\text{sire(species)}}$</th>
<th>$P_{\text{sire(species)}}$</th>
<th>$\text{MS}_{\text{species}}$</th>
<th>$P_{\text{species}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chirp Rate</td>
<td>0.334 [292]</td>
<td>0.662 [23]</td>
<td>0.006*</td>
<td>0.383 [15]</td>
<td>0.721</td>
<td>8.403 [2]</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Pulses per Chirp</td>
<td>0.814 [292]</td>
<td>1.613 [23]</td>
<td>0.005*</td>
<td>1.067 [15]</td>
<td>0.794</td>
<td>39.652 [2]</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Condition</td>
<td>0.00309 [292]</td>
<td>0.00305 [23]</td>
<td>0.479</td>
<td>0.00383 [15]</td>
<td>0.303</td>
<td>0.00006 [2]</td>
<td>0.984</td>
</tr>
<tr>
<td>Pronotum</td>
<td>0.106 [292]</td>
<td>0.273 [23]</td>
<td>0.00015*</td>
<td>0.400 [15]</td>
<td>0.200</td>
<td>16.426 [2]</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* $P$ value with significant effect.

Table 9. Variance components, as percentages.

<table>
<thead>
<tr>
<th></th>
<th>$\text{VC}_{\text{error}}$</th>
<th>$\text{VC}_{\text{dam(sire(species))}}$</th>
<th>$\text{VC}_{\text{sire(species)}}$</th>
<th>$\text{VC}_{\text{species}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chirp Rate</td>
<td>73%</td>
<td>7%</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>Pulse Rate</td>
<td>23%</td>
<td>0%</td>
<td>0%</td>
<td>77%</td>
</tr>
<tr>
<td>Frequency</td>
<td>8%</td>
<td>1%</td>
<td>0%</td>
<td>91%</td>
</tr>
<tr>
<td>Pulses per Chirp</td>
<td>64%</td>
<td>8%</td>
<td>0%</td>
<td>24%</td>
</tr>
<tr>
<td>Condition</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Pronotum</td>
<td>77%</td>
<td>0%</td>
<td>0%</td>
<td>23%</td>
</tr>
</tbody>
</table>
Song Traits Within and Among Species

Chirp Rate (CR)—Sibships varied significantly within all three species. *G. lineaticeps* and “*G. staccato*” have hardly diverged in chirp rate from one another, but have a higher chirp rate than in *G. personatus* (Figure 3A). The error variance for chirp rate was substantial but apparently not due greatly to diet (non-significant except for diet x sibship in “*G. staccato*”: Table 5).

Pulse Rate (PR)—Pulse rate behaved differently than chirp rate. The three species all differed in pulse rate, and sibship did not vary significantly in any species (Figure 3B). Notably, pulse rate always diverged among species and yet has been under stabilizing selection within species (Blankers et al. 2015).

Frequency—Like chirp rate, frequency is similar between *G lineaticeps* and “*G. staccato*”, whereas *G. personatus* has diverged (Figure 3C). Also like chirp rate, sibships varied significantly for frequency in all three species; however, the percent variance due to dams within sires was very small, as it was for pulse rate, suggesting the ghost of stabilizing selection past. Diet x sibship was significant for *G. lineaticeps* (Table 5), but the error term was only 8%, presumably because of species divergence.

Pulses Per Chirp—Unlike the other three song traits, pulses per chirp has not diverged much among species (Figure 3D). Sibships varied significantly for “*G. staccato*” and for *G. personatus* but not for *G. lineaticeps* (Table 6). Dams within sires varied significantly for “*G. staccato*” (Table 7). Probably, there has been less stabilizing
selection than in, say, chirp rate. As it did for chirp rate, dams within sires displayed a noticeable percentage (8%), probably exaggerated as a percentage because of the low divergence among species. The error variance was substantial with a possible contribution due to diet in *G. personatus* (Table 6).

**Figure 3.** Four song characteristics, means and standard deviations based on 10 sibships in *G. lineaticeps*, 10 sibships in “*G. staccato*”, and 16 sibships in *G. personatus*. Percentages are variance components from pure model II nested ANOVAs (Table 8).
DISCUSSION

Introduction

Investigating causes of variation in signaling among these three sister species of *Gryllus* has lead to new insights with empirical support for what drives trait variation in related species. In exploring environmental causes, manipulating diet had a limited effect on morphology and song traits as experimental diets “failed” to instigate a major effect on either in some species. In exploring genetic causes, family groups (sibships) had a major and consistent effect on song traits consistently within a species, consistently among all three species, and aided in characterizing the specific sources of that variation (sire, dam, species). Each of these factors and the nuances of their observed effects can be described further to better appreciate their role in the observed trait variation of these three sister species and potential causes therein.

Diet Treatments

Males reared on the high-quality diet showed significant differences in mass and condition from males reared on the low-quality diet in *G. personatus* and “*G. staccato*”. However, the diets had no significant effect on components of calling song, except for pulses per chirp in *G. personatus*. This may have been a result of individual crickets compensating when reared on the poor diet. High-nutrition diets were consumed at a constant rate between regularly scheduled diet checks, whereas low-nutrition diets appeared to have been consumed in half that time, necessitating supplemental feedings. Thus, it is possible no consistent effect of diet was observed in my study because crickets compensated for the low-nutrition diet by consuming it in greater quantities. My
experimental design with *ad libitum* feeding did not allow me to test conclusively whether diet had an effect on traits of calling song, but related research on calling in *Gryllus* has shown that diet can have an effect on sexually selected song traits, most notably chirp rate (Wagner & Hoback 1999, Wagner & Harper 2003). Such effects have been found in studies that controlled for diet quantity. Wagner & Hoback (1999) found that an average male *G. lineaticeps* could consume a mean (± SE) diet of 26.6 ± 0.2 mg in a day. This standard quantity was used to formulate diets of nutritious and non-nutritious quality to investigate the effect of a variable diet on signal traits, with poor diets being diluted with non-nutritive cellulose to maintain constant quantity (Wagner & Harper 2003). Crickets may also allocate resources differently depending upon life stage. Maklakov et al. (2008) showed that adult *Teleogryllus commodus* crickets fed high-carbohydrate diets had higher signaling effort, whereas juvenile crickets provided with high-protein diets invested those nutrients in achieving greater adult body sizes. Crickets in this study were reared on diets that varied in relative amounts of proteins, carbohydrates, and fats, which may have been allocated differently as was observed with *T. commodus* (Maklakov et al. 2008). Diets differed in impact given that diets in my study yielded a significant difference in mass but not pronotum width and had almost no significant effect on song traits. It is possible that protein was more important in early development to increase body mass but not pronotum width (the observed morphological effects). While carbohydrates were more important during adulthood to fuel calling effort, carbohydrates did not affect song traits once calling occurred (the observed effects on song). In essence, experimental diets provided throughout the life of crickets in my study were probably ineffective at inducing qualitatively different diet treatments because
crickets either ate more and/or metabolized diet components at different developmental stages to optimize their use for growth and calling effort. For future studies, controlling the quantity of high- or low-nutrition diet provided and imposing those diets only at later life stages would be prudent.

**Quantitative Genetics Within Species**

Many potential sources of genetic variation could be responsible for the effects observed in my study. Given the design of my study, these sources derive from either the sire (additive genetic variance, dominance, epigenetics) or from the dam (additive as well as maternal effects, including dominance, epigenetics). I analyzed this by forming full sibling groups (sibships); however, I did not conduct half-sibling analyses due to lack of statistical power. Sibship had a consistently significant effect for song traits across all three species studied but not necessarily in ways that were anticipated. While specific sources of variation in the phenotypes measured do not address all potential sources of environmental and genetic variation, such as dominance, epigenetics, maternal effects, and non-additive genetic effects, like epistasis (Kruuk 2004), they provide a means of differentiating the relative importance of those sources in what was observed in this study.

Sibship affected chirp rate and frequency significantly in all three species. Both chirp rate and frequency are vital components of male calling song in crickets. Calling song is important to long-range communication in crickets for phonotaxis (Otte 1974), enabling females to recognize calling song patterns (Stabel et al. 1989) and exhibit positive phonotaxis towards signals originating from conspecifics (Doherty 1985). Traits
within calling song are used by females to discriminate conspecific from hetero-specific males (frequency) (Thorson et al. 1982, Gerhardt 1994) and to assess the quality of conspecific males for reproduction (chirp rate; Wagner 2000).

Sibship did not affect pulse rate and condition in all three species. Pulse rate is an important determinant of female response to male calling song allowing females to hone in on conspecific males through discrimination of the rate of pulses (Walker 2000), similar to frequency. Since pulse rate was not affected by sibship in all three species, this suggests genetics may affect traits important for species song recognition differently from one another. Alleles involved in song recognition could be more or less fixed in each species with pulse rate and frequency genetic variation following the species tree and split with speciation events. If the divergence in pulse rate is not owed to genetics, then it is possible that it may be owed to environmental effects although not those explored in the current study. Condition was most likely not affected in any of the three species by sibship because crickets optimized body condition off of the “failed” experimental diets regardless of sibship. However, GEI interactions revealed how some sibships were affected equally on both diets and others were affected positively or negatively, denoting sibships are not inherently identical in response even if those differences are not statistically different.

Sibship affected pulses per chirp in two of the three species, *G. personatus* and “*G. staccato*”. An indicator of male phenotypic quality under stabilizing selection, similar to chirp rate, pulses per chirp follow the pattern observed with quality indicator song traits, with a marginally non-significant effect in *G. lineaticeps*. However, unlike other quality indicating traits, neither sire nor dam served as a significant source of variation, with the
exception of dams in “G. staccato”. Within all three species, sire did not have a
significant effect for nearly all of the traits measured, except for an effect on condition in
G. lineaticeps. Conversely, the effect of dams within sires was highly significant, and
was further supported by calculating variance components. Variance components give
three possible estimates of heritability (sire, dam, and species) (Becker, 1992). The sire
estimate is typically the preferred estimate as it is free from possible maternal and/or
dominance variance, while the best estimates of additive genetic variance and heritability
are the genotypic estimates (the mean of the sire and dam estimates) (Roff 2007). Given
the limited effect of sire, these findings suggest that the observed genetic variation may
be due to dams through maternal effects. Maternal effects occur when the phenotype of
the mother/dam affects the phenotype of her offspring, in ways additional to the additive
effects of the genes she has passed on (Mousseau & Fox 1998). These maternal effects
also may be environmentally or genetically determined and possess the capacity to
generate covariance between siblings, which may be mistaken for additive genetic
variance unless explicitly modeled (Kruuk 2004). Again, given sire had little to no effect,
yet sibship had such a consistently strong effect on song traits, maternal effects are a
prime contender as the source of genetic variation in these three species of crickets.

Song Traits Within and Among Species

Across all three species of Gryllus, measured song traits (chirp rate, pulse rate,
frequency, and pulses per chirp) and body condition responded similarly to causes of
variation with little to no exception. While the environmental causes were overall in-
effective, song traits responded to genetic causes of variation in a consistent fashion and
with a strikingly similar range of variation among species. These similarities potentially derive not just from relatedness but from selective pressures known to shape acoustic signals and their associated traits. As evidenced in Figure 3A and 3D, quality indicating traits (chirp rate and pulses per chirp) exhibited a wide range of variation among sibships in all three species, indicating a wide range of trait expression within a species. In Figure 3B and 3C, species recognition traits (pulse rate and frequency) exhibited a narrow range of variation among sibships concentrated around discrete trait values in all three species. The dueling constraints expressed by natural and sexual selection force male signals to respond dynamically to female choice and help to illuminate how different traits responded in my study.

Selective pressures incur heavy energetic costs through increased allocation to signal trait expression, imposing a handicap that honestly reveals male condition by limiting deceptive signaling by lower quality males (Zahavi 1975). Traits expressed in this manner provide females with a basis for assessing potential high-quality and low-quality direct benefits through materials and resources that improve offspring production or indirect benefits through heritable genetic gains. (Tolle & Wagner 2011). These traits serve as reliable signals for evaluating a potential mate’s condition and associated costs and benefits to female fitness (Kokko et al. 2003). This condition-dependent expression also reveals different fitness potential for male signal traits in response to environmental condition in the presence of environmental heterogeneity in which the effect of environment is different for different genotypes. The assessment of quality signaling by females is predictive of environmental condition, immunity, rearing environment (Leonard & Hedrick 2010) manifesting itself through female preference and choosiness...
(Judge et al. 2014). Given the observed significance of sibship on those traits associated with qualitative assessment (chirp rate and pulses per chirp) and the limitations of environmental discrepancy between diets, further exploring the role of dams as a source of variation would be prudent but only if more discrete environments can be approximated to enable full expression of these signals of quality.

Condition-dependent expression can affect male signaling by informing female mate choice of the phenotype and associated genotype that is best suited for the environment in which it occurs, which represents a difference in the heritability of traits that could impact the viability of offspring (Andersson 1986). A number of studies have shown that males exhibit phenotypic variation in sexually selected traits: eye-stalk length in stalk eyed flies (David et al. 2000, Cotton et al. 2004); male advertisement displays of red sage grouse (Vergara 2012); the ultrasonic calls of lesser waxmoths (Rodriguez & Greenfield 2003); and the calling song components of some *Gryllus* cricket species (Scheuber et al. 2003, Judge et al. 2008). These pronounced plastic responses denote genotype-by-environment interaction (GEI), which has become a reliable means of investigating condition-dependent trait expression as genotypes have differential phenotypic expression depending on the environment within which they are expressed (Ingleby et al. 2010) and carry a high degree of genetic correlation across environments that can be used to estimate the degree to which the expressed phenotype is attributable to the same set of alleles (Via & Lande 1985). However, while the literature provides a straightforward and reliable assessment of how condition dependence can be expressed and potentially investigated in a variety of mating systems, investigating condition dependence through my study species was not clearly demonstrated. Using the
experimental high-quality and low-quality diets as proxies for qualitatively different environments, mean trait values for male calling song traits (Figure 3A-D) and body condition of sibships for each species were used to generate GEI reaction norms (Appendix D) to compare condition-dependent expression. There was no consistent pattern of expression among traits within or among species, even for a trait like chirp rate, which is known to be condition dependent (Wagner & Hoback 1999, Scheuber et al. 2003). Some traits were weakly affected by diet, denoting some plasticity, but none of the effects were statistically significant with the exception of pulses per chirp in *G. personatus*. Thus, the variable responses observed for song traits and body condition illustrate an inconclusive assessment of condition dependence compared with that of the established literature.

**Divergence Among Species**

Assessing causes of variation and their impact on the response variables in this study are highly informative to characterizing how different signal trait variation is within this sister species complex. Yet, investigating trait variation in this manner also provides insight into how these three species have diverged from their ancestral population into the distinct species that exist today. According to Gray et al. (2015), these three species have distributions across the American Southwest, abutting one another in a west-to-east orientation, yet remain isolated from one another due to natural geographic boundaries. Where these species occur today reveals part of how they may have diverged from one another, but examination of song traits reveals another facet to speciation within this complex given how far they have diverged in some traits and how similar they remain in others.
Song trait variation exhibits patterns of divergence similar to those observed for female preference functions. Just as female preference functions for song traits can be open or closed, so too can male traits be either static or dynamic. As outlined in Gray et al. (2015), traits that are static match with closed preference functions as they are less likely to vary while traits that are dynamic match with open preference function to encompass the potential variation. Two song traits in particular in all three species follow a similar pattern: frequency is a static trait under stabilizing selection while chirp rate is a dynamic trait under moderate/weak directional selection (Tolle & Wagner 2011, Gray et al. 2015, Hennig et al. 2016). Given the divergent patterns in all three species for frequency (Gray et al. 2015) and the directional selection toward exaggerated values of chirp rate (Tolle & Wagner 2011), these selective pressures known to shape the genetics of populations and sexually selected characters could support the consistent effect of sibship on these two traits in particular through some form of genetic correlation. Static traits like frequency and pulse rate have low coefficients of variation while dynamic traits like chirp rate and pulses per chirp have higher coefficients of variation, each owing to the selective pressures impressed upon both kinds of traits. However, while both frequency and chirp rate have similar sibship effects, pulse rate and pulses per chirp do not. This apparent discrepancy in the effect of sibship on static and dynamic traits suggests some form of genetic correlation. There is evidence for components of calling song being phenotypically correlated in field crickets (Scheuber et al. 2003, Blankers et al. 2016). Studies of selection on such correlated traits of calling song show that although they may be dissimilar, they might be under multivariate stabilizing selection (Brooks et al. 2005), which would account for the effects observed in my study. Among these three
species, pulse rate is a static song trait exhibiting divergence among all three species (Gray et al. 2015).

According to work by Hennig et al. (2016), the similarity in male song traits among these three species has been interpreted as a form of phylogenetic conservatism within this group from a recent common ancestor with any observed differences deriving from divergence from the same ancestor. It is likely that the current geographic distribution of these cricket species represent an evolutionary history of divergence under allopatric conditions, which is further supported by mitochondrial and nuclear DNA sequences (Gray et al. 2015). According to Gray et al. (2015), evolutionary conservation of directional female preferences for dynamic song components with divergence in female preference for static song components are suggested causes for the observed divergent patterns of molecular genetic variation. Given the results of my study, general patterns for these three species can now include how sexually selected signal traits respond to genetic variation. Despite not being able to more definitively identify sources of genetic variation, my study provides a path forward to identify other potential sources of variation through assessment of heritability, which can tease apart the relative importance of sires and dams within sibship overall.

Conclusion

The findings of this study have lead to some preliminary generalizations about the potential role of genetic and environmental factors on signal traits and how they are likely to vary across related species. Environmental factors on signal traits were found to not be a significant source of the observed variation, either due to the environments being too
similar or the crickets being able to optimize whichever environment they were reared in. Conversely, genetic factors on signal traits were found to be consistently significant for traits important to species recognition (frequency) and traits that convey information about male quality (e.g., chirp rate, pulses per chirp) across all three species. While this study focused solely on the causes of male trait variation in these three sister species, the observed patterns provide a roadmap for future investigations into the variation of female preference for these male traits as well. A similar investigatory framework could determine how environmental and genetic factors affect female preference functions in a similar manner as they do males in both conspecific and related heterospecific species. Such an investigation could yield a fuller exploration of sexual selection and condition-dependence in these three species and provide findings that would be complementary to those which have been explored here.
LITERATURE CITED


**APPENDIX A: GRYLLUS LINEATICEPS DESCRIPTIVE FIGURES**

**Figure 4.1** *G. lineaticeps* mean mass (±SE) did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 0.51, P = 0.477$).

**Figure 4.2** *G. lineaticeps* mean (±SE) pronotum width did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 0.03, P = 0.867$).
**Figure 4.3** *G. lineaticeps* mean (±SE) record age did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 0.54, P = 0.465$).

**Figure 4.4** *G. lineaticeps* mean (±SE) body condition did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 1.87, P = 0.175$).
Figure 4.5 *G. lineaticeps* mean (±SE) pulse rate did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 0.27, P = 0.607$).

Figure 4.6 *G. lineaticeps* mean (±SE) chirp rate did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 2.96, P = 0.089$).
**Figure 4.7** *G. lineaticeps* mean (±SE) call frequency did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 1.26, P = 0.265$).

**Figure 4.8** *G. lineaticeps* mean (±SE) pulses per chirp did not differ for crickets fed high and low quality diet treatments ($F_{1,91} = 0.31, P = 0.578$).
Figure 4.9 *G. lineaticeps* mean (±SE) mass per diet per sibship.

Figure 4.10 *G. lineaticeps* mean (±SE) pronotum width per diet per sibship.
Figure 4.11 *G. lineaticeps* mean (±SE) record age per diet per sibship.

Figure 4.12 *G. lineaticeps* mean (±SE) body condition per diet per sibship.
Figure 4.13 *G. lineaticeps* mean (±SE) pulse rate per diet per sibship.

Figure 4.14 *G. lineaticeps* mean (±SE) chirp rate per diet per sibship.
Figure 4.15 *G. lineaticeps* mean (±SE) call frequency per diet per sibship.

Figure 4.16 *G. lineaticeps* mean (±SE) pulses per chirp per diet per sibship.
APPENDIX B: “GRYLLUS STACCATO” DESCRIPTIVE FIGURES

**Figure 5.1** “G. staccato” mean mass (±SE) differed significantly for crickets fed high and low quality diet treatments ($F_{1,184}=4.57$, $P = 0.034$).

![Mass Diagram](image)

**Figure 5.2** “G. staccato” mean (±SE) pronotum width did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,184}=2.16$, $P = 0.143$).

![Pronotum Diagram](image)
Figure 5.3 “G. staccato” record age (±SE) did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,184} = 4.57, P = 0.034$).

Figure 5.4 “G. staccato” mean (±SE) condition did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,184} = 2.41, P = 0.123$).
Figure 5.5 “G. staccato” mean (±SE) pulse rate did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,184} = 0.06, P = 0.814$).

Figure 5.6 “G. staccato” mean (±SE) chirp rate did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,184} = 0.09, P = 0.764$).
Figure 5.7 “G. staccato” mean (±SE) frequency did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,184} = 1.12$, $P = 0.291$).

Figure 5.8 “G. staccato” mean (±SE) condition did not differ significantly for crickets fed high and low quality diets ($F_{1,184} = 0.14$, $P = 0.712$).
**Figure 5.9** “G. staccato” mean (±SE) mass per diet per sibship.

**Fig. 5.10** “G. staccato” mean (±SE) pronotum width per diet per sibship.
Fig. 5.11 “G. staccato” mean (±SE) record age per diet per sibship.

Fig. 5.12 “G. staccato” mean (±SE) body condition per diet per sibship.
Figure 5.13 “G. staccato” mean (±SE) pulse rate per diet per sibship.

Figure 5.14 “G. staccato” mean (±SE) chirp rate per diet per sibship.
Figure 5.15 “G. staccato” mean (±SE) frequency per diet per sibship.

Figure 5.16 “G. staccato” mean (±SE) pulses per chirp per diet per sibship.
**Figure 6.1** *G. personatus* mean mass (±SE) differed for crickets fed high and low quality diet treatments ($F_{1,162}= 4.73$, $P = 0.031$).

**Figure 6.2** *G. personatus* mean pronotum width (±SE) did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,162}= 1.31$, $P = 0.255$).
Figure 6.3 *G. personatus* mean record age (±SE) differed significantly for crickets fed high and low quality diet treatments ($F_{1,162} = 5.85$, $P = 0.017$).

Figure 6.4 *G. personatus* mean condition (±SE) differed significantly for crickets fed high and low quality diet treatments ($F_{1,162} = 3.92$, $P = 0.050$).
Figure 6.5 *G. personatus* mean pulse rate (±SE) did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,162} = 1.54, P = 0.216$).

Figure 6.6 *G. personatus* mean chirp rate (±SE) did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,162} = 1.54, P = 0.216$).
Figure 6.7 *G. personatus* mean frequency (±SE) did not differ significantly for crickets fed high and low quality diet treatments ($F_{1,162} = 2.04, P = 0.155$).

Figure 6.8 *G. personatus* mean pulses per chirp (±SE) differed significantly for crickets fed high and low quality diet treatments ($F_{1,162} = 13.05, P = 0.0004$).
**Figure 6.9** *G. personatus* mean mass per diet per sibship (±SE).

**Figure 6.10** *G. personatus* mean pronotum width per diet per sibship (±SE).
Figure 6.11 *G. personatus* mean record age per diet per sibship (±SE).

Figure 6.12 *G. personatus* mean condition per diet per sibship (±SE).
Figure 6.13 *G. personatus* mean pulse rate per diet per sibship (±SE).

Figure 6.14 *G. personatus* mean chirp rate per diet per sibship (±SE).
Figure 6.15 *G. personatus* mean frequency per diet per sibship (±SE).

Figure 6.16 *G. personatus* mean pulses per chirp per diet per sibship (±SE).
APPENDIX D: ALL _GRYLLUS_ GEI REACTION NORMS

**Figure 7.1** Chirp rate in _G. lineaticeps_ sibships response to Diet A and Diet B \[F_{1,63} = 3.65, P = 0.061\].

**Figure 7.2** Pulse rate in _G. lineaticeps_ sibships response to Diet A and Diet B \[F_{1,63} = 0.390, P = 0.535\].
Figure 7.3 Frequency in *G. lineaticeps* sibships response to Diet A and Diet B [$F_{1,63} = 3.59$, $P = 0.063$].

Figure 7.4 Pulses per chirp in *G. lineaticeps* sibships response to Diet A and Diet B [$F_{1,63} = 0.435$, $P = 0.512$].
Figure 7.5 Condition in *G. lineaticeps* sibships response to Diet A and Diet B [$F_{1,63} = 1.085, P = 0.302$].

Figure 8.1 Chirp rate in “*G. staccato*” sibships respond to Diet A and Diet B [$F_{1,142} = 1.14, P = 0.287$].
Figure 8.2 Pulse rate in “G. staccato” sibships response to Diet A and Diet B [$F_{1,142} = 0.0615, P = 0.804$].

Figure 8.3 Frequency in “G. staccato” sibships response to Diet A and Diet B [$F_{1,142} = 1.676, P = 0.197$].
**Figure 8.4** Pulses per chirp in “*G. staccato*” sibships response to Diet A and Diet B \[F_{1,142} = 0.253, P = 0.616\].

**Figure 8.5** Condition in “*G. staccato*” sibships response to Diet A and Diet B \[F_{1,142} = 3.44, P = 0.066\].
Figure 9.1 Chirp rate in *G. personatus* sibships response to Diet A and Diet B \(F_{1,120} = 0.693, P = 0.407\).

Figure 9.2 Pulse rate in *G. personatus* sibships response to Diet A and Diet B \(F_{1,120} = 2.55, P = 0.113\).
Figure 9.3 Frequency in *G. personatus* sibships response to Diet A and Diet B \([F_{1,120} = 1.51, P = 0.221]\).

Figure 9.4 Pulses per chirp in *G. personatus* sibships response to Diet A and Diet B \([F_{1,120} = 14.6, P = 0.0002]\).
Figure 9.5 Condition in *G. personatus* sibships response to Diet A and Diet B
\[ F_{1,120} = 2.21, \ P = 0.140 \].