

**A test of the redundant signals and multiple
messages hypotheses in two species of field crickets,
Gryllus texensis and *Gryllus assimilis***

By

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Abstract

Multiple sexual signals may indicate separate aspects of male condition (multiple messages) or independently signal overall condition (redundant signals). Traits acting as multiple messages should only respond to specific changes in condition (those for which they signal) and females should prioritize male signals indicative of the aspects of condition in which they are interested. Redundant signals should respond similarly to any change in condition and females should weigh all signals equally. I tested whether different components of the male's long distance acoustic signal act as multiple messages or redundant signals in Texas, *Gryllus texensis*, and Jamaican, *G. assimilis*, field crickets. I manipulated male juvenile and adult condition independently via dietary limitation and measured the effects on calling behavior. In both species, call components appeared to be acting as multiple messages. I tested whether female condition affects which call components are most important to them. *Gryllus texensis* females prioritized differently depending on condition. *Gryllus assimilis* females exhibited priorities, but were unaffected by condition. Overall, the multiple messages hypothesis was supported.

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General Introduction

Many species possess multiple sexual signals (Møller & Petrie, 2002). These signals can be morphological, acoustic, olfactory, tactile, behavioural, etc. They can exist in the same sensory modality (multicomponent signals) or in different sensory modalities (multimodal signals). Despite their prevalence in nature, the reason for the occurrence of multiple sexual signals remains unclear and highly debated (Candolin, 2003).

Sexual signals are traits which occur in one sex, and are used in mate selection by the opposite sex. Since it is generally males that exhibit sexual signals, and females that exhibit mate choice, I will refer only to male traits and female choice, though the reverse commonly occurs in nature. Females generally prefer the most extravagant and/or energetically expensive male trait (Ryan 1988; Andersson 1994). However, despite distinct female preferences for male trait expression, extensive variation persists in sexually selected signals (Andersson, 1994; Andersson & Simmons, 2006). In fact, most sexually selected traits exhibit higher variation than morphological traits (Pomiankowski & Møller, 1995). The reason for the maintenance of this variation, despite females consistently applying directional selection upon these traits, remains unclear (Andersson, 1994; Andersson & Simmons, 2006).

One possible explanation of the maintenance of variation in sexually selected traits is condition-dependence (Rowe & Houle, 1996). Males who are able to obtain the most resources will be in better condition, and can therefore afford to invest in an expensive sexual trait. A female who chooses such a mate may then receive either direct benefits, such as increased paternal care, or higher sperm count, or indirect genetic benefits as a result, or both. Therefore, a female who assesses a male's sexual signal, will be able to assess both his condition, and the potential benefits he has to offer.

The occurrence of multiple sexual traits presents a dilemma. In theory, multiple female preferences for such traits should not evolve, since each additional trait strongly increases the overall cost of female choice (Pomiankowski & Iwasa, 1993; Møller & Pomiankowski, 1993; Iwasa & Pomiankowski, 1994). Instead, females should select for one main cue that is the most honest, reliable and detectable indicator of condition of all the available traits (Schluter & Price, 1993; Johnstone, 1996). Additional traits would then evolve only through Fisherian selection. Two hypotheses have been put forward to address the dilemma of why multiple sexual signals persist: the redundant signals hypothesis and the multiple messages hypothesis (Møller & Pomiankowski, 1993).

Redundant Signals and Multiple Messages

The redundant signals hypothesis states that each trait is an independent signal of overall condition (Møller & Pomiankowski, 1993). Since all traits are independent of one another, they will differ in the direction and magnitude of their errors in signaling overall condition. Therefore, a female must assess all of the traits in order to obtain the most accurate estimate of overall male condition. Additionally, redundant signals may collectively improve the efficacy of signal transmission or reception (Nelson & Poesel, 2007). For example, male spadefoot toads, *Scaphiopus couchii*, exhibit multiple sexual signals: acoustic signals (calls) and sexually dimorphic coloration (Vásquez & Pfennig, 2007). These two signals were both found to be correlated to overall condition. Since calling often occurs in large aggregations, it may become quite difficult for females to assess males. Coloration that provides a redundant cue to acoustic signaling may,

therefore, aid females in locating high quality mates in a noisy environment. In this way, redundant signals may actually reduce the cost of choice for females.

The multiple messages hypothesis, on the other hand, proposes a mechanism for multiple sexual signals to evolve, even if exhibiting multiple preferences is costly (van Doorn & Weissing, 2004). This hypothesis states that each trait signals a different aspect of male condition, or signals condition at different stages of life (Møller & Pomiankowski, 1993). For example, Scheuber et al. (2003a, b) found that two aspects of the male acoustic sexual signal in field crickets, *Gryllus campestris*, were each indicative of male condition at a different stage of life. By manipulating either juvenile or adult male condition, they found that one acoustic parameter, carrier frequency, was indicative of juvenile condition, while a different acoustic parameter, chirp rate, was indicative of adult condition. A female that assesses all of a male's traits can evaluate several aspects of his condition, and will therefore be in a position to select the most suitable mate for her particular needs.

Correlations between Multiple Signals

Many studies have attempted to distinguish between the redundant signals hypothesis and the multiple messages hypothesis by examining the correlations between multiple sexual signals within individuals (reviewed in Candolin, 2003). The premise behind this approach is that redundant signals should all be correlated, since they are indicative of, and thus correlated to, the same overall condition, whereas multiple messages should not necessarily be correlated since they are indicative of different aspects of condition (van Doorn & Weissing, 2004). For example, Higgins and

Waugaman (2004) examined three components of the long distance acoustic signal (call) of the Texas field cricket, *Gryllus texensis* (pulse rate, trill length and intertrill interval). They found strong correlations between all three components, and therefore concluded that these components must be redundant signals. Conversely, Buchanan and Catchpole (1997) investigated twelve possible sexual signals in the sedge warbler, *Acrocephalus schoenobaenus*, and found three to be correlated with mating success: repertoire size, song flighting and territory size. None of the three variables were correlated with each other. The authors took this lack of correlation to mean support for the multiple messages hypothesis.

However, this correlative approach is based on an incorrect, though intuitive, interpretation of the original hypotheses (Møller & Pomiankowski, 1993). Although redundant signals should each be correlated with overall condition, they provide independent estimates, and as such need not be correlated with each other (van Doorn & Weissing, 2004). In fact, several studies have demonstrated traits that appear to act as redundant signals, but which are not correlated to each other. For example, Vásquez and Pfennig (2007) found that male coloration and calling behaviour in the spadefoot toad were both correlated to overall condition (ie. act as redundant signals). However, the two signals were not correlated to each other. Conversely, other studies have shown that multiple traits can be correlated, and yet can still be indicative of different aspects of condition (multiple messages). For example, Møller and Petrie (2002) investigated the role of the train, a multicomponent signal, in male blue peafowl, *Pavo cristatus*. They found that the three components in question – longest feather length, number of ocelli and average ocelli diameter – were all positively correlated with each other. However,

despite the correlations, each trait component differed in the extent to which it was indicative of three components of immunocompetence (cell-mediated immunity, humoral immunity and heterophil-lymphocyte ratio). As such, correlations or lack thereof between multiple sexual signals cannot be used to distinguish between the redundant signals hypothesis and the multiple messages hypothesis.

Two more effective methods to discriminate between the redundant signals and multiple messages hypotheses have been described in the literature. The first is to directly manipulate different aspects of condition, or condition at different stages throughout life, and monitor the subsequent changes in the expression of each sexual signal (e.g. Scheuber et al., 2003a; b). The second is to test for differences in the way that females prioritize between these signals (van Doorn & Weissing, 2004). I provide details on both of these discrimination methods in the next two sections.

Response of Multiple Signals

Both the redundant signals and multiple messages hypothesis require that multiple traits be condition-dependent. However, these signals should react differently to changes in specific aspects of condition, depending on whether they are redundant signals or multiple messages. Since redundant signals are indicative of the same overall condition, all traits should change in the same direction in response to any change in condition (Møller & Pomiankowski, 1993). For example, if an organism possessing two sexual traits (Trait 1 and Trait 2) is in good overall condition, then both traits should be highly exaggerated (Table 0.1). If any aspect of condition, such as juvenile condition, is decreased, then the organism's overall condition will decrease as well. If Traits 1 and 2

are redundant signals, they should both decrease their expression in response to this reduction in overall condition (Table 0.1). Conversely, the multiple messages hypothesis predicts that multiple traits should be fairly unresponsive to most changes in condition, but very responsive to changes in the specific aspects of condition of which they are indicative (Møller & Pomiankowski, 1993). For example, if Trait 1 is indicative of juvenile condition, and Trait 2 is indicative of adult condition, then decreasing juvenile condition should only result in a reduction in the expression of Trait 1. Trait 2 should not change in expression because adult condition was not affected. Conversely, if only adult condition is decreased, only Trait 2 should decrease in expression (Table 0.1).

Table 0.1 *Predicted changes in the expression of two hypothetical sexual signals in response to changes in two separate aspects of condition.*

Hypothesis	Aspect of Condition 1	Aspect of Condition 2	Overall Condition	Expression of Trait 1	Expression of Trait 2
Redundant Signals	Decrease	--	Decrease	Decrease	Decrease
	--	Decrease	Decrease	Decrease	Decrease
Multiple Messages	Decrease	--	Decrease	Decrease	No effect
	--	Decrease	Decrease	No effect	Decrease

To date, very few studies have explicitly manipulated condition in the manner required to test the effects on multiple signals. For example, in male bowerbirds, ultraviolet-violet (UVV) brightness (measured as feather reflectance in the UVV spectrum), and bower quality were found to be significantly correlated with different aspects of male condition (Doucet & Montgomerie, 2003). Feather UVV brightness was correlated with endoparasite load, while bower quality was correlated with ectoparasite load. However, these relationships were purely correlative. It is possible that the signals

were actually indicative of other, unmeasured, aspects of condition, which were correlated to the aspects which were actually measured. It is also possible that both of these measures of immunity are correlated to overall immunity. Without manipulating both endo- and ectoparasite load separately, it is impossible to conclude whether these aspects of condition are directly affecting the expression of these two sexual signals.

Similarly, multiple feather ornaments in collared flycatchers, *Ficedula albicollis*, are potentially indicative of male condition at different times of the year (Hegyi et al., 2007). Forehead patch size was found to be correlated with winter climate, while wing patch size was found to be correlated with summer climate. Since climate can have a great effect on male condition, these traits may be indicators of condition at separate life stages. However, these relationships were purely correlative; differences in trait expression may not have been due to climate itself, but to another variable related to climate, such as food availability. Without direct manipulation of each variable, while controlling for all others, it is impossible to definitively uncover such relationships.

One of the few studies which did directly manipulate different aspects of condition separately involved condition at different life stages of the field cricket, *G. campestris* (Scheuber et al., 2003a; b). Scheuber et al. (2003a) first reduced adult condition by limiting food availability during adulthood, then measured the subsequent effects on different parameters of the male acoustic long-distance mate attraction signal (call). They found that in food-limited adult males, chirp rate was decreased (ie. less attractive) compared to males who had been provided food *ad libitum*. However, they found that another call component, carrier frequency, was unaffected. When they decreased juvenile condition only, by food-limiting juvenile males (Scheuber et al.,

2003b), they found that carrier frequency was increased (ie. became less attractive, since females prefer lower frequencies), but chirp rate was unaffected compared to control males. This series of studies successfully supported the multiple messages hypothesis. It demonstrated that the two different call components were indicative of different aspects of condition by directly manipulating those aspects.

Multiple Female Preferences

Most studies have not tested for female preference for the traits in question. Without testing for female preference, such studies fail to confirm a key prediction of both the redundant signals and multiple messages hypotheses: that multiple female preferences can evolve (Møller & Pomiankowski, 1993; Johnstone, 1996; van Doorn & Weissing, 2004). For example, as mentioned above, Møller and Petrie (2002) found that different train components were correlated to different aspects of immunocompetence in the peacock, male blue peafowl, *Pavo cristatus*, possibly indicating the multiple messages hypothesis. However, they did not test for female preference for these components. With the exception of one component, it is unknown whether females actually select for these components. Without this information it is impossible to state that these components are even used in sexual selection. It is unfortunate that the study did not investigate the slew of male blue peafowl traits for which females are known to select (e.g. blue ventral colour, green dorsal colour, exaggerated crest, white facial skin patch, long spurs, etc.; Møller & Petrie, 2002).

In addition, females may prioritize some traits over others. For example, Smith and Roberts (2003) artificially varied three components of the mating call of the quacking

frog, *Crinia georgiana*: call rate, dominant frequency and number of pulses in the first note called. When they varied one component at a time, they found that females preferred high calling rate, average dominant frequency and high number of pulses in the first note called. However, when they manipulated two variables at a time, they uncovered a hierarchy of female preference between traits. They found that females preferred a song with an average dominant frequency (which was previously found to be preferable) even if it had a low call rate (which was found to be less attractive) over a song that had a high call rate (preferable) but that had a high or low dominant frequency (not preferable). As such, they found that multiple female preferences were affected by the relative expression of each song component (Smith & Roberts, 2003).

van Doorn and Weissing (2004) assert that the only way to confirm the multiple messages hypothesis is to demonstrate that females weigh multiple signals differently. Because redundant signals are all indicative of the same overall condition (Møller & Pomiankowski, 1993), none should be more accurate than the rest, otherwise there would simply be selection on the most reliable, honest and detectable signal (Schluter & Price, 1993; Johnstone, 1996). Therefore, females should not prioritize any signals over others. However, if multiple traits are acting as multiple messages, then some females should prioritize certain traits over others, while other females will prioritize different traits. Since multiple messages are indicative of different aspects of condition (Møller & Pomiankowski, 1993), some signals may indicate direct benefits to the female, while others indicate indirect, genetic benefits (Candolin, 2003). Females should, therefore, exhibit different priorities for signals depending on which benefits they are seeking. For example, female barn swallows, *Hirundo rustica*, that are in good condition prefer males

that have long tails, which is indicative of high genetic quality (Soler et al., 1998). However, these males are poor nest-builders, and do not provide as much direct benefit as other males. Conversely, females that are in poorer condition tend to prefer short-tailed males. Such males have lower genetic quality, but build better nests. It therefore appears that females in good condition can afford to prioritize genetic quality over direct benefits. It is important to note that in this case, females appeared to be choosing mates based on only one trait, tail length, which was indicative of genetic quality. However, it is also possible that they were also selecting males based on another, unmeasured, trait that was indicative of nest-building effort.

A likely, but thus far untested possibility is that, in the case of multiple messages, short-term, dynamic traits (those which vary greatly over time) signal direct benefits, while long-term, static traits (those that do not vary much over time) signal indirect genetic benefits (Gerhardt, 1991; Hill et al. 1999; Kokko et al. 1999; Candolin, 2003). This is because the nature of an honest signal of condition is likely to be similar to the particular aspect of condition to which it is related. In other words, it is likely that an aspect of male condition, such as those mainly determined by genetic quality, which does not vary much over time, are more likely to be signaled by traits that also do not and cannot vary greatly over time. Conversely, a more variable aspect of condition, such as current male nutritional status, is more likely to be signaled by a trait which is also highly variable over time. For example, bower quality was related to ectoparasite load, while feather UVV brightness was correlated to endoparasite load in bowerbirds (Doucet & Montgomerie, 2003). These traits, and the respective aspects of condition for which they signal, vary at different rates over time. Ectoparasites, such as lice, can be easily

acquired and removed and, therefore, the ectoparasite load of a single individual can vary greatly over a short period of time. Likewise, bower quality, which signals ectoparasite load, can also change easily from one day to the next, making it a suitable signal for that aspect of male condition (Doucet & Montgomerie, 2003). Blood parasite load, on the other hand, involves immune response and therefore changes more slowly over time. Likewise, plumage condition, which was indicative of blood parasite load, has also been found to vary more slowly over time, as it generally only changes between moults (Doucet & Montgomerie, 2003). In this species, a female may choose between the direct benefit of lowering her immediate exposure to ectoparasites, which can easily be transferred between individuals, or the indirect benefit of obtaining high quality immune resistance to endoparasites for her offspring. A female in poor condition may choose to lower her immediate risk of being exposed to ectoparasites by choosing a male with high bower quality, even if he exhibits less preferable feather condition. A female in good condition, on the other hand, may prefer to mate with a male with better feather condition (indicating immune function), even if his bower quality is relatively low (indicating high ectoparasite load), since she will be better able to resist the transfer of ectoparasite, or better able to remove them.

Study Organism

I used the Texas field cricket, *Gryllus texensis*, and Jamaican field cricket, *Gryllus assimilis*, to test whether male secondary sexual traits respond differently to changes in separate aspects of condition (multiple messages hypothesis) and whether females that differ in body condition prioritize these traits differently. Crickets were an ideal model

organism for this project because their mating system has been studied extensively, both in the context of multiple sexual signals, and in the field of sexual selection in general.

Male crickets produce a long-distance acoustic signal (call) to attract mates. This call is a multicomponent signal, consisting of several acoustic parameters (e.g. pulse rate, number of pulses per trill, carrier frequency, etc.), each of which has the potential to act as a separate sexual signal. Many of these parameters have been previously found to vary with different aspects of condition, or with condition at different life stages in other related cricket species. For example, Scheuber et al. (2003a, b) found that, in the long-distance call of the field cricket, *G. campestris*, chirp rate decreased when adult condition was decreased, and carrier frequency increased (due to decreased wing size) when juvenile condition was decreased. This manipulative study showed that chirp rate and carrier frequency alone were indicative of male adult and juvenile condition, respectively. Trait expression also tends to decrease when different aspects of condition are decreased. This is not surprising, given calling behaviour is energetically expensive. For example, Hedrick (2005) found that when adult male field crickets, *G. integer*, were fasted for two days, their mass decreased (indicating a decrease in condition) as did their long-distance calling bout duration (the average length of time they would call for continuously). Moreover, Hedrick (2005) found that the decrease in mass was proportional to the reduction in bout duration. This suggests that bout duration is an accurate signal of current condition in this species.

Female crickets exhibit distinct preferences for many of these parameters, most of which involve an increase in overall calling (Wagner & Hoback, 1999). For example, female Texas field crickets, *G. texensis*, prefer males that spend the most time calling

throughout the night (Cade & Cade, 1992), have the largest average number of pulses per trill (Gray & Cade, 1999), and exhibit shorter intercall intervals (Wagner et al., 1995). Similarly, female variable field crickets, *G. lineaticeps*, prefer males with higher chirp rates and longer chirp durations (Wagner & Hoback, 1999).

Female crickets receive different benefits by mating with males that exhibit preferable traits. For example, female variable crickets, *G. lineaticeps*, that were mated to males with higher chirp rates (preferred) gained the direct benefit of greater lifetime fertility (Wagner & Harper, 2003). The same study also found that females who were mated to males that produced longer chirp durations (preferred) gained the direct benefit of increased longevity (Wagner & Harper, 2003). However, this study only varied one trait at a time. It did not look for interactions between female preferences.

Female crickets have been shown to prioritize some male signals (and thus, some aspects of condition) over others. In a follow-up to the previous study, Wagner and Basolo (2007) found that when both chirp rate and chirp duration were varied simultaneously, females still preferred higher chirp rates, but did not prefer a call with a higher pulse duration (preferred) if the chirp rate was low. Their study suggests that females prioritized chirp rate, and its associated benefits (greater lifetime fertility), over pulse duration. Similarly, Scheuber et al. (2004) found that female *G. campestris*, preferred low carrier frequency and high chirp rates, but prioritized carrier frequency (the static, long-term signal) over chirp rate (the dynamic, short-term signal). Carrier frequency, as the less variable, long-term signal, may be more indicative of overall male suitability to the environment and thus could indicate indirect genetic benefits to the female. Chirp rate, on the other hand, being more indicative of short-term fluctuations in

condition may advertise direct benefits, such as higher sperm count (Andersson, 1994; Candolin, 2003). In both aforementioned cricket studies, females prioritized the long-term signal. However, females were fed *ad libitum*, and were thus in relatively good body condition. Females in poorer condition may prioritize the short-term signal over the long-term signal because of their more immediate need for direct benefits over indirect genetic benefits. To my knowledge, no study has yet experimentally tested the effect of female condition on her prioritization of multiple sexual signals.

Project Goals

I used the Texas field cricket, *G. texensis*, and the Jamaican field cricket, *G. assimilis*, to test how different components of the males' calls responded to changes in condition at the juvenile and adult stages (Chapter 1). I used field-captured males to quantify the natural variation in signaling components and how this variation was correlated with body size and condition. I also conducted experiments using laboratory reared crickets where I manipulated the body condition of juveniles and adults via dietary limitation and then assessed how condition influenced signaling components. If the calling components are redundant signals, then they should respond similarly to reductions in condition at both life stages. However, if the calling components act as multiple messages, then only one, or a few, should respond to a decrease in condition at the juvenile stage, while a different one, or few, should respond to a decrease in condition at the adult stage.

As an essential follow up to this study, I tested whether females that differ in body condition prioritize these call components differently (Chapter 2). The redundant signals

hypothesis predicts that females should not prioritize any of the signals, as each is indicative of the same overall condition, and none should be more reliable or honest than another. Conversely, the multiple messages hypothesis predicts that females in good condition should prioritize different signals than females in poor condition.

Chapter 1 – Male Calling Behaviour

Methods

1a – Natural Variation

Adult male and female *G. texensis* and *G. assimilis* of unknown age, calling history, and mating experience were collected at night from fields and parking lots in Austin, Texas, USA, Sep 26-Oct 4, 2007 (Field Season 1), and Sep 12-Sep 26, 2008 (Field Season 2). Crickets were separated by species and sex into communal 68 l plastic bins, given food and water *ad libitum*, and provided with crumpled paper towel for shelter. Females were provided with wet dirt in which to lay eggs.

During Field Season 1, approximately 200 field-captured *G. texensis* males were placed into individual 500 ml containers, along with *ad libitum* food and water. Their long-distance mate attraction calls (if they were observed to produce any) were individually recorded for at least two minutes using a handheld Zoom Recorder between the hours of 20:00 h and 06:00 h. Male calls consist of individual pulses of sound, which are grouped into trills (approx. 20-40 pulses each, *G. texensis*) or chirps (approx. 7-11 pulses each, *G. assimilis*). Spike2 audio software was used to measure mean pulse duration, mean pulses per trill/chirp, mean interpulse duration (time between pulses), mean intertrill/interchirp duration (time between trills/chirps), mean pulse rate (pulses per minute), mean trill/chirp rate (trills/chirps per minute) and the dominant frequency (carrier frequency of the call) of each individual recording (Figure 1.1).

Males which had been individually recorded were then monitored by the Electronic Acoustic Recorders (EARs) for at least 24 hours following recording. This system monitored each individual's temporal calling pattern. Each container was

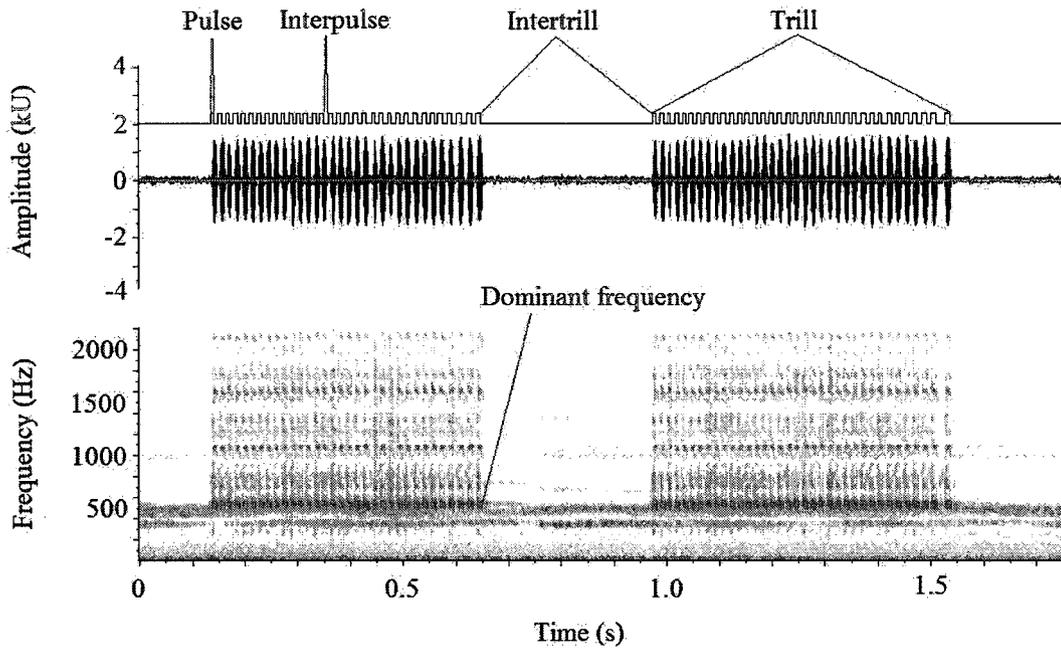
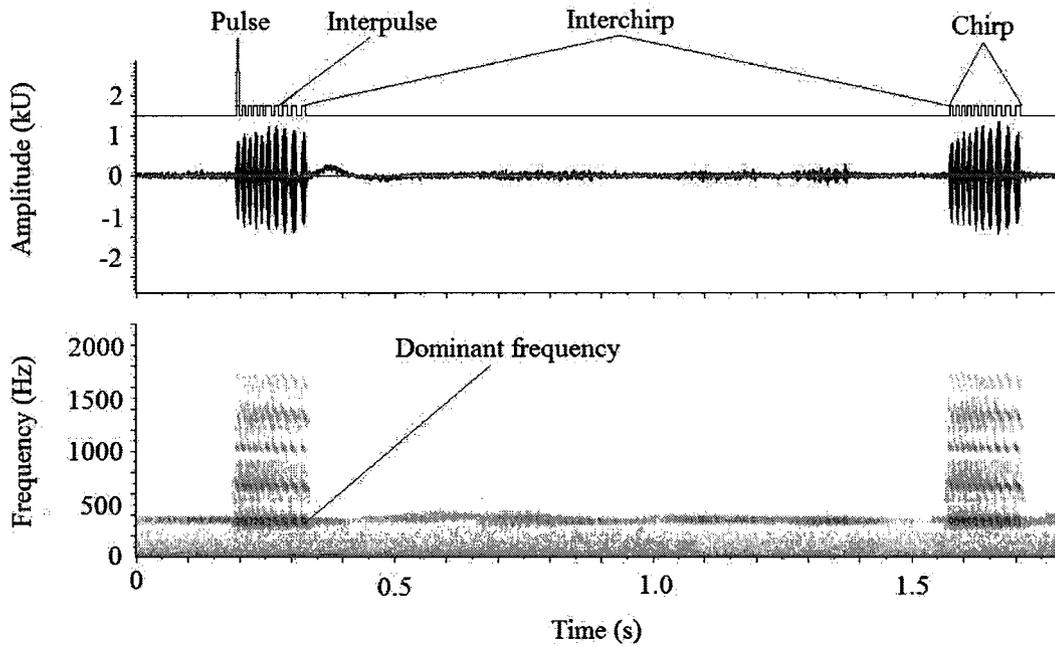
separated by acoustic foam, to prevent sound contamination across containers, and was monitored with its own microphone, hung approximately 5 cm above the cricket. Each microphone took eight readings per second. If the reading was above a certain threshold (ie. the cricket was calling) the system scored that reading as a 1. If the reading was not above that threshold, the system scored it as a 0. Each second, the eight readings were added together, producing a score from 0 to 8. Thus, the raw data from the EARs are scores, between 0 and 8, for every individual every second throughout the course of the night. Raw data from the EARs were analyzed using a Matlab algorithm to determine the average time spent calling per night.

I obtained a measure of dry mass and pronotum area for each recorded male. Pronotum area was measured from highly magnified photographs taken with a Zeiss Axio Observer inverted microscope of the frozen bodies using AxioVision software. Dry weight was obtained following at least 24 h in a drying oven at 200°C.

Statistical Analyses

All statistical analyses were performed with JMP 7 statistical software (SAS Institute Inc., 100 SAS Campus Drive, Cary, U.S.A.). Pronotum area was used as an index of structural body size (Scheuber et al., 2003b). Since body size is determined by juvenile condition, I performed linear regressions of each calling parameter onto pronotum area in order to determine which calling parameter (or parameters) was an indicator of juvenile condition. The residuals of a regression of dry mass onto pronotum area were used as quantitative measures of condition (Gray & Eckhardt, 2001). Since body condition is related to current nutritional status, I performed linear regressions of

each calling parameter onto condition in order to identify which parameter (or parameters) was an indicator of adult condition.

(a) *G. texensis* long-distance mate attraction call.(b) *G. assimilis* long-distance mate attraction call.Figure 1.1 Several parameters of the long-distance mate attraction calls of (a) *G. texensis* and (b) *G. assimilis*.

1b – Effects of Diet on Male Calling Behaviour

Prior to the start of the experiment, crickets were raised in communal 68 l plastic containers (containers were separated by species). A 10 cm x 15 cm hole was cut in each lid, with wire mesh glued to it, to provide the crickets with light and air. In 2008, communal containers were housed in the basement insect rearing room of the Nesbitt Building at Carleton University, Ottawa, Ontario, Canada. In 2009, communal containers were relocated to a greenhouse bay in the same building. Lights above the wire mesh windows were controlled on timers to provide a 12 h:12 h L:D cycle. Crickets were fed food and water *ad libitum*, and were provided with egg cartons for shelter. Upon reaching the fourth to sixth instar, juvenile males were placed into individual 500 ml plastic containers. They were provided with water *ad libitum* and a crumpled piece of paper towel for shelter.

Dietary Manipulations

All males were given powdered rat chow (Harlan Teklad Rodent diet no. 8604) in 1” x 1” plastic weighing dishes. They were provided with fresh water at all times. If a male’s moulted exoskeleton was discovered in his container, it was left undisturbed, regardless of the dietary treatment; in this way, all males were able to consume all of their moults if they wanted. Males were checked every two days in order to determine the approximate date of final moult (ie. the first day of adulthood).

From Feb-Mar 2008 (Winter 2008), 1st-generation male offspring (n = 98) of the adult *G. texensis* collected during Field Season 1 were placed onto one of two dietary treatments upon reaching adulthood. The first, a “low” treatment, consisted of giving, on

average, half (by mass) of what the cricket would normally eat per day (corrected for body mass). Males on the “high” treatment were given *ad libitum* food. Food masses for the low treatment were estimated by measuring the amount of food consumed daily, on average, by 15 pilot males placed on the high treatment for one week. Males were removed from the experiment once they had been recorded.

From May-Nov 2008 (Summer 2008), 2nd-generation male offspring of the adult *G. assimilis* collected during Field Season 1 were randomly assigned to one of four dietary treatments. Each treatment consisted of either a high (high) or low (low) juvenile diet, followed by a high (high) or low (low) adult treatment (resulting in a factorial design of juvenile diet-adult diet: HH, HL, LH, LL). The low dietary treatments (both juvenile and adult) consisted of a continuous cycle of *ad libitum* food for two days, followed by two days of fasting. The high dietary treatment consisted of *ad libitum* food at all times. On fasting days, males on the low diet were given empty dishes. Males were kept on the juvenile diets until reaching adulthood. They were then kept on their adult diet until they were removed from the experiment. This experiment was replicated from Jan-Jul 2009 using 1st-generation offspring (Winter 2009) of the adult *G. assimilis* collected during Field Season 2. *Gryllus assimilis* males in the Summer 2008 replicate were removed from the experiment once they had been recorded, or on Nov. 9 2008, the day the experiment was terminated. Males in the Winter 2009 replicate were removed from the experiment at 28 days of adult age.

Once males had been removed from their respective experiments, they were humanely euthanized by freezing at -20°C for at least 30 min. All bodies were preserved in a -20°C freezer. I obtained a measure of dry mass and pronotum area for each

recorded male. Pronotum area was measured from highly magnified photographs taken with a Zeiss Axio Observer inverted microscope of the frozen bodies using AxioVision software. Dry weight was obtained following at least 24 h in a drying oven at 200°C.

Calling Parameters

Male *G. texensis* (Winter 2008) and *G. assimilis* (Summer 2008) long-distance mate attraction calls were recorded for at least two minutes, using a handheld Zoom Recorder (as described above) between the hours of 20:00 h and 05:00 h. *Gryllus texensis* were recorded at 6-32 days of adult age (15.8 ± 6.9 days, mean \pm SD, $n = 24$), and *G. assimilis* were recorded at 10-94 days of adult age (43.8 ± 21.7 days, mean \pm SD, $n = 51$).

Winter 2009 *G. assimilis* males were recorded using the New Electronic Acoustic Recorders (NEARs). Males were placed into the system upon reaching adulthood. The system consists of 96 individually recording microphones. Each microphone is also coupled with a single LED light that provided the males with the same 12 h:12 h L:D cycle as the rearing room. Each microphone is contained in an acoustically isolated enclosure (a cooler box made of 2" thick Styrofoam that is internally lined with 1" thick acoustic foam) to reduce sound contamination from neighboring males. The microphones are continuously monitored by a computer program called Cricket (developed specifically for the Bertram lab by Cambridge Electronic Design Ltd., Unit 4, Science Park, Milton Road, Cambridge, UK). Cricket automatically analyzes the sound wave recorded by the microphone and calculates, in real time, all of the calling parameters described above, as well as the call amplitude.

Statistical Analyses

Raw Zoom recordings and NEARs data were manipulated and summarized for data analysis using Spike2 audio software. All data were then analyzed using JMP 7 statistical software (SAS Institute Inc., 100 SAS Campus Drive, Cary, U.S.A.). Pronotum area was used as an index of structural body size (Scheuber et al., 2003b). The residuals of a regression of dry mass on pronotum area were then used as a measure of condition (Gray & Eckhardt, 2001). The effects of adult diet on *G. texensis* body size, condition and each calling parameter were analyzed using one-way ANOVAs. For *G. assimilis*, the effects of juvenile and adult diet on body size, condition, and calling behaviour were assessed using two-way ANOVAs. Because there was such a large age range in the Summer 2008 replicate, where necessary, calling parameters were adjusted to control for the effects of age. Where significant or near significant effects of diet were observed, I tested for a correlation between that call parameter and measures of body size and/or condition using linear regressions.

Results

1a – Natural Variation

G. texensis

Of the 36 *G. texensis* males that were recorded during Field Season 1, seven called for less than five minutes per night (as measured by the EARs). These males were categorized as the typically silent “satellite” males, and were excluded from all calling behaviour analyses. Two of the remaining males exhibited unusually high numbers of pulses per trill (77.3 and 105.3). These males were possibly *G. rubens*, a morphologically identical species inhabiting the same region, and were also excluded from all calling behaviour analyses (**Note:** excluding these two individuals did not affect the significance level or direction of any of the relationships described below).

The natural variation in *G. texensis* for each of the measured calling parameters is displayed in Table 1.1. Both dominant frequency and pulse duration were significantly correlated with body size (pronotum area; dominant frequency: Figure 1.2, Table 1.2; $r^2 = -0.189$, $F = 5.8321$, $p = 0.0234$; pulse duration: Figure 1.3, Table 1.2; $r^2 = 0.46$, $F = 21.4106$, $p < 0.0001$). They were also almost significantly correlated to each other ($r^2 = 0.129$, $F = 3.6977$, $p = 0.0660$, $DF = 1$, $n = 27$). No other calling parameters were significantly correlated with body size (Table 1.2). There was a non-significant correlation between trill duration and body condition (Figure 1.4, Table 1.3; $r^2 = 0.111$, $F = 2.8692$, $p = 0.1038$). No calling parameters were significantly correlated with body condition (Table 1.3).

Table 1.1 *The natural variation for all calling parameters for G. texensis males trapped and recorded during Field Season 1 (n = 27). These values were later used to construct ecologically-relevant calls for the female preference trials (Chapter 2).*

Calling parameter	Mean	Std Dev	Min	Max
Mean pulse duration (ms)	7.131	1.400	4.230	10.32
Mean interpulse duration (ms)	6.808	2.452	3.729	13.83
Mean pulses per trill	29.37	7.973	18.66	51.12
Mean trill duration (ms)	396.8	107.0	246.8	745.7
Mean intertrill duration (ms)	192.4	92.25	117.1	570.0
Dominant frequency (Hz)	5559	304.6	4742	6031
Mean pulse rate (min ⁻¹)	4473	580.5	2899	5427
Mean trill rate (min ⁻¹)	106.9	24.62	55.48	159.6

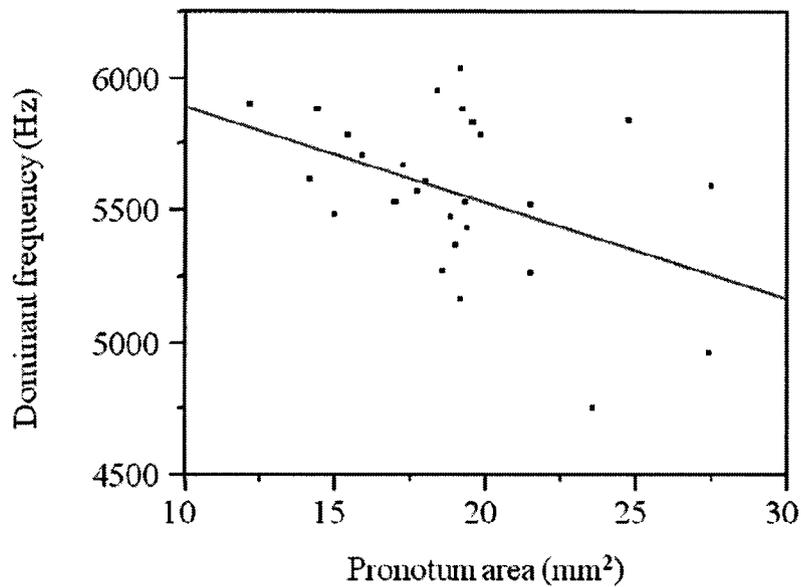


Figure 1.2 *Linear regression of dominant frequency onto pronotum area (body size) for G. texensis males trapped and recorded during Field Season 1 ($r^2 = 0.189$, $F = 5.8321$, $p = 0.0234$, $DF = 1$, $n = 27$).*

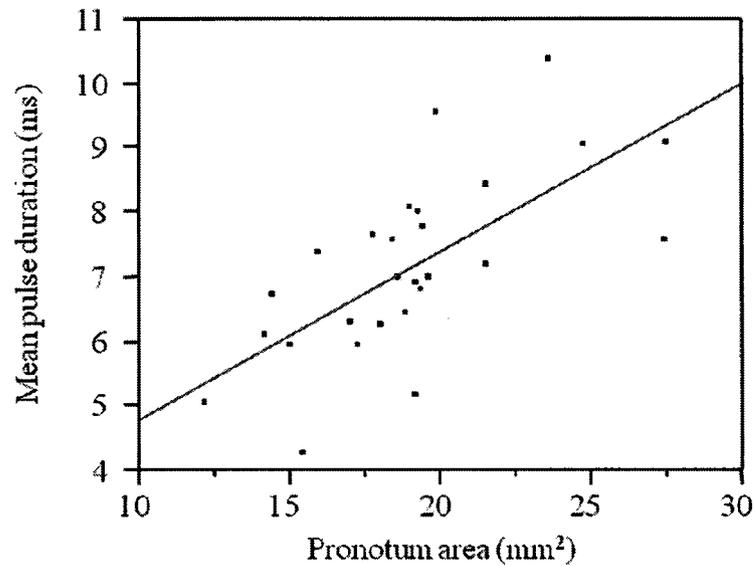


Figure 1.3 *Linear regression of mean pulse duration onto pronotum area (body size) for G. texensis males trapped and recorded during Field Season 1 ($r^2 = 0.46$, $F = 21.4106$, $p < 0.0001$, $DF = 1$, $n = 27$).*

Table 1.2 *Results of linear regressions of each calling parameter onto pronotum area (body size) for G. texensis males trapped and recorded during Field Season 1 ($DF = 1$, $n = 27$ for all calling parameters).*

Calling parameter	r^2	F	p
Mean pulse duration (ms)	0.461	21.4106	< 0.001**
Mean interpulse duration (ms)	0.088	2.4129	0.1329
Mean pulses per trill	0.018	0.4682	0.5001
Mean trill duration (ms)	0.007	0.1713	0.6825
Mean intertrill duration (ms)	0.126	3.6146	0.0689
Dominant frequency (Hz)	0.189	5.8321	0.0234*
Mean pulse rate (min ⁻¹)	0.017	0.4400	0.5132
Mean trill rate (min ⁻¹)	0.044	1.1516	0.2935

(*) denotes $p < 0.05$

(**) denotes $p < 0.01$

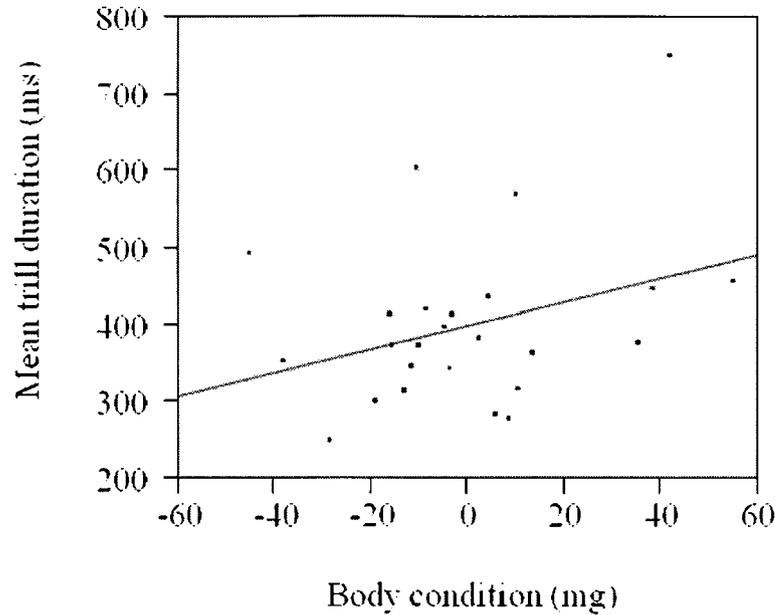


Figure 1.4 *Linear regression of mean trill duration onto body condition for G. texensis males trapped and recorded during Field Season 1 ($r^2 = 0.111$, $F = 2.8692$, $p = 0.1038$, $DF = 1$, $n = 25$).*

Table 1.3 *Results of linear regressions of each calling parameter onto body condition for G. texensis males trapped and recorded during Field Season 1 ($DF = 1$, $n = 25$ for all calling parameters).*

Calling parameter	r^2	F	p
Mean pulse duration (ms)	0.018	0.4127	0.5269
Mean interpulse duration (ms)	0.003	0.0788	0.7814
Mean pulses per trill	0.057	1.3822	0.2518
Mean trill duration (ms)	0.111	2.8692	0.1038
Mean intertrill duration (ms)	< 0.001	0.0016	0.9686
Dominant frequency (Hz)	0.038	0.9031	0.3518
Mean pulse rate (min^{-1})	0.044	1.0584	0.3143
Mean trill rate (min^{-1})	0.078	1.9541	0.1755

1b – Effects of Diet on Male Calling Behaviour

G. texensis

In the Winter 2008 replicate (fed high: ad lib, or low: 50% (mass), as adults) *G. texensis* adults on the high diet produced significantly more pulses per trill, and subsequently significantly longer trill durations than males on the low diet (pulses per trill: Figure 1.5; $r^2 = 0.178$, $F = 5.6341$, $p = 0.0253$; trill duration: Figure 1.6; $r^2 = 0.261$, $F = 9.1708$, $p = 0.0055$). Adult diet did not have a significant effect on the other calling parameters (Table 1.4).

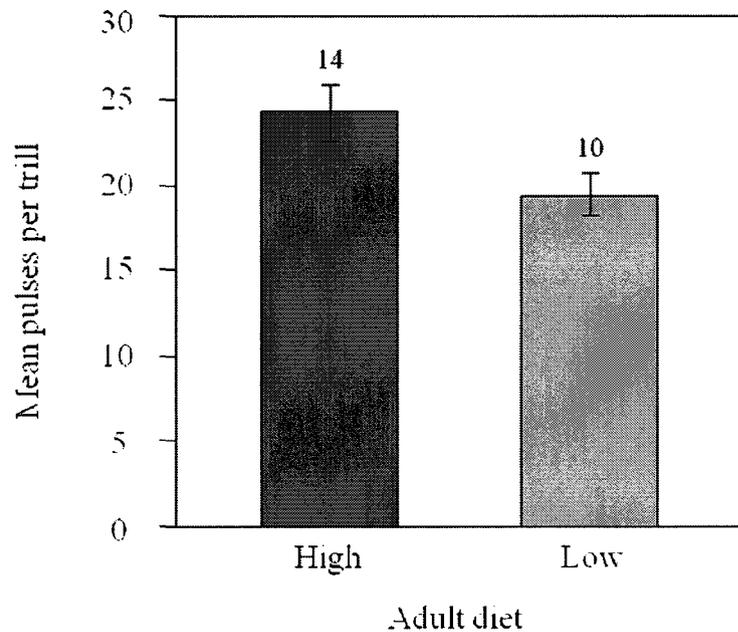


Figure 1.5 *Effect of adult diet on the mean number of pulses per trill for G. texensis males recorded during the Winter 2008 replicate ($r^2 = 0.362$, $F = 12.5$, $p = 0.0019$, $DF = 1$).*

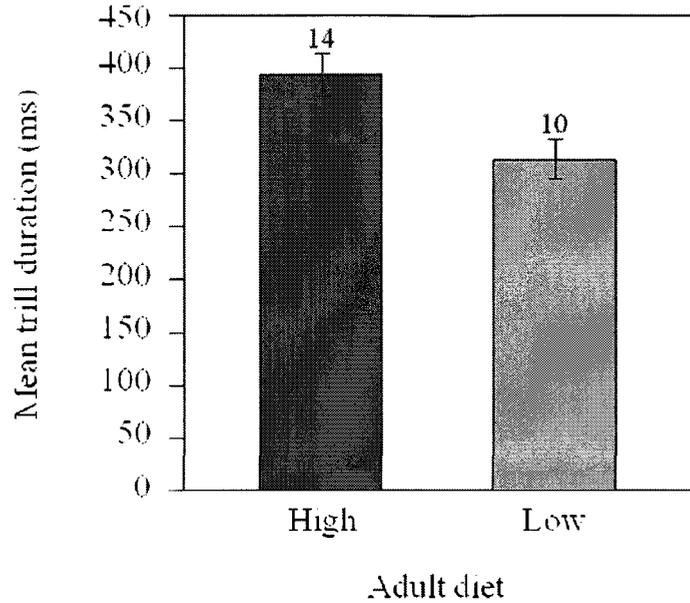


Figure 1.6 *Effect of adult diet on mean trill duration for G. texensis males recorded during the Winter 2008 replicate ($r^2 = 0.448$, $F = 17.8$, $p = 0.0003$, $DF = 1$). Numbers above bars represent sample sizes.*

Table 1.4 *Results of one-way ANOVAs comparing calling parameter expression between adult diets (high vs. low) for G. texensis males recorded during the Winter 2008 replicate ($DF = 1$, $n_{High} = 13$, $n_{Low} = 15$ for all calling parameters).*

Calling parameter	r^2	F	p
Mean pulse duration (ms)	0.133	3.9959	0.0562
Mean interpulse duration (ms)	0.065	1.7971	0.1917
Mean pulses per trill	0.178	5.6341	0.0253*
Mean trill duration (ms)	0.261	9.1708	0.0055**
Mean intertrill duration (ms)	0.001	0.0209	0.8861
Dominant frequency (Hz)	0.007	0.1728	0.6811
Mean pulse rate (min^{-1})	0.001	0.0281	0.8682
Mean trill rate (min^{-1})	0.108	3.1537	0.0875

(*) denotes $p < 0.05$

(**) denotes $p < 0.01$

G. assimilis

Summer 2008. In the Summer 2008 replicate (fed high: ad lib, or low: 2 days on-off, as juveniles and adults; recorded individually by handheld Zoom recorder) juvenile diet alone was found to have a effect on pronotum area; males that had been on the high diet as juveniles were significantly larger as adults than males that had been on the low juvenile diet (Figure 1.7; juvenile diet: $F = 4.8036$, $p = 0.0305$). At the time of recording, body condition was significantly affected by an interaction between juvenile and adult diet (Table 1.5; juvenile diet * adult diet: $F = 5.5810$, $p = 0.0223$). This relationship did not remain when condition effects was calculated using dry mass (Table 1.5; ANOVA: $r^2 = 0.030$, $F = 1.1048$, $p = 0.3505$).

Pulse rate was the only calling parameter significantly affected by juvenile diet alone (Tables 1.5 & 1.7, Figure 1.8; juvenile diet: $F = 4.4282$, $p = 0.0403$). It, therefore, appeared that pulse rate was indicative of juvenile condition. This was further supported by the near significant positive relationship between pulse rate and pronotum area (Figure 1.9; $r^2 = 0.059$, $F = 3.1918$, $p = 0.0800$). Pulse rate was not affected by adult age ($r^2 = 0.004$, $F = 0.2151$, $p = 0.6447$, $DF = 1$, $n = 55$), and was, therefore, unadjusted (and further suggests that it is a signal of past condition).

Interchirp duration was the only calling parameter affected by adult diet alone (Tables 1.5 & 1.7, Figure 1.10; adult diet: $F = 4.4577$, $p = 0.0397$). This caused a corresponding non-significant trend for males on the low adult diet to call at higher chirp rates than males on the high adult diet (Table 1.5; adult diet: 2.0649 , $p = 0.1568$). No calling parameters were correlated with body condition (either at the time of recording, at four weeks of adult age or as measured by dry mass following euthanasia).

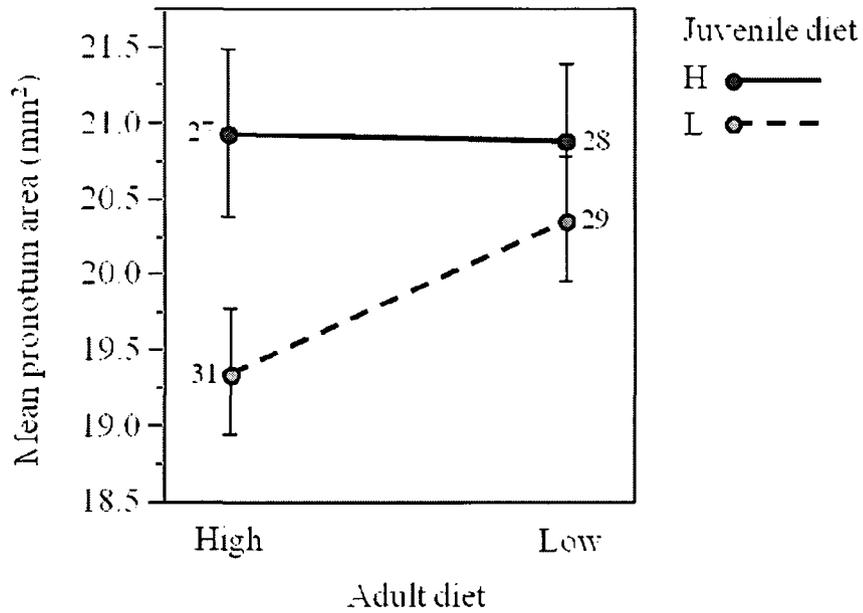


Figure 1.7 *Effect of diet on pronotum area of G. assimilis males from the Summer 2008 replicate (juvenile diet: $F = 4.8036$, $p = 0.0305$, $DF = 1$). Numbers within figure are sample sizes. Bars indicate standard error.*

Table 1.5 *Summary of the effects of juvenile and adult diet on pronotum area, body condition (at time of recording), body condition (as calculated from dry mass), and all of the male call parameters for G. assimilis males from the Summer 2008 replicate.*

Body/call parameter	Diet	F	p	Least Square Mean		n
				High	Low	
Pronotum area (mm ²)	Juvenile	4.8036	0.0305*	20.90	19.86	115
	Adult	0.9779	0.3249	20.15	20.62	
	Juvenile*Adult	1.2834	0.2597			
Condition at recording (mg)	Juvenile	1.6688	0.2027	-8.94	8.38	51
	Adult	0.2357	0.6295	-3.53	-2.98	
	Juvenile*Adult	5.5810	0.0223*			
Condition (by dry mass) (mg)	Juvenile	0.1441	0.7050	1.03	-1.15	113
	Adult	1.6953	0.1957	3.66	-3.78	
	Juvenile*Adult	1.6893	0.1964			
Pulse duration (ms)	Juvenile	3.0165	0.0885	7.75	8.46	55
	Adult	2.3874	0.1285	8.43	7.79	
	Juvenile*Adult	3.9648	0.0518			
Interpulse duration (ms)	Juvenile	0.2564	0.6148	6.70	6.45	55
	Adult	2.5061	0.1196	6.18	6.98	
	Juvenile*Adult	4.5028	0.0387*			
Dominant frequency (Hz)	Juvenile	1.6150	0.2096	3682.30	3631.40	55
	Adult	0.0269	0.8705	3653.60	3660.10	
	Juvenile*Adult	0.0024	0.9608			
Chirp duration (ms)	Juvenile	0.2877	0.5940	122.64	124.55	55
	Adult	1.2053	0.2774	125.55	121.64	
	Juvenile*Adult	1.2332	0.2720			
Interchirp duration (ms)	Juvenile	0.0882	0.7676	1137.72	1108.25	55
	Adult	4.4577	0.0397*	1227.74	1018.23	
	Juvenile*Adult	1.0541	0.3094			
Pulses per chirp (PPC)	Juvenile	0.5942	0.4443	8.97	8.79	55
	Adult	1.9300	0.1708	9.04	8.72	
	Juvenile*Adult	2.4240	0.1257			
Age-adjusted PPC	Juvenile	0.3487	0.3487	8.98	8.78	55
	Adult	1.7972	0.1860	9.02	8.73	
	Juvenile*Adult	0.4175	0.5211			
Pulse rate (min ⁻¹)	Juvenile	4.4282	0.0403*	4393.50	4250.44	55
	Adult	0.0213	0.8846	4326.90	4317.00	
	Juvenile*Adult	0.2350	0.6299			
Chirp rate (min ⁻¹)	Juvenile	0.5556	0.4595	51.34	56.11	55
	Adult	2.0649	0.1568	49.12	58.33	
	Juvenile*Adult	0.7234	0.3990			

(*) with value emboldened denotes $p < 0.05$.

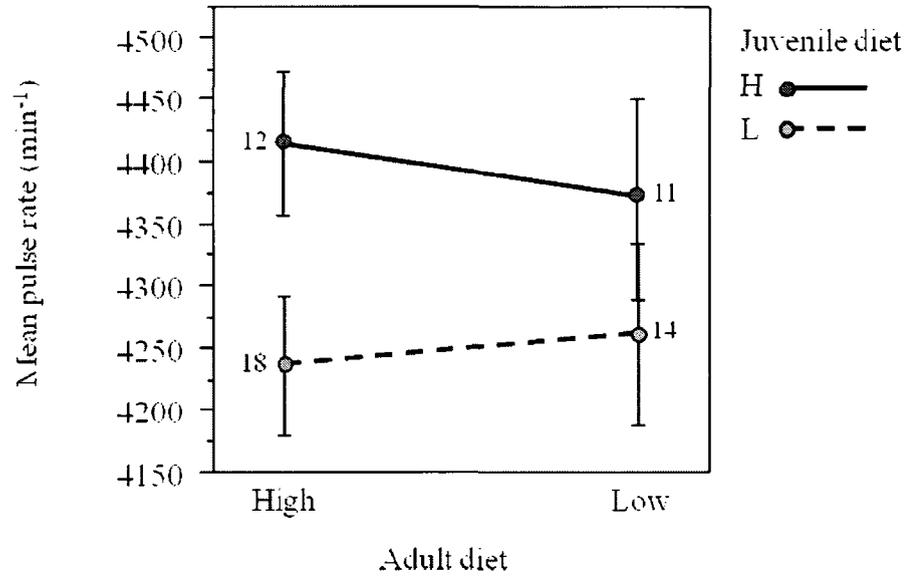


Figure 1.8 *Effect of diet on the pulse rate of G. assimilis male calls from the Summer 2008 replicate (juvenile diet: $F = 4.4282$, $p = 0.0403$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

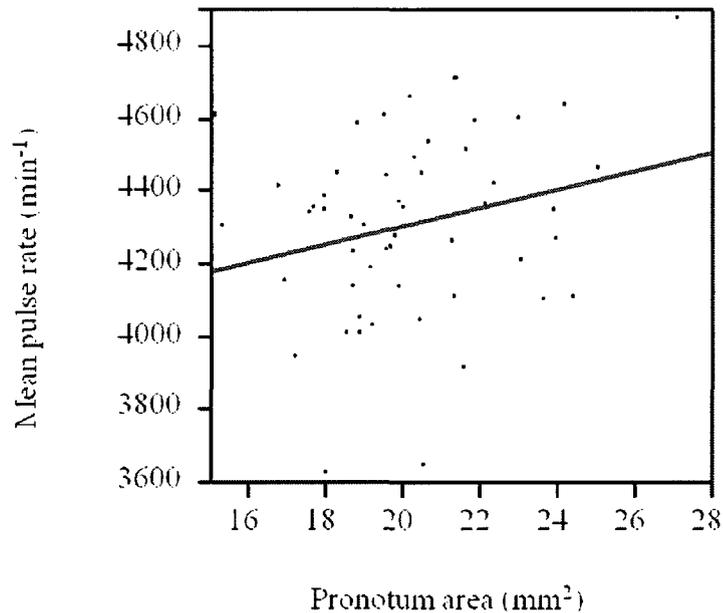


Figure 1.9 *Effect of pronotum area on the mean pulse rate of G. assimilis male calls from the Summer 2008 replicate ($r^2 = 0.059$, $F = 3.1918$, $p = 0.0800$, $DF = 1$, $n = 53$).*

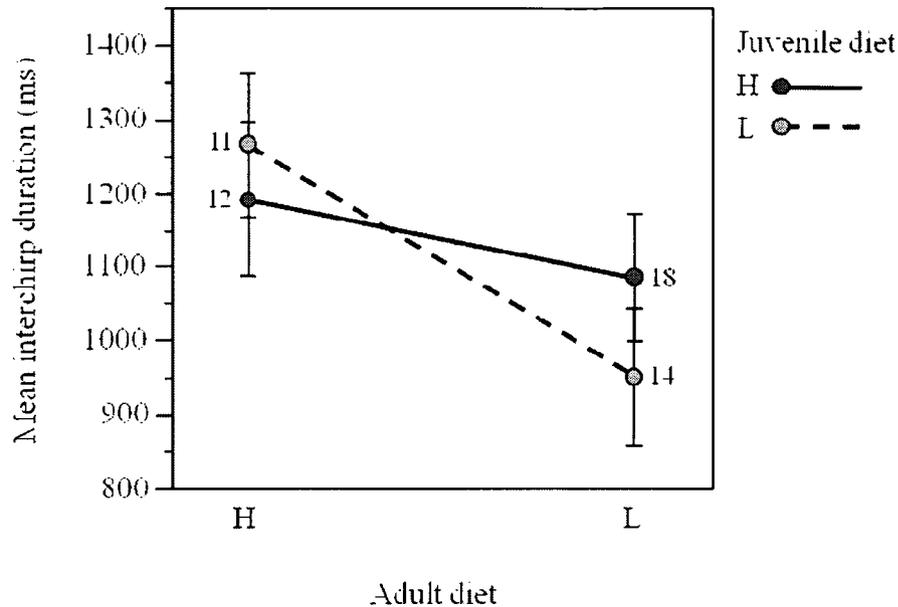


Figure 1.10 *Effect of diet on the mean interchirp duration of G. assimilis male calls from the Summer 2008 replicate (adult diet: $F = 4.4577$, $p = 0.0397$, $DF = 1$). Numbers within figure are sample sizes. Bars indicate standard error.*

Winter 2009. In the Winter 2009 replicate (fed high: ad lib, or low: 2 days on-off, as juveniles and adults; recorded 24 h per day in NEARs) pronotum area was again affected by juvenile diet alone, though the relationship was only near significant this time (Tables 1.6 & 1.7, Figure 1.11; juvenile diet: $F = 3.0006$, $p = 0.0869$). Condition (as calculated from dry mass) was affected by adult diet alone; males on the high adult diet were in significantly better condition than males on the low adult diet (Tables 1.6 & 1.7, Figure 1.12; adult diet: $F = 17.2963$, $p < 0.0001$).

In agreement with the first replicate, pulse rate was again affected by juvenile diet; males that had been on the high diet as juveniles called at significantly higher pulse rates than males that had been on the low juvenile diet (Tables 1.6 & 1.7, Figure 1.13; juvenile diet: $F = 4.9092$, $p = 0.0282$). The mean number of pulses per chirp was affected

by adult diet; males on the high adult diet exhibited near significantly more pulses per chirp than males on the low adult diet (Tables 1.6 & 1.7, Figure 1.14; adult diet: $F = 1.89$, $p = 0.0606$). Taken together, these two effects likely explain why chirp duration was significantly affected by both juvenile (Tables 1.6 & 1.7; juvenile diet: $F = 5.1529$, $p = 0.0246$) and adult diet (Tables 1.6 & 1.7: adult diet: $F = 4.1408$, $p = 0.0436$). All other things being equal, the higher pulse rates of males that had been on the high juvenile diet likely contributed to shorter chirp durations in the same group, while more pulses per chirp for males on the high adult diet likely contributed to longer chirp durations in that group.

Dominant frequency was significantly affected by both juvenile (Tables 1.6 & 1.7; juvenile diet: $F = 3.9393$, $p = 0.049$) and adult diet (Tables 1.6 & 1.7; adult diet: $F = 5.2539$, $p = 0.0233$). There was also a non-significant effect of an interaction between juvenile diet and adult diet (Tables 1.6 & 1.7; juvenile diet * adult diet: $F = 3.5449$, $p = 0.061$).

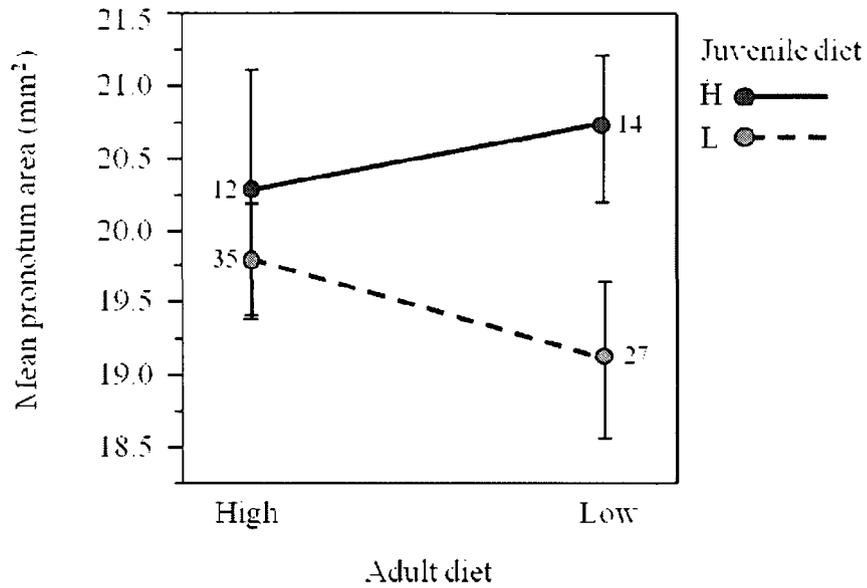


Figure 1.11 *Effect of diet on the mean pronotum area of G. assimilis males from the Winter 2009 replicate (juvenile diet: $F = 3.0006$, $p = 0.0869$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

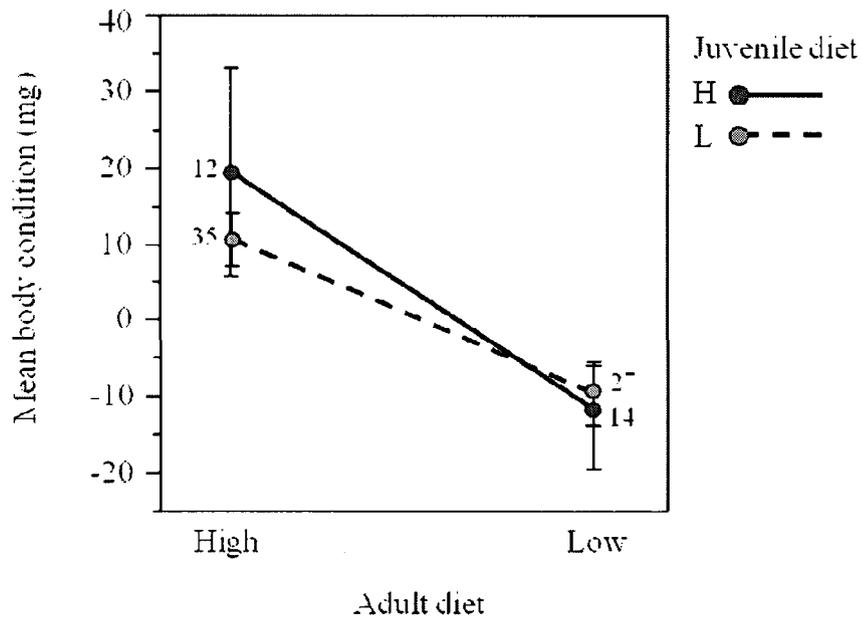


Figure 1.12 *Effect of diet on body condition (as calculated from dry mass) of G. assimilis males from the Winter 2009 replicate (adult diet: $F = 17.2963$, $p < 0.0001$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

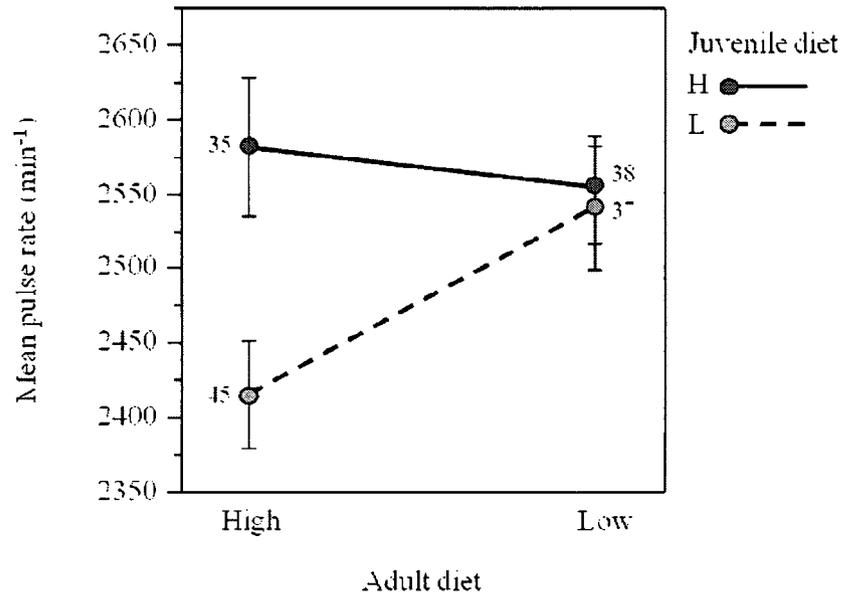


Figure 1.13 *Effect of diet on the mean pulse rate of G. assimilis male calls from the Winter 2009 replicate (juvenile diet: $F = 4.9092$, $p = 0.0282$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

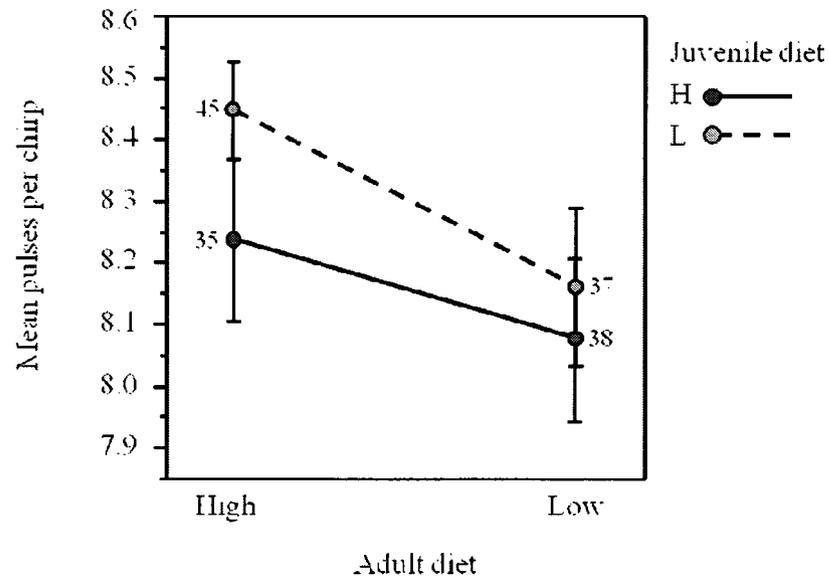


Figure 1.14 *Effect of diet on the mean pulses per chirp of G. assimilis male calls from the Winter 2009 replicate (adult diet: $F = 1.89$, $p = 0.0606$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

Table 1.6 *Summary of the effects of juvenile and adult diet on pronotum area, body condition (as calculated from dry mass), and all of the male call parameters for G. assimilis males from the Winter 2009 replicate.*

Body/call parameter	Diet effect	F	p	Least Squares Mean		N
				High	Low	
Pronotum area (mm ²)	Juvenile	3.0006	0.0869	20.49	19.46	88
	Adult	0.0456	0.8315	20.04	19.91	
	Juvenile*Adult	0.9397	0.3351			
Condition (by dry mass) (mg)	Juvenile	0.2325	0.6309	3.52	15.04	88
	Adult	17.296	< 0.0001**	0.5	-11.02	
	Juvenile*Adult	0.8188	0.3681			
Pulse duration (ms)	Juvenile	2.4981	0.1161	10.1	10.43	155
	Adult	2.6163	0.1079	10.44	10.1	
	Juvenile*Adult	2.0008	0.1593			
Interpulse duration (ms)	Juvenile	2.7000	0.1024	16	16.55	155
	Adult	0.0004	0.9835	16.28	16.27	
	Juvenile*Adult	0.8248	0.3652			
Pulses per chirp	Juvenile	1.5656	0.2128	8.16	8.3	155
	Adult	3.5750	0.0606	8.34	8.12	
	Juvenile*Adult	0.3121	0.5772			
Chirp duration (ms)	Juvenile	5.1529	0.0246*	192.9	203.6	155
	Adult	4.1408	0.0436*	203	193.4	
	Juvenile*Adult	2.4759	0.1177			
Interchirp duration (ms)	Juvenile	0.2715	0.6031	1048.2	1087.8	155
	Adult	1.0933	0.2974	1107.7	1028.3	
	Juvenile*Adult	0.0098	0.9213			
Dominant frequency (Hz)	Juvenile	3.9393	0.0490*	4227.1	4065.5	155
	Adult	5.2539	0.0233*	4052.9	4239.6	
	Juvenile*Adult	3.5449	0.0617			
Pulse rate (min ⁻¹)	Juvenile	4.9092	0.0282*	2566.9	2478.3	155
	Adult	1.4341	0.2330	2498.6	2546.5	
	Juvenile*Adult	3.7273	0.0554			
Chirp rate (min ⁻¹)	Juvenile	0.6407	0.4247	52.22	49.89	155
	Adult	2.1008	0.1493	48.94	53.17	
	Juvenile*Adult	0.2298	0.6324			

Emboldened values denote $p < 0.10$.

(*) denotes $p < 0.05$.

(**) denotes $p < 0.01$

Table 1.7 *Summary of the effects of the low diet (ie. a decrease in condition) at the juvenile and/or adult life stages of G. assimilis males.*

Replicate	Call parameter	Life stage		
		Juvenile	Adult	Juv*Adult
Summer 2008	Pulse duration	-	-	-
	Interpulse duration	-	-	Depends*
	Dominant frequency	-	-	-
	Chirp duration	-	-	-
	Interchirp duration	-	Decrease*	-
	Pulses per chirp (PPC)	-	-	-
	Age-adjusted PPC	-	-	-
	Pulse rate	Decrease*	-	-
	Chirp rate	-	-	-
Winter 2009	Pulse duration	-	-	-
	Interpulse duration	-	-	-
	Dominant frequency	Decrease*	Increase*	Depends
	Chirp duration	Increase*	Decrease*	-
	Interchirp duration	-	-	-
	Pulses per chirp	-	Decrease	-
	Pulse rate	Decrease*	-	Depends
	Chirp rate	-	-	-

All correlations noted are $p < 0.10$

(*) denotes $p < 0.05$

Conclusions

G. texensis

The long-distance call parameters of male *G. texensis* appear to be acting as multiple messages. If the call parameters were acting as redundant signals, one would expect several call parameters to be associated with both life stages (juvenile and adult). Instead, I found that each life stage was associated with a different independent call parameter; the dominant frequency (and corresponding pulse duration) of the male's call was the calling parameter most correlated with body size (and thus juvenile condition) in the field, and the number of pulses per trill was the call parameter most affected when adult condition was manipulated in the lab. No other call parameters were strongly associated with either life stage.

Since cricket pronotum area is known to be influenced by past condition, one would expect that any call parameters which are correlated with pronotum area to also be influenced, and thus be indicative of past condition. During my observations of natural variation in the field, I found both dominant frequency and pulse duration to be significantly correlated with pronotum area and thus potential indicators of past condition. Dominant frequency was found to decrease with increasing pronotum size. This finding agrees with past studies of other cricket species; larger males produce calls at lower dominant frequencies than smaller males due to their correspondingly larger harps (resonant structures located on the forewings) (Bennet-Clark, 1999; Martin et al., 2000; Bailey et al., 2001). Females also tend to prefer larger males, as well as calls that occur at lower dominant frequencies (Scheuber et al., 2004), which is consistent with my

finding that dominant frequency decreases with increasing body size. I also found a significant negative correlation between dominant frequency and pulse duration; males that called a lower frequencies exhibited longer pulse durations. Since these two call parameters were not independent of one another, I concluded that either was a suitable candidate for an indicator of past (juvenile) condition. I chose dominant frequency, as it agrees with the current literature. However, it should be noted that whenever dominant frequency is altered, pulse duration almost always is as well.

In the same group of individuals, I also found a non-significant positive correlation between body condition and trill duration. Female preference for various call parameters, and calling effort in general, tends to increase with increasing energy expenditure (Wagner & Hoback, 1999) (a characteristic typical of an honest signal; Gerhardt, 1991). Thus, males in good current condition may invest energy into increasing the duration of their trills. Trill duration can be affected by changes in pulse duration, interpulse duration, pulse rate and the number of pulses per trill. Thus, while not entirely conclusive, there were indications one or more of these parameters was indicative of adult condition.

When I manipulated current male condition in the lab, I found that males that were fed the unlimited “high” diet produced significantly more pulses per trill and, as a result, significantly longer trill durations, than males fed the limited “low” diet. Thus, either of these two parameters could be indicative of current male condition. However, since neither pulse duration, interpulse duration nor pulse rate (the other three factors contributing to trill duration) were affected by adult diet, I concluded that the number of

pulses per trill was the call parameter signaling current (adult) male condition in *G. texensis*.

G. assimilis

The parameters of the call of *G. assimilis* also appeared to be acting as multiple messages. If they were acting as redundant signals, one would expect to see them affected in the same direction by a decrease in either juvenile or adult condition. However, when juvenile and adult male *G. assimilis* condition were manipulated independently, I found that most of the call parameters were only affected by a decrease in one or the other, but not both. Furthermore, those few that were affected by both were always affected in opposite directions. For example, when juvenile condition was decreased, dominant frequency tended to be lower. However, when adult condition was decreased, dominant frequency tended to be higher. Similarly, when juvenile condition was decreased, chirp duration tended to be longer, but when adult condition was decreased, chirp duration tended to be shorter. Therefore, the parameters of the call of *G. assimilis* appear to be acting as multiple messages; they respond weakly, or not at all, to most changes in condition, but respond strongly to those aspects of condition for which they signal.

Pulse rate was the only calling parameter significantly affected by juvenile diet alone in both replicates. Males that were on the high diet as juveniles produced calls with higher pulse rates than males that were on the low juvenile diet. This finding was consistent with past studies which have found that female crickets prefer calls which are more energetically costly (e.g. Wagner, 1996; Hoback & Wagner, 1997). Pulse rate was

also unaffected by the age at which the male was recorded. This further supports its role as an indicator of past condition, as it does not change throughout the adult's life. It, therefore, appeared that pulse rate was an honest indicator of juvenile condition. This was additionally supported by the near significant positive relationship between pulse rate and pronotum area. In both replicates I found that adult males that had been on the high juvenile diet were larger (as measured by pronotum area) than males that had been on the low juvenile diet (though this relationship was less significant in the second replicate). This confirmed my assumption that male body size is highly influenced by past (ie. juvenile) male condition.

In the first replicate, interchirp duration was the only calling parameter affected by adult diet alone. However, the relationship did not occur in the direction predicted; males on the high adult diet exhibited longer interchirp durations than males on the low adult diet. This suggests that males on the low adult diet were chirping more often, an action which requires increased energy expenditure. This was supported by a non-significant trend for males on the low adult diet to call at higher chirp rates than males on the high adult diet. Since males on the high diet likely expended less energy by calling at longer interchirp durations and, subsequently, lower chirp rates, interchirp duration was not a good candidate for an honest signal of current male condition.

Another candidate emerged in the second replicate. Here the number of pulses per chirp was affected by adult condition alone, though not quite significantly; males on the high adult diet produced more pulses per chirp than males on the low adult diet. This was supported by the fact that chirp duration was also significantly longer for males on the high adult diet. As with *G. texensis*, chirp duration could have been affected by any

or a combination of changes to pulse duration, interpulse duration, pulse rate or the number of pulses per chirp. However, none of the other three parameters were affected by adult diet thus the number of pulses per chirp appears to be an honest signal of current male condition in *G. assimilis*.

Summary

The parameters of the male's long distance call appeared to be acting as multiple messages in both species. In *G. texensis* males, dominant frequency was found to be the calling parameter most indicative of juvenile condition, and the number of pulses per trill was found to be most indicative of adult condition. In *G. assimilis* males, pulse rate was found to be most indicative of juvenile condition, and the number of pulses per chirp was found to be most indicative of adult condition. Together, these results provide strong support for the multiple message hypothesis put forth to explain why multiple sexual signals persist.

Chapter 2 – Female Preference Prioritization

Methods

Cricket colonies were housed in the same manner as described in Chapter 1. Females were removed from the colonies and placed into their own 500 ml plastic containers. Two female preference experiments were carried out using *G. texensis*: GT Experiment (Exp) 1, completed Feb-May 2009 and GT Exp 2, completed May-Jun 2009 (see below for details). Another experiment was carried out using *G. assimilis* and was completed Feb-Jun 2009. GT Exp 1 and *G. assimilis* females were transferred to individual containers at approximately the fourth to sixth instar stages. GT Exp 2 females were transferred upon reaching adulthood. All individuals used were lab-reared 2nd- and 3rd-generation offspring of adult *G. texensis* and *G. assimilis* captured in Austin, TX during Field Season 2 (see Chapter 1).

All females were provided with *ad libitum* water, and a piece of crumpled paper towel for shelter. They were fed powdered rat chow (Harlan Teklad Rodent diet no. 8604) in 1" x 1" plastic weighing dishes. Females that were isolated as juveniles were randomly assigned to one of four dietary treatments (HH, HL, LH, or LL – see Chapter 1). Females that were isolated as adults were randomly assigned to either an adult high or adult low diet; these diets were manipulated in the same way as the high and low portions of the four diets listed above.

Trials

At 14-21 days of adult age, females were run through a two-way choice test (Wagner & Reiser, 2000; Smith & Roberts, 2003). Each female was placed under a small

plastic enclosure in the centre of a 100.3 cm x 51.4 cm x 17.8 cm (L x W x H) rectangular arena (plastic Sterilite “Ultra” Underbed box; Figure 2.1). A speaker was placed in each corner of the arena. The speakers were placed 6 cm from the sides so that the female did not contact a speaker simply as the result of walking along the wall. Two of the speakers, opposite to each other, broadcast two synthesized male calls (see below for details). The speaker location of each call was randomized between these two speakers. The other two speakers broadcast nothing. This was done as an extra control to ensure that females were actually attracted to the synthetic calls, and not just the dark corners of the arena.

The female was allowed to acclimatize in the enclosure for three minutes. The first two minutes passed in silence. During the third minute, the calls were broadcast. After acclimation period, the enclosure was lifted. The female was then allowed to explore the arena for five minutes, during which time the calls continued to be broadcast. All of the trials were recorded using a Sony digital camcorder.

Two synthetic male calls were constructed for each round of female preference tests (Tables 2.1 & 2.2). Each pair of calls varied in the male call parameters identified in Chapter 1: one which was most indicative of past (juvenile) male condition, and one which was most indicative of present (adult) male condition. These two parameters were manipulated simultaneously within each call. The first call was indicative of a male that had been in good past condition but was in poor present condition. This will be referred to as the juvenile call (Juv Call), in reference to the life stage at which the male was in good condition. The other call was indicative of a male that had been poor past condition, but was now in good present condition (Adult Call). All other independent

call parameters were held constant at the population means for that species. The Juv Call and Adult Call parameters were manipulated by taking the population mean and adding or subtracting (based on the effects of juvenile and adult diet observed in Chapter 1) 1 SD accordingly.

The juvenile parameter used for *G. texensis* was dominant frequency and the adult parameter was number of pulses per trill. The *G. assimilis* call parameters indicative of juvenile and adult condition were pulse rate and the number of pulses per chirp, respectively (see Chapter 1). Trait attractiveness was assumed to increase with increasing pulses per trill/chirp (due to increasing energetic demands) (Wagner, 1996; Hoback & Wagner, 1997). Increasing dominant frequency was assumed to decrease in attractiveness, since larger, more attractive males call at a lower dominant frequency (Scheuber et al., 2003a, 2004). Due to very high natural variation, in GT Exp 1 the difference in the number of pulses per trill between the Juv Call and the Adult Call was extremely large (19 and 43 pulses per trill, respectively). This may have accounted for the overall female preference for the Adult Call observed in GT Exp 1 (see below). The same calls were modified to decrease this difference so that the numbers of pulses per trill were 22 and 30 for the Juv Call and Adult call, respectively in GT Exp 2. All other independent call parameters were unchanged; naturally, both chirp and interchirp duration were affected by these changes. Since only adult diet had an effect on female preference in GT Exp 1 (see Results below), only adult diet was manipulated in GT Exp 2.

Statistical Analyses

Videos were viewed using Windows Media Player software and scored for female choice. A piece of acetate was placed on the computer monitor and an identically-sized rounded cone was drawn around each speaker. When any part of the female was viewed to be inside the cone, she was scored as attending that speaker. I recorded: (1) the first speaker touched, (2) the amount of time to reach the first speaker, and (3) the amount of time spent at each speaker.

As such, the “preferred” call was scored in two different ways: (1) the first speaker touched and (2) the speaker at which the female spent the most time. I tested whether these two methods agreed using a two-tailed Fisher’s exact test. All time-spent data were non-normally distributed and were square-root transformed (so as not to exclude the zeros). Where both juvenile and adult diet were manipulated, I performed two-way ANOVAs to assess whether juvenile and adult diet had an effect on body size, condition, which call was chosen (both by time spent and by first speaker chosen), and the amount of time spent at each call. Where only adult diet was manipulated, I performed one-way ANOVAs to test whether diet had an effect on the same variables.

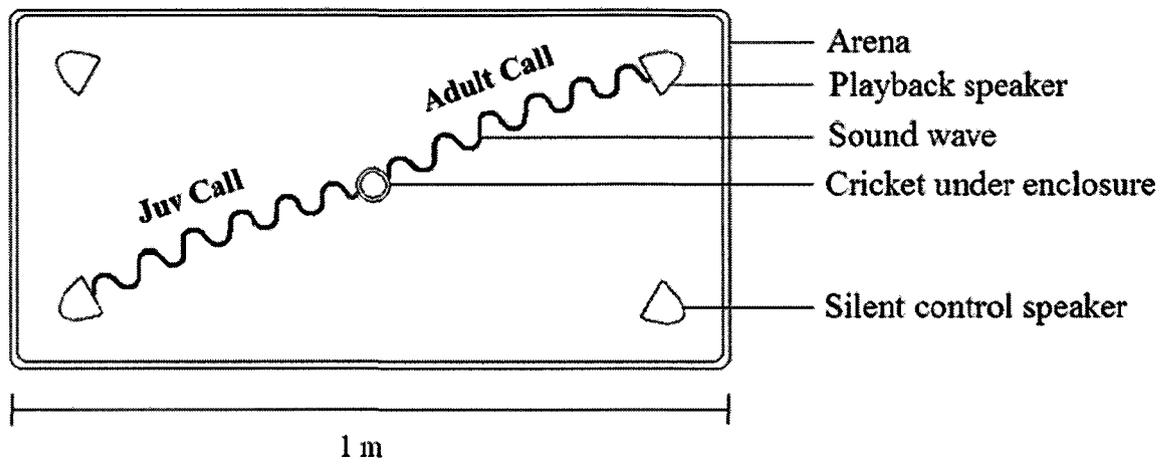


Figure 2.1 *Arena used in female preference trials.*

Table 2.1 Relative values for the male call parameters indicative of juvenile and adult condition in the synthesized test calls used during female preference trials.

Species	Call parameter	Juv Call	Adult Call
<i>G. texensis</i>	Juvenile: Dominant frequency (Hz)	Low	High
	Adult: Pulses per trill	Low	High
<i>G. assimilis</i>	Juvenile: Pulse rate (min^{-1})	High	Low
	Adult: Pulses per chirp	Low	High

Table 2.2 Male call parameters of synthesized calls used during female preference trials. Emboldened values are the juvenile and adult parameters for which female preference was assessed. Note that some other parameters vary between calls due to their dependence on the focal parameters.

Species	Experiment	Call	Pulse duration (ms)	Interpulse duration (ms)	Pulses per trill/chirp	Trill/chirp duration (ms)	Intertrill/interchirp duration (ms)	Dominant frequency (Hz)	Pulse rate (min^{-1})	Trill/chirp rate (min^{-1})
<i>Gryllus texensis</i>	GT Exp 1	Juv	6.17	7.07	19	243.26	428.7	5189.19	4524.58	89.29
		Adult	4.27	8.93	43	559.67	114.04	5853.66	4524.58	89.29
	GT Exp 2	Juv	6.17	7.07	22	283.7	389.02	5189.19	4524.58	89.29
		Adult	4.27	8.93	30	387.03	285.21	5853.66	4524.58	89.29
<i>Gryllus assimilis</i>	-	Juv	8.17	5.02	8	100.5	1019.95	3652.43	4550	53.55
		Adult	8.17	6.62	10	141.28	979.17	3652.43	4058	53.55

Results

G. texensis

GT Exp 1

Female *G. texensis* pronotum area was affected by juvenile diet; surprisingly, there was a near significant trend for females that had been on the high diet as juveniles to have smaller pronotum areas than females that had been on the low juvenile diet (Figure 2.2; juvenile diet: $F = 3.4887$, $p = 0.0674$). Body condition was affected by adult diet; females on the high adult diet were in significantly better condition than females on the low adult diet (Figure 2.3; adult diet: $F = 6.9829$, $p = 0.0108$).

Of the 64 successful female preference trials, five females never approached either of the calls. These females were excluded from all preference analyses as they did not prioritize either call parameter. There was good agreement between the two methods of scoring female choice; the first call approached by a female was a good predictor of which call at which she would spend the most time (Fisher's Exact test, two-tailed: $p = 0.0005$, $DF = 1$, $n = 40$). There was no significant difference in pronotum area or body condition of females that chose the Juv Call versus those that chose the Adult Call (pronotum area: first speaker touched $r^2 = 0.001$, $F = 0.0253$, $p = 0.8745$, $DF = 1$, $n = 36$; time spent $r^2 = 0.007$, $F = 0.3429$, $p = 0.5608$, $DF = 1$, $n = 52$; body condition: first speaker touched $r^2 = 0.033$, $F = 1.1528$, $p = 0.2905$, $DF = 1$, $n = 36$; time spent $r^2 = 0.004$, $F = 0.2185$, $p = 0.6422$, $DF = 1$, $n = 52$).

The amount of time that female *G. texensis* spent at the Juv Call was affected by adult diet; females on the high adult diet spent significantly more time at the Juv Call than females on the low adult diet (Figure 2.4; adult diet: $F = 4.2658$, $p = 0.0436$). There was no effect of diet on the amount of time spent at the Adult Call (ANOVA: $r^2 = 0.018$, $F = 0.3438$, $p = 0.7938$, $DF = 3$, $n = 59$). However, females in general tended to spend more time at the Adult Call than the Juv Call (overall: paired $t = 4.759$, $p < 0.0001$, $n = 59$), and this trend did not differ between diets (though it was not significant for females on the HH diet) (Figure 2.5). Therefore, while it appeared that all females preferred the Adult Call, females on the high adult diet also paid some attention to the Juv Call.

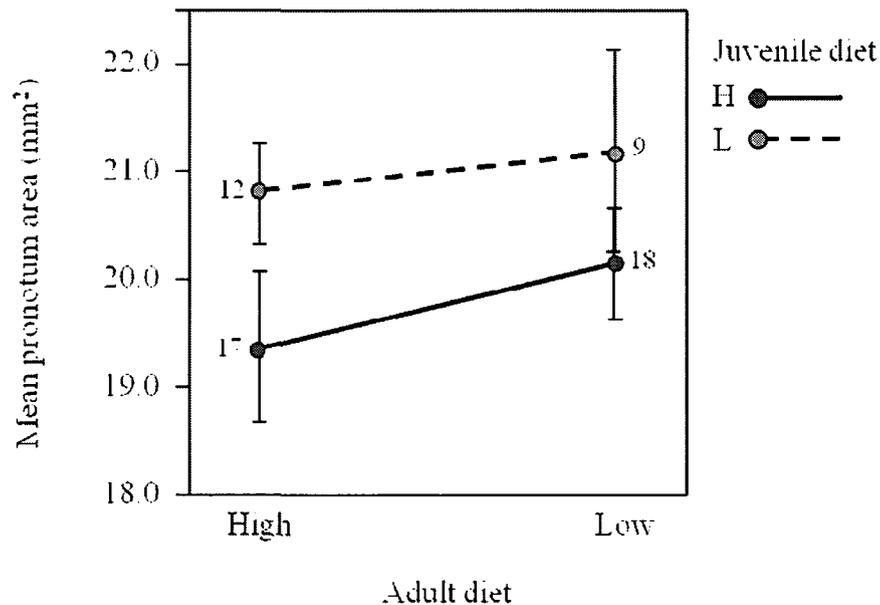


Figure 2.2 *Effect of diet on the pronotum area of *G. texensis* females from GT Exp 1 (juvenile diet: $F = 3.4887$, $p = 0.0674$, $DF = 1$). Numbers within figure are sample sizes. Bars indicate standard error.*

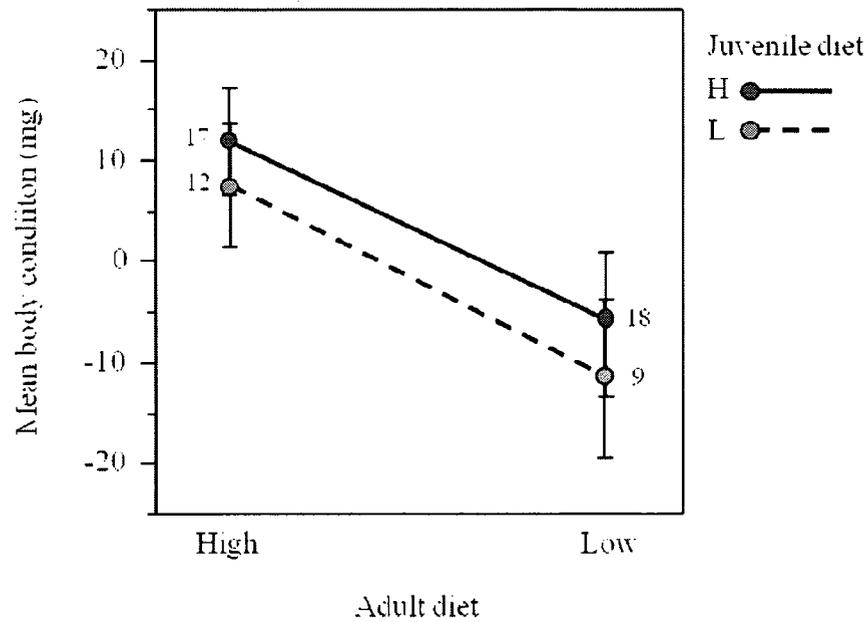


Figure 2.3 *Effect of diet on the body condition of G. texensis females from GT Exp 1 (adult diet: $F = 4.2658$, $p = 0.0436$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

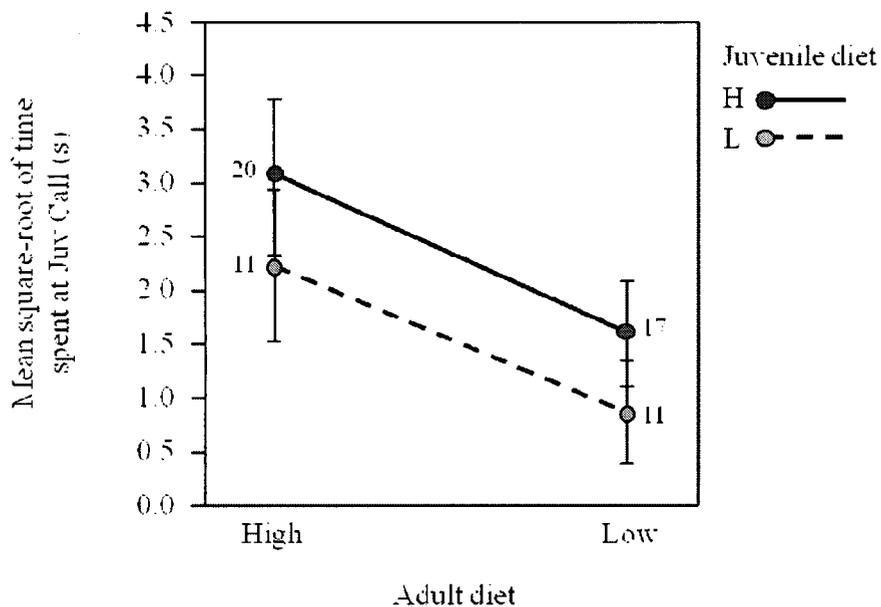


Figure 2.4 *Effect of diet on mean time spent (square-root transformed) at the Juv Call by G. texensis females from GT Exp 1 (adult diet: $F = 6.9829$, $p = 0.0108$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

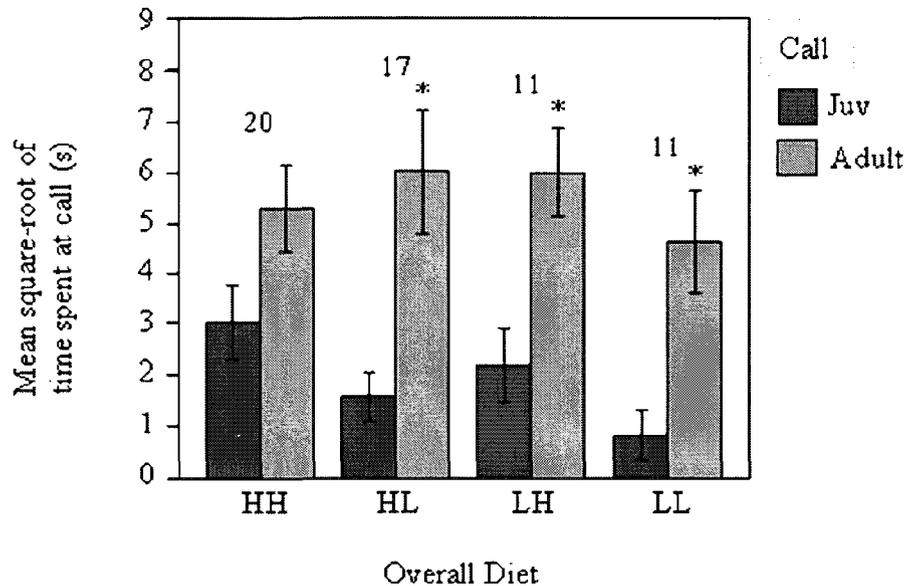


Figure 2.5 Time spent (square-root transformed) at the Juv Call and Adult Call by overall diet for *G. texensis* females from GT Exp 1 (overall: paired $t = 4.759$, $p < 0.0001$, $n = 59$). Numbers above bar represent sample sizes for that diet. Stars indicate significant within-diet differences ($\alpha = 0.05$).

GT Exp 2

There was no significant difference in pronotum area between diets (ANOVA: $r^2 = 0.077$, $F = 1.9173$, $p = 0.1794$, $DF = 1$, $n = 25$). This time, there was no difference in the amount of time, overall, that females spent at the Juv Call and Adult Call (overall: paired $t = 0.976$, $p = 0.3396$, $n = 23$). There was a weak trend for females on the high diet to spend more time at the Juv Call than females on the low diet (Figure 2.6; ANOVA: $r^2 = 0.077$, $F = 1.7429$, $p = 0.2010$), and a weak trend for females on the low diet to spend more time at the Adult Call than females on the high diet (Figure 2.7; ANOVA: $r^2 = 0.079$, $F = 1.8105$, $p = 0.1928$), but these trends were not significant. Power analyses revealed that each test had relatively low power and that more than twice

the sample size ($n = 53.2$ and $n = 51.3$ for time spent at Juv Call and Adult Call, respectively) would be required to see an effect of diet.

Females on the high diet did not spend significantly more time at either call (paired $t = -0.548$, $p = 0.5947$, $n = 12$), but females on the low diet spent significantly more time at the Adult Call than the Juv Call (Figure 2.8; paired $t = 2.707$, $p = 0.0220$).

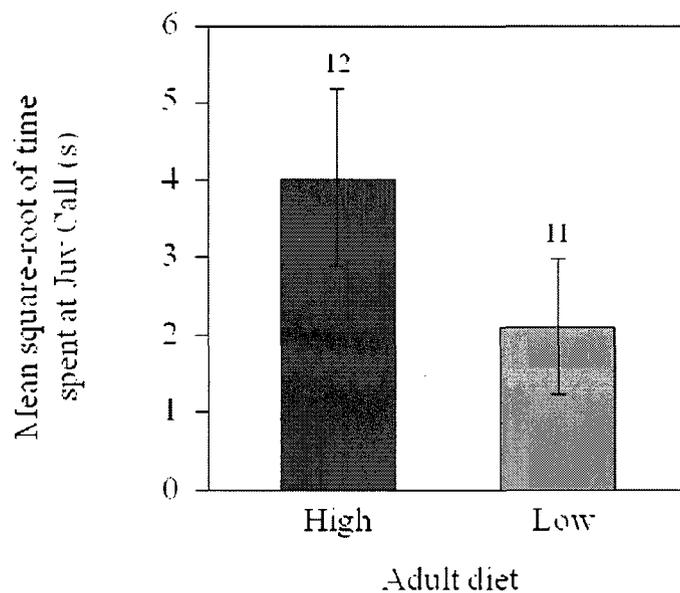


Figure 2.6 *Effect of adult diet on time spent (square-root transformed) at the Juv Call by G. texensis females from GT Exp 2 (ANOVA: $r^2 = 0.077$, $F = 1.7429$, $p = 0.2010$, $DF = 1$). Numbers above bar represent sample sizes.*

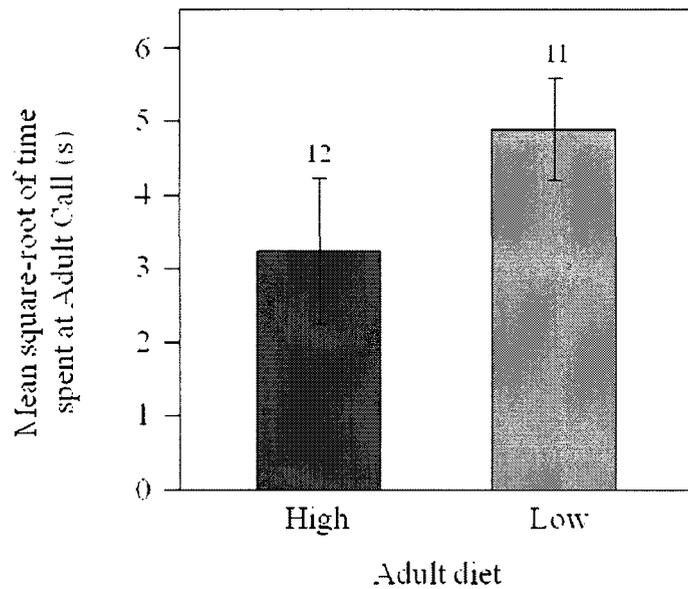


Figure 2.7 *Effect of adult diet on time spent (square-root transformed) at the Adult Call by G. texensis females from GT Exp 2 (ANOVA: $r^2 = 0.079$, $F = 1.8105$, $p = 0.1928$, $DF = 1$). Numbers above bar represent sample sizes.*

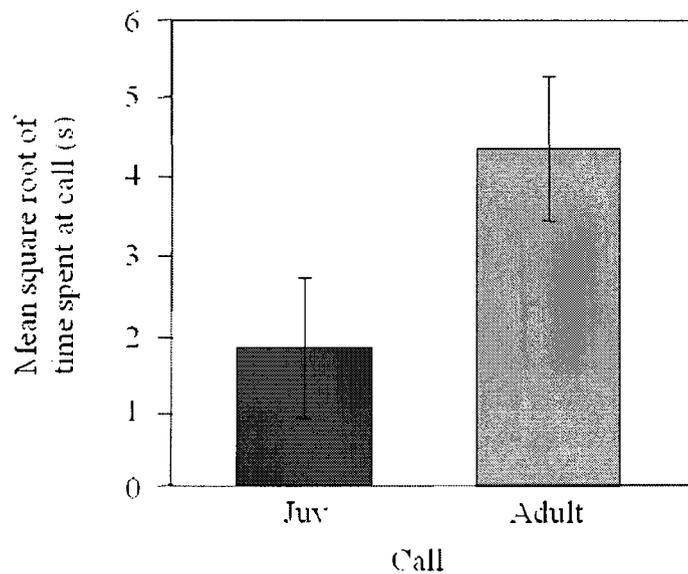


Figure 2.8 *Mean time spent (square-root transformed) at the Juv Call and Adult Call for G. texensis females on the low adult diet from GT Exp 2 (paired $t = 2.707$, $p = 0.0220$, $n = 11$).*

G. assimilis

Female *G. assimilis* pronotum area was affected by juvenile diet; females that had been on the high diet as juveniles had almost significantly larger pronotum areas than females that had been on the low juvenile diet (Figure 2.9; juvenile diet: $F = 2.7527$, $p = 0.0987$). Body condition was affected by adult diet; females on the high adult diet were in significantly better condition than females on the low adult diet (Figure 2.10; adult diet: $F = 5.41$, $p < 0.0001$).

Of the 190 females run successfully through female preference trials, 18 video files were corrupted and thus never analyzed. Of the remaining females, 52 never approached either of the calls during the five-minute trial. These females were excluded from all analyses involving the amount of time spent at a call, as they did not prioritize either call parameter. As with *G. texensis*, there was good agreement between the two methods of scoring female choice; the first call approached by a female was a good predictor of the call at which she would spend the most time (Fisher's Exact test, two-tailed: $p < 0.0001$). There was no significant difference in pronotum area or body condition of females that chose the Juv Call versus those that chose the Adult Call (pronotum area: first speaker touched $r^2 = 0.014$, $F = 1.1796$, $p = 0.2807$, $DF = 1$, $n = 83$; time spent $r^2 < 0.0001$, $F = 0.0022$, $p = 0.9630$, $DF = 1$, $n = 111$; body condition: first speaker touched $r^2 = 0.001$, $F = 0.0433$, $p = 0.8359$, $DF = 1$, $n = 58$; time spent $r^2 = 0.0001$, $F = 0.0078$, $p = 0.9298$, $DF = 1$, $n = 72$).

The amount of time that female *G. assimilis* spent at the Juv Call was affected by juvenile diet; females that had been on the high diet as juveniles spent near significantly

more time at the Juv Call than females that had been on the low juvenile diet (Figure 2.11; juvenile diet: $F = 3.0915$, $p = 0.0815$). There was no effect of diet on the amount of time spent at the Adult Call (ANOVA: $r^2 = 0.005$, $F = 0.1651$, $p = 0.9197$). However, there was a non-significant trend for females in general to spend more time at the Juv Call than the Adult Call (overall: paired $t = -1.676$, $p = 0.0965$, $n = 112$).

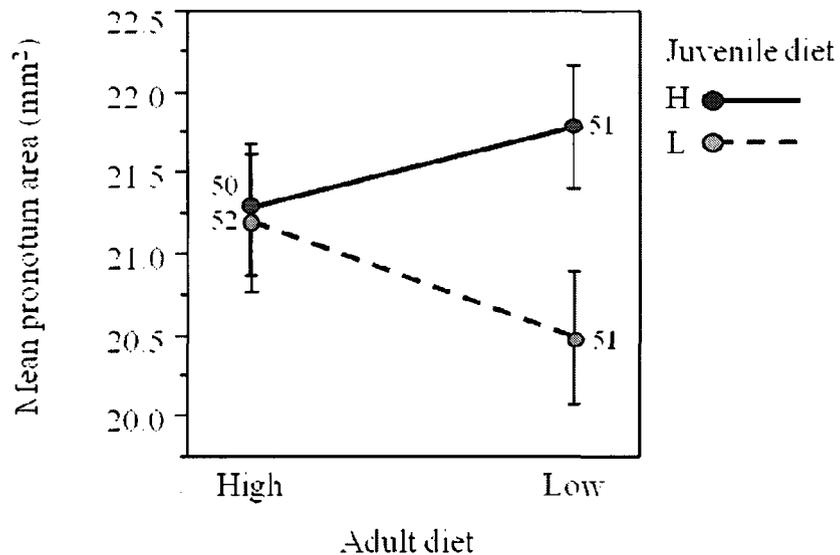


Figure 2.9 *Effect of diet on pronotum area of G. assimilis females (juvenile diet: $F = 2.7527$, $p = 0.0987$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

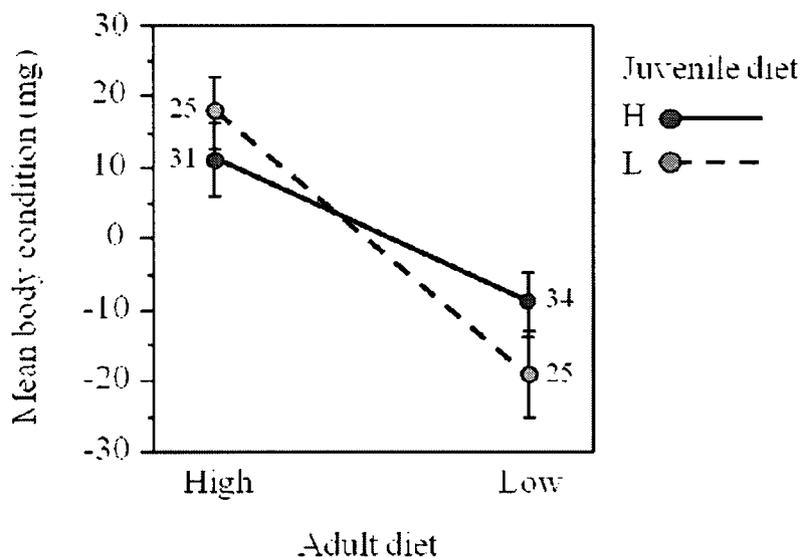


Figure 2.10 *Effect of diet on body condition of G. assimilis females (adult diet: $F = 5.41$, $p < 0.0001$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

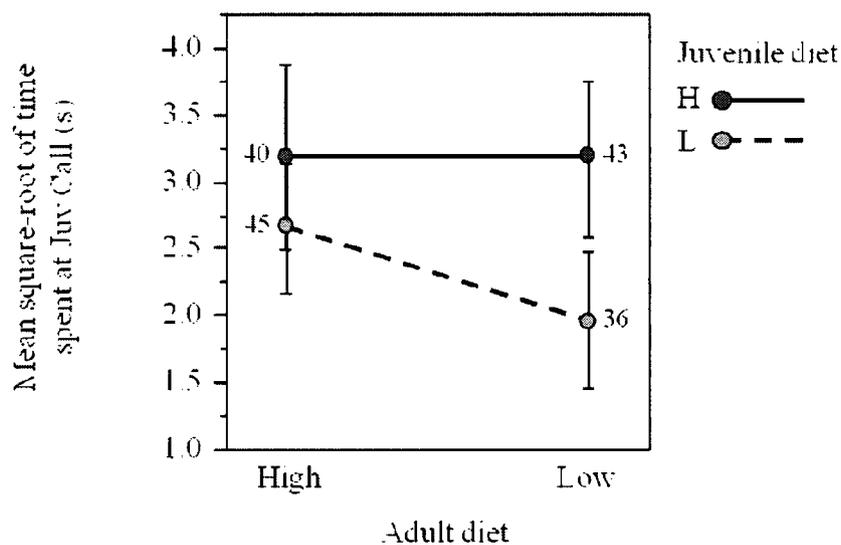


Figure 2.11 *Effect of diet on time spent (square-root transformed) at Juv Call by G. assimilis females (adult diet: $F = 5.41$, $p < 0.0001$, $DF = 1$). Numbers within figure are samples sizes. Bars indicate standard error.*

Conclusions

G. texensis

There were some indications that *G. texensis* females that differed in body condition also differed in which male signal they prioritized. In the first experiment, while all females in general spent more time at the adult call than the juvenile call, females on the high adult diet also spent significantly more time at the juvenile call than females on the low adult diet. Females on the high adult diet were also in better condition than females on the low adult diet. Therefore, it appeared that, while all females prioritized the adult signal, females in good condition additionally selected based on the juvenile signal.

It is likely that the reason that females in general preferred the adult call over the juvenile call was due to the values of the adult signal (pulses per trill) used in the test calls. Due to the high natural variation for that signal, the values used were 19 and 43 pulses per trill for the juvenile and adult calls, respectively. It appears that 43 pulses per trill was so much more attractive than 19 that, no matter how attractive the juvenile signal (dominant frequency) was, females almost always prioritized the adult signal (pulses per trill) over the juvenile signal (dominant frequency).

In the second experiment the distance between the two test values of pulses per trill was decreased. As expected, the trend for females in general to prioritize the adult parameter (pulses per trill) was no longer observed. Instead, there was a non-significant trend for females on the high adult diet to spend more time at the juvenile call than females on the low adult diet. There was also a non-significant trend for females on the

low adult diet to spend more time at the adult call than females on the high adult diet. While these trends were not significant, it is notable that decreasing the distance between the two adult signal values made the adult call less attractive overall. This time, only females on the low diet spent significantly more time at the adult call than the juvenile call. Females on the high adult diet were, once again, in significantly better condition than females on the low adult diet. Therefore, there were some indications that females in good condition prioritized the juvenile male signal, while females in poor condition prioritized the adult signal. However, larger sample sizes are needed to demonstrate this definitively.

G. assimilis

Female condition did not appear to influence which male signal a female will prioritize in *G. assimilis*. There was a non-significant trend for females in general to spend more time at the juvenile call than the adult call. Females that had been on the high diet as juveniles also spent more time at the juvenile call than females that had been on the high juvenile diet, but there were no other effects of diet on the amount of time spent at either call. Females which had been on the high diet as juveniles were also larger than females which had been on the low juvenile diet. It therefore appeared that, while females in general prioritized the juvenile signal (pulse rate) over the adult signal (pulses per chirp), this trend was strongest for large females.

Summary

Female condition in *G. texensis* appeared to affect which male signal, and thus which aspect of condition, females prioritized. There were weak tendencies for females in good condition to prioritize juvenile male condition, and its associated signal, and for females in poor condition to prioritize adult male condition.

Female condition in *G. assimilis* did not appear to have an effect on whether juvenile or adult male condition was more important during mate choice. All females appeared to prioritize past condition, particularly the largest females.

If multiple male signals do act as multiple messages, then each signal should be indicative of a different aspect of male condition, or of male condition at a different life stage (e.g. juvenile vs. adult). As such, not all females will prioritize the same aspect of male condition and thus the same male signal. However, if multiple male signals act as redundant signals, no one signal should be more detectable, honest, or reliable than the rest. Thus, females should never prioritize one signal over any of the others.

Females of both species exhibited priorities for one signal over the other, supporting the multiple messages hypothesis. Furthermore, *G. texensis* females prioritized different male signals depending on their own body conditions, providing additional support for this hypothesis.

General Discussion

Male Calling Behaviour

The long-distance call parameters of both *G. texensis* and *G. assimilis* appeared to be acting as multiple messages. Those parameters which were affected by a decrease in juvenile condition were not affected in the same way by a decrease in adult condition, as would be predicted for redundant signals (Møller & Pomiankowski, 1993). Rather, most responded to either a decrease in one aspect of condition or the other, but not both. Many of the parameters did not respond at all. Furthermore, the few that did respond to decreases in both juvenile and adult condition were always affected in opposite ways by the two life stages.

It is noteworthy that adult condition was signaled by the number of pulses per trill in *G. texensis*, and by its analogue, the number of pulses per chirp, in *G. assimilis*. The dynamic nature of pulse number (ie. the fact that it is relatively simple for individuals to increase or decrease the number of pulses per trill/chirp) makes it an ideal honest indicator of current condition (Gerhardt, 1991), which can be just as highly temporally variable. Similarly, the indicators of past condition in *G. texensis* and *G. assimilis*, dominant frequency and pulse rate respectively, are less dynamic since they are more physiologically-limited. This makes them ideal indicators of past condition (Gerhardt, 1991), which is similarly fixed and static.

Dominant frequency is determined mainly by body size, and is due both to the size of a male's harp (the resonant structure found on the male forewings) (Bennet-Clark, 1999; Martin et al., 2000; Bailey et al., 2001) and the speed of wing closure (Martin et al., 2000). Adult structural body size in crickets, as well as other insects, is influenced by past condition and does not change significantly after the insect's final moult. Dominant

frequency and pulse duration were negatively correlated in both species. This is partly due to forewing size (Bennet-Clark, 1999; Martin et al., 2000; Bailey et al., 2001). Smaller forewings have proportionally smaller harps which produce higher dominant frequencies; they also have proportionally smaller files which take less time to scrape, resulting in short pulse durations. The speed of wing closure, the part of the wing-beat during which the scraper hits the file, producing a single pulse of sound, also contributes to the negative correlation between dominant frequency and pulse duration (Martin et al., 2000). When a male closes his wings faster, the teeth of the file are hit at a faster rate, resulting in a higher frequency. Faster wing closure also produces a shorter pulse, since the entire file is scraped in less time. Thus, both forewing size and the speed of wing closure influence dominant frequency and pulse duration. Juvenile condition may limit forewing development, as well as the development or function of the neurological pathways, or body structures associated with wing closure, thereby affecting these call components.

In *G. lineaticeps*, the energetic cost of calling was found to increase with increasing pulse duration (Hoback & Wagner, 1997). This is interesting since I found pulse duration to be negatively correlated with dominant frequency. This implies that lower frequency pulses (ie. those with longer pulse durations) are more expensive to produce. Therefore, it is possible that since larger individuals call at lower dominant frequencies, they are forced to reduce effort in other call parameters due to the increased energy required to produce calls at these frequencies. This implication is intriguing, since larger males also require proportionally more resources than smaller males to remain in good condition. This means that for two males of equal body condition, the

larger of the two will have fewer resources available to invest in signals of current body condition. If this is the case, females should pay attention to both signals and judge them relative to one another, not independently, in order to accurately assess both aspects of male condition. This may provide one explanation for why females pay attention to multiple signals.

Pulse rate was identified as the signal of juvenile male condition in *G. assimilis*. Studies in other cricket species have also found pulse rate to be determined by juvenile environment. *Gryllus rubens* males that were reared at a higher temperature produced calls which had significantly higher pulse rates than those produced by individuals reared at a lower temperature (Walker, 2000). While Walker (2000) did not quantify male body size or condition, it is likely that males that had been reared at a higher temperature were in better condition as juveniles. Development time for crickets, and other insects, decreases with increasing temperature (to a point). It is likely that insects reared at a higher temperature matured faster due to increased juvenile condition of such individuals. However, I did not test this hypothesis.

Additionally, pulse rate has been found to increase with increasing ambient temperature; this is due to increases in the rate at which the cricket either opens or closes its wings, though it appears to be affected mainly by wing opening (Martin et al., 2000). Since the temperature while calling did not differ between individuals (all males were recorded in the same greenhouse bay and all dietary treatments overlapped), temperature does not explain the difference I observed in pulse rate between male *G. assimilis* on the high and low juvenile diets. Instead, males that were on the high juvenile diet may be, physiologically, better suited as adults to call at a higher pulse rate (ie. open or close their

wings at a faster rate). Since insect metabolism increases with increasing ambient temperature, I hypothesize that the increases in pulse rate observed here may be similarly due to increases in either neural or muscular development or function. However, this remains to be tested.

In many cricket species, males also produce a second acoustic signal; once a female has been attracted via the long-distance call and has made physical contact, males switch to a short-range courtship call. This call is structurally quite different from the long-distance call (e.g. Wagner & Reiser, 2000). In one species, it has also been found to be more than twice as energetically expensive as the long-distance call (Hack, 1998). For the sake of simplicity, I did not include courtship calls in my studies (or any other of a number of potential sexual signals, e.g., cuticular hydrocarbons, coloration, fighting ability, etc). Since females exhibit distinct preferences for several aspects of the males' long-distance calls (e.g. Cade & Cade, 1992; Gray & Cade, 1999; Wagner et al., 1995; Wagner & Hoback, 1999), and males which produce the most energetically costly long-distance calls attract more females (Wagner & Hoback, 1999), I was certain that sexual selection was indeed acting upon the long-distance call, making it a suitable subject for my studies.

However, the structural and energetic differences between the two call types suggest that they signal different aspects of male condition. Unlike the long-distance call, parameters of the courtship call have not been found to be affected by male juvenile or adult condition. For example, Gray and Eckhardt (2001) found that in *G. texensis*, neither juvenile nor adult diet had an effect on three adult male courtship call parameters. Similarly, Wagner and Reiser (2000) found that there was no effect of a poor diet (cut by

33% with non-nutritive cellulose) on courtship chirp rate in the variable field cricket, *G. lineaticeps*. This suggests that the courtship call is not indicative of the same aspects of condition as the long-distance call.

In fact, some parameters of the courtship call have been found to vary with other measures of male quality. In general, increased trait expression has been found to be correlated with high immune function, while immune insults have resulted in a decrease in calling effort. For example, Rantala and Kortet (2003) found that in *G. bimaculatus*, high tick rate and long high-frequency tick duration were correlated with a high encapsulation rate (cellular response to a foreign object in the body). Similarly, Tregenza et al. (2006) found that inducing an encapsulation response by implanting a foreign object in the body reduced the propensity of male Australian crickets, *Teleogryllus oceanicus*, to produce a courtship call. It appears that courtship calls may signal overall immune function as well as current immune status. As such, females may be able to gain further information on male condition from the courtship call. Future studies should examine the relationship between the long-distance and courtship calls and which aspects of male condition each signals, as well as the relative importance that females place upon them, particularly in light of female condition.

Female Preference

There were indications that female *G. texensis* condition had an effect on which signals, and thus which aspects of male condition, a female prioritized; females in good condition appeared to prioritize past condition (and its associated signal) over present male condition, while those in poor condition prioritized present over past condition.

Female condition in *G. assimilis* did not affect whether females prioritized either past over present condition, or vice versa; females in general appeared to prioritize past condition.

It is possible that female *G. texensis* on the low adult diet only appeared to prioritize the number of pulses per trill (adult signal) over dominant frequency (juvenile signal). Instead, the food limitation may have caused a disruption in the development or maintenance of their auditory pathways that prevented or restrained females on the low adult diet from hearing the lower dominant frequency of the juvenile call. While this hypothesis remains to be tested, support for it would not necessarily rule out the existence of female prioritization; instead, it may provide a proximate explanation for my observation that females in poor condition prioritized the number of pulses per trill over dominant frequency.

The results of the *G. texensis* experiments agree with those of other cricket species which have found that females in good condition prioritize long-term static signals over short-term dynamic signals. Scheuber et al. (2004) found that female *G. campestris* prioritized dominant frequency, which is indicative of juvenile condition, over chirp rate, which is indicative of adult condition. Some authors have suggested that long-term signals are more likely indicative of genetic suitability to the environment, and thus indirect benefits, while short-term signals are more indicative of the direct benefits a male has to offer (Gerhardt, 1991; Hill et al., 1999; Kokko et al., 1999; Candolin, 2003). Females in good condition may be able to forego the direct benefits offered by males in good current condition, and instead may opt to select a mate possessing higher genetic

benefits. Conversely, a female in poor condition may choose a mate offering higher immediate benefits regardless of his genetic quality.

While condition did not affect female *G. assimilis* preferences for past or present male condition, size did. Females that had been on the high juvenile diet were larger as adults, and spent more time at the juvenile call than females that had been on the low juvenile diet. This appears to be a case of size-assortative mating. Juvenile condition affected body size in both males and females, and is known to do so in insects in general. Thus, large females tended to spend more time than smaller females attending the call that was indicative of a large male. However, there was also an overall tendency for all females to spend more time at the juvenile call (that of the “large” male). This may be because all female *G. assimilis* prioritize past male condition over present male condition. If this is the case, it provides reason to reject the redundant signals hypothesis, which predicts that no signal should be more detectable, reliable or honest than any other (Møller & Pomiankowski, 1993); as such, females should not prioritize any signal over another. However, it is also possible that females were unable to detect a difference between the high and low versions of the adult signal (pulse number), 10 and 8 pulse per chirp, respectively. Had the difference between these two values been greater, it is possible that there would have been stronger female preference exhibited for the adult signal as well. Alternatively, perhaps females do not discriminate between calls based on pulse number, either because they do not select mates based on current male condition, or because they are selecting for current condition based on another signal. Further work should be conducted to determine whether female *G. assimilis* exhibit preferences for pulse number.

I did not explicitly investigate the benefits that females may receive by mating with males in good past versus present condition. Males in good current condition may be able to produce more or better sperm (Wagner & Hoback, 1999). They may also be less likely to be infected with parasites, thus decreasing the female's chances of contracting them during mating (e.g. Borgia & Collis, 1990; Doucet & Montgomerie, 2003). These, and other benefits, may also be heritable, conferring similar advantages to the female's offspring. Alternatively, Hedrick (2005) suggests that females may acoustically locate to males that exhibit attractive condition-dependent signals in order to find locations with favourable environmental conditions, either for their own benefit, or for the benefit of their offspring. Wagner and Harper (2003) found that female *G. lineaticeps* that mated with males that produced calls with preferred chirp rates and durations received fertility, fecundity and life-span benefits as a result. Further study should be conducted to determine which benefits, if any, are received by female *G. texensis* and *G. assimilis* which mate with males producing calls indicative of good juvenile and adult condition.

Body Size and Condition

Adult insect body size is influenced by past condition and is stable following final moult. Therefore, I predicted that body size would be influenced by juvenile diet alone. Overall, body size was affected by juvenile diet alone in both male and females of both species; individuals that had been on the high diet as juveniles were larger, on average, than those that had been on the low juvenile diet, with one exception. In the first *G. texensis* female preference experiment, the opposite was observed; females that had been

on the high diet as juveniles were smaller, on average, than those that had been on the low juvenile diet. Since this one occurrence is an exception to all other observations of body size, I believe it is likely due to stochastic chance. It is possible that females on the low juvenile diet happened to be larger to begin with than females on the high juvenile diet. As such, they may have gained proportionally less weight as juveniles, but still moulted into larger adults, due to their larger initial sizes. However, juvenile initial weight and weight gain were not monitored in this group, so this is merely speculation.

Condition was measured as the residuals of a regression of mass onto pronotum area (Gray & Eckhardt, 2001). As such, body size is effectively eliminated from measures of condition. I therefore predicted that condition would mainly be influenced by adult diet. Overall, condition was affected by adult diet alone in males and females of both species; individuals on the high adult diet were in better condition than those on the low adult diet, again with one exception. In the Summer 2008 replicate, there was no effect of adult diet on body condition (as calculated from dry mass) in male *G. assimilis*. This result is likely due to the fact that these males were euthanized following individual recordings, which occurred over a large variety of ages (10-94 post final moult). The effects of diet on condition may have been confounded by the effects of age. Had condition in this group been measured over a smaller age range, it is likely that it would have conformed to the other results observed.

Conclusions

Overall, the multiple messages hypothesis was confirmed in these two species of crickets. There was strong evidence that male signals acted as multiple messages in both

species. In *G. texensis*, there was some evidence that females that differed in body condition prioritize male signals differently. *Gryllus assimilis* females also exhibited priorities, though these were unaffected by body condition.

Females that exhibit multiple preferences for traits which act as multiple messages can gain reproductive benefits by obtaining more information about a potential mate's condition. They can assess males over a variety of characteristics and choose a mate which is most suitable for their particular needs (ie. one which can provide the benefits they most require). These reproductive benefits can then offset the potential increased cost of exhibiting multiple preferences.

However, I would caution those attempting to distinguish between multiple messages and redundant signals that the two are not as distinct from one another as they have been portrayed. The assumption that since redundant signals are indicative of the same overall condition, they should also be equally honest, detectable and reliable may be unfounded. Even redundant signals must develop along different pathways. For instance, beak length and feather colour are certainly influenced by different resources, even if they are both affected by overall condition. As such, redundant signals will always be most affected by certain changes in condition: those, for which they are most indicative. When defined in this way, the two hypotheses seem to be describing the same phenomenon. The only time that redundant signals will be truly redundant is when they are completely dependent on one-another and the value of one can be calculated from the value of the other. For example, we can always predict the proportion of black hair on a zebra if we know the proportion of white hair. However, to label these two dependent

traits as separate, redundant signals results in a somewhat hyperbolic example of parsing what is more likely a single signal into several.

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