Analysis of male bioacoustic calls and male mating success in the Bunchgrass Grasshopper, *Pseudopomala brachyptera*

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ABSTRACT
Sexual selection and the mate-choice mechanism of the Bunchgrass Grasshopper *Pseudopomala brachyptera* were studied with particular regard to biometric measurements (femur, tegmen, and pronotum length, mass, total length, width, height, and volume) and bioacoustic call parameters (syllable length, number of pulses/syllable, syllable:pause duration ratio, and dominant power frequency). Male and female *P. brachyptera* were collected over a three week period in July 2006 on the National Bison Range in western Montana and were used for bioacoustic call characterization and in male mating success experiments. Four days of experimentation revealed that females select males of greater mass ($P = 0.0052$) but with smaller tegmen ($P = 0.0409$). Examination of masses of mated and unmated males suggests that a fixed threshold mating strategy has been adopted by the females rather than a “best-of-n” strategy. No significant, biologically relevant relationships were found among call parameters and biometrics, suggesting that bioacoustic calls of males function to attract females and convey male species, location, and general health, but are not used directly in mate selection.

Keywords: sexual selection, female mate choice, mating success, acoustic communication, *Pseudopomala brachyptera*, Orthoptera, National Bison Range

INTRODUCTION

Sexual selection is the evolutionary effect of differential reproductive success that “arises from variations among individuals in traits that affect success in competition over mates and fertilization” (Andersson 1994). Both natural selection and sexual selection (as an essential component of natural selection) result in “the evolution of phenotypic properties that tend to increase the reproductive success and gene propagation of individuals” (Searcy &
Andersson 1986). These oft-exaggerated secondary sexual characteristics intuitively appear to be detrimental to survival in terms of increased predation risks and energy expended in their production. However, these same traits seem to promote success in reproductive competition and thus increase the fitness of an individual. A more fit individual is able to pass more genetic material to the next generation, which apparently outweighs any costs to survival these traits might have. Fit males, who are able to successfully reproduce, pass on any genetic benefits to his offspring, such as improved survivorship or attractiveness to potential mates (Thornhill & Alcock 1983). Fit females are better able to select genetically fit mates and pass those good genes to her offspring – and thus increase her own fitness (Thornhill & Alcock 1983).

Intuitively, sexual selection can change gene frequencies in a population and thus can be a significant force in evolution. For example, if a particular displayed characteristic is favored by mate selection, directional selection can occur within a population, effectively shifting frequencies of particular alleles. Simultaneously, distinctive courtship displays help distinguish populations of closely related species. In this way, mating signals allow individuals to correctly identify conspecifics and to select a mate based on preferred signal characteristics, notably demonstrated among orthopterans in American, Australian, and European cricket species by Walker (1957), Hill et al. (1972), and Shuvalov and Popov (1973), respectively. The divergence in mating signals and their receivers provides the basis for premating barriers between populations or subspecies that can eventually result in speciation, as seen in three geographically separated populations of the gomphocerine grasshopper Chorthippus parallelus (Dagley et al. 1994).
Sexual selection operates as both intrasexual competition among males and as intersexual selection through female mate choice. Intrasexual selection occurs as males compete with each other to attract and gain access to females and successfully reproduce. Intersexual selection involves females selecting a mate based on a particular set of displayed characteristics that are presumably indicative of male fitness. Some systems exist in which females are found to reject males of the wrong species or reject males of their own species that are undesirable and to choose a mate based on an attractive courtship behavior, particular morphological characteristics, or some kind of material benefit offered to the female by the male (Thornhill & Alcock 1983). Male attributes that are attractive to females apparently serve as the basis for female choice of mate, and these traits are often correlated with male mating success. This principle of female mate choice relies heavily on the assumption that fitness-related genetic differences among males are detected by females and used to discriminate among potential mates (Thornhill & Alcock 1983).

Many species exhibit various mechanisms of female mate choice, the most prevalent of which are the fixed threshold strategy and the “best-of-n” strategy. Females operating with a fixed-threshold strategy have an internal standard for a particular male characteristic that is functioning as a proxy for fitness. A female compares the fitness of males she encounters to her internal threshold and only selects a mate if he exceeds this particular fitness value (Janetos 1980). According to the “best-of-n” strategy, females do not have absolute standards but rather assess several males relative to each other and select the best one. Janetos found that this mechanism “yields the highest average fitness of males chosen at all n’s examined” and is particularly effective at selecting fit mates when n is greater than five (1980).
Furthermore, some mating systems operate through assortative mating, in which males and females of similar biometric measures (or which are in relatively similar ranking among their gender’s distribution) tend to mate with each other. Some non-calling grasshopper species have been found to use assortative mating based on size, a common occurrence in insects (Thornhill & Alcock 1983, Dagley et al. 1994, Castillo et al. 1999). Positive assortative mating in relation to body size can be explained by mate choice in which large males are more successful in contests and thus gain access to larger females, who produce more eggs (Ridley 1983, Castillo et al. 1999).

Favored displayed characteristics are typically morphological (in the form of plumage, horns, etc.) or behavioral (dances, flight, or calling songs). Bioacoustic calls are a dynamic means by which many species of bird, frog, and insect attract and select mates. Searcy and Andersson present numerous examples demonstrating how song is used by animals as a means of sexual selection with evidence that males and females respond preferentially to songs with particular attributes and that “mating success is related to the same song attributes” (1986). Calls are used to attract mates from a distance while also providing information about the caller. From the songs, receivers can assess the time and energy it would take to locate the caller based on call amplitude, while temporal song characteristics can reveal species. Acoustic calls are thought to be subject to selective forces at work in natural and sexual selection despite the fact that they have no ecological adaptation to promote survival but instead are involved in intraspecific social competition, especially for mates (Darwin 1871, West-Eberhard 1984). Song is common component of the mating systems of many Orthopteran species and, like other characteristics subject to sexual selection, is likely to play a significant role in the initial stages of pre-mating isolation (Ewing 1989).
Calls in many Orthopteran species are thought to be indicative of male size, relative amount of energy expended in calling, and the health of the calling male, particularly his physiological health and the absence of deleterious mutations (Thornhill & Alcock 1983). Male mating calls "designate an individual's mating type and are, in effect, used by females to assess the genotype of potential mates" (Otte 1977). For example, song patterns in grasshoppers and their perception mechanism have been found to be genetically controlled (von Helversen and von Helversen 1975a, b). Subtle differences distinguish individual callers due to variation in calling persistence, rate of song production, call duration, and intensity of calls. Songs differ in many distinct parameters, particularly frequency (kHz), syllable length, number of pulses/syllable, and syllable:pause duration ratio (Figure 1). Females use these bioacoustic distinctions to identify and assess individuals when selecting a mate (Klappert and Reinhold 2003).

Investigations of male call parameters, biometrics, and mating success have not yet been conducted in the Bunchgrass grasshopper, *Pseudopomala brachyptera*. Like many slant-faced grasshoppers of the subfamily Gomphocerinae, male *P. brachyptera* have a row of pegs on their inner femur used to produce calls when moved across the raised medium radial vein of the tegmen in an action known as stridulation (Ewing 1989, Johnson 2003, von Helversen et al. 2004). Male *P. brachyptera* songs are created by 10-20 individual leg strokes and produce a "sibilant sh-sh-sh sound of increasing intensity" (Otte 1970). The *P. brachyptera* mating system has yet to be thoroughly examined, and it is probable that male bioacoustic calls play a role in the attraction and selection of mates. As these grasshoppers do not fly (Laws, personal communication) to escape predators, anything an individual does to
increase its risk of predation, such as calling, should be outweighed by some fitness advantage, such as mate attraction.

The aim of this study is to characterize several call parameters thought to be important in male song assessment by females (particularly dominant power frequency (kHz), call length (s), the number of pulses/syllable, and syllable:pause duration ratio) with regard to male biometrics and to determine whether females mate non-randomly with respect to bioacoustic and/or biometric measurements in attempt to describe female mate preference. While *P. brachyptera* do not have a duetting mating system similar to that of *Chorthippus biguttulus* in which females respond to male calls with stridulations of their own conveying female acceptance of a male for mating (von Helversen and von Helversen 1994, Klappert and Reinhold 2003, von Helversen et al. 2004), comparing successfully mated males and unsuccessful males can provide evidence of female mate choice. It is expected that a relationship between some biometric measure (male size measurements: mass, femur length, pronotum length, tegmina length, total length, width, height, volume) will correlate with bioacoustic parameters (dominant power frequency, syllable length, number of pulses/syllable, syllable:pause duration ratio). Additionally, it is expected that females mate non-randomly with respect to male biometrics (male size measurements: mass, femur length, pronotum length, tegmina length, total length, width, height, volume).

METHODS

Bioacoustics

A sample of grasshoppers of the species *Pseudopomala brachyptera* were collected, by sweeping and stalking individual grasshopper with aerial insect nets, as they were found in
a typical habitat on the National Bison Range, Moiese, Montana in mid-July. Males and females were kept in separate terraria, and males, in groups of 2-3 at a time, were introduced into the female cage for a ten minute period to induce male calling. The song of any calling male from either terrarium was recorded using an AudioTechnica AT-815 Shotgun condenser microphone connected with 1/4" TRS to a M-AUDIO MicroTrack 24/96 solid state digital recorder formatted to linear PCM WAV (uncompressed files) at 48kHertz / 16-bit sampling.

Calling individuals were removed, and biometric measurements were recorded. Mass of each male to the nearest 0.01g was measured using an OHAUS CS1000 electronic scale. Femur, tegmen, and pronotum length were measured with a dial caliper to the nearest 0.1mm. Volume (total length x width at widest point x height at greatest distance) was measured with a dial caliper to the nearest 0.1mm to give overall size (mm³). The biometric measurements of non-calling males were also recorded in order to determine the distribution of the collected male population. Call recordings were transferred to a PC and analyzed using Raven 1.2 Bioacoustics software. Three calls (syllables) of each individual caller were analyzed based on call length (s), number of pulse/syllable, and syllable:pause duration ratio, and dominant power frequency (kHz) (particularly highest sustained frequency, lowest sustained frequency, and delta frequency – the difference between highest and lowest frequency). The mean bioacoustic measurements of these three syllables were used for comparisons.

Male Mating Success

Male and female *P. brachyptera* were collected separately from the call analysis procedure and put into a single terrarium with males outnumbering females 2:1 (approximately 5F:10M) during each of four days of experimentation. Mating pairs were removed from the terrarium, and male and female biometric parameters were measured in
order to compare successfully mated males to unsuccessful males. Biometric parameters
(femur, tegmen, and pronotum length, mass, total length, width, height, and volume) of mated
females were measured and were used, in association with the biometrics of her mate, to test
for assortative mating. Biometrics of successfully mated males were compared to males
remaining unmated males.

RESULTS

Over three weeks in July 2006, 97 males and 28 females of the slant-face grasshopper
species *P. brachyptera* were collected. Of these males, 28 songs of individual males were
recorded and their bioacoustic parameters assessed, and 46 males were involved in the mate
choice experiment: 22 successful and 24 unsuccessful. Data for call analysis and for the
mating choice experiment were analyzed using the statistical program JMP IN 5.1 (SAS
Institute Inc).

Comparisons of population biometric measures revealed several significant – or nearly
significant – relationships; the most biologically notable were femur length and tegmen length
\( R^2 = 0.14, n = 97, \text{P} = 0.0002; \text{figure 2} \) and tegmen length and mass \( R^2 = 0.12, n = 97, \text{P} =
0.0006; \text{figure 3} \). All significant relationships among biometric measurements were positive
correlations (Table 1).

Bioacoustics

Analysis of the calls of 28 individual males consisted of determining and comparing
several features of song: number of pulses/syllable, syllable length, syllable:pause duration
ratio, and dominant power frequency. Calls were comprised of syllables, typically 0.22 s long
\( \pm 0.12 \text{s SD} \), with an average of 3.79 pulses in each syllable \( \pm 2.03 \text{ SD} \), and an average
syllable:pause duration ratio of 2.37 (±0.83). The mean frequency range was from 4574.98 Hz (±703.85 Hz SD) to 19880.31 Hz (±1517.32 Hz SD), with the mean delta frequency of 15305.34 Hz (±1817.21 Hz SD) (Figure 4). No biologically remarkable significant relationships were found in linear regression analyses of bioacoustic parameters (Table 2).

Linear regressions comparing song parameters and biometrics was used to discern any relationships by which females could gauge male size (a possible proxy for fitness) (English 2001) by assessing his song parameters. Femur length and syllable length were positively significantly related ($R^2 = 0.1572, n = 28, P = 0.0367$; Figure 5). Table 3 contains the results for all correlation analyses run between call parameters and biometrics.

**Male Mating Success**

For the male mating success experiment, the experimental group of 46 males was initially compared to the entire collected male population in order to verify that this sample did not differ from the larger population sample, and was not found to be significantly different for any biometric measurement except mass (Table 4). Males used in the mating experiment had significantly greater masses than the overall collected male population (Student’s t-test: $t = 3.14, df = 141, P = 0.002$). Within the experimental group, biometrics of mated and unmated males were compared using Student’s t-tests to find any difference in biometric measurements between mated and unmated males that might be related to male mating success. Mated males has significantly greater masses than males not found mated (Student’s t-test: $t = 2.94, df = 44, P = 0.0052$; Figure 6), while unmated males had significantly longer tegmina than mated males (Student’s t-test: $t = 2.11, df = 44, P = 0.04$).

Successfully mated males did not have significantly greater mass than the unmated males on a daily basis in the mate-choice experiments ($P \geq 0.11$). Based on these results,
males from each day of experimentation were then divided by mass into an upper half and a lower half to determine whether these daily experimental groups were variable enough for differences in mass to be detected. For each of four days, there was a significant difference between the upper half and the lower half, however successfully mated males were generally found in both the upper and lower half (P ≤ 0.005 for all days; Figure 7).

Linear regression was used to test for assortative mating, comparing biometric measurements of associated males and females from 21 mated pairs. Linear regression compared male and female femur, tegmen, and pronotum length, mass, total length, height, width, and volume. No significant correlative relationships were found between the biometrics of males and their female mates (P ≥ 0.10 in all comparisons).

DISCUSSION

Comparisons of biometric measurements of the entire collection of populated males revealed significant positive relationships between femur length and tegmen length and between tegmen length and mass. While tegmen and femur are both involved in the sound production mechanism for P. brachyptera, a causative relationship between femur length and tegmen length seems unlikely; rather, greater overall size appears to manifest itself in these two biometric measures. The tegmen functions as the protective covering for the delicate hind-wing (typically used for flying in other Orthopteran species) and thus could comprise a considerable amount of an individual’s total mass. Therefore, it follows that males with longer tegmina would be more massive.

Bioacoustics
Bioacoustic analysis of call parameters found several significant relationships; however, these results were not biologically relevant as these parameters by their nature are auto-correlated. Fewer than expected significant relationships were found in analysis of biometric measurements of calling males and bioacoustic parameters of their song. Femur length was significantly positively related to syllable length. The femur is responsible for sound production in stridulation as pegs on the inner side of the femur are dragged against the tegmen mid-vein, and syllable length has been shown to correlate closely with peg-row length in other gomphocerinae species (Reynolds 1980, Dagley 1988, Dagley et al. 1994). Therefore, based on the mechanism of sound production of grasshoppers, it is intuitive that longer femurs would function in the production of longer syllables and that this relationship was found.

In the Gomphocerinae species Nightingale Grasshopper (Chorthippus biguttulus), female choice is based on male calls, which presumably contain information reflecting their quality. Females determine the relative quality of the male based on several bioacoustic parameters of the call, including frequency, total syllable duration, and syllable:pause duration ratio (Klappert & Reinhold 2003). Amplitude and syllable:pause duration ratio may be indicative of fitness because larger males tend to produce louder calls and because it is more costly energetically to produce longer calls with shorter pauses, which are preferred by females (Klappert & Reinhold 2003). Male mating success has been linked to loudness and/or amount of song produced, both of which are typically associated with male body size, “a factor often implicated in mate choice with females almost invariably preferring larger males” (Ewing 1989). However, song intensity is not always involved in mate choice, as shown in the gomphocerine grasshopper Chorthippus brunneus by Butlin et al. (1985).
Amplitude was not considered in this study due to the inherent difficulties in maintaining a constant distance from calling males in this experimental set-up. Syllable:pause duration ratio was not found to be biologically significantly related to any bioacoustic or biometric parameter. While frequency, total syllable duration and number of pulses/syllable were not found significantly related to any biometric measurement that was important in male-mating success experiments (mass and femur length), further examination of call parameters could elucidate the role of bioacoustic calls in the *P. brachypiera* mating system, with particular consideration to the calls of mated vs. unmated males.

**Male Mating Success**

In mate choice experiments, the experimental population was found to be similar to the total male population in all biometrics, save mass. The experimental population was only a small portion of the total male population (38 of 108 males), and all of these were collected during the last days of the three week collection period. Because grasshoppers continue to fluctuate in mass due to feeding and egg or spermatophore production during the adult stage (Laws, personal communication), it is probable to assume that male population in the field increased in mass over three week of collection. Therefore, the difference in mass between the entire collected male population and the experimental group that was caught latest in the collection period can be attributed to growth or gamete production over the course of the season.

To determine what – if any – biometric measures might be responsible for male mating success, successfully mated males were compared to their unmated counterparts. Mated males were found to be significantly more massive. Females may prefer males with greater mass because size often functions as a proxy for fitness due to increased energy
reserves, increased mating agility or desiccation resistance or correlation with larger spermatophore or increased fertilization (English 2001). In species of *Conocephalus* and *Orchelimum* katydids, females actively select relatively larger mates because male size is directly proportional to spermatophore size (Gwynne 1982). Additionally male size is a reliable indicator of male social dominance and resource gathering skills (Thornhill and Alcock 1983). Males with relatively greater mass out-compete other males or are preferentially selected by females. With greater access to females, these larger males are able to pass on more of their genes to future generations and are thus more fit (Fisher 1958).

Unmated males had significantly longer tegmina than mated males. It seems that females select against tegmina length, as tegmina (the protective leathery forewings) are adaptive only for protection of hind-wings; however, this species does not fly or even have full wings (Laws, personal correspondence). Instead tegmina need only be long enough to effectively produce song. In the male mating success experiment, males that were selected as mates by females were more massive and had shorter tegmina than their unmated counterparts. These results suggest that, in mate selection, females look for a balance between the positive selective pressures of mass and the negative selective pressure of tegmen length. It is necessary that a tegmen is long enough to produce calls but not so long that production of this relatively minor feature is energetically wasteful. Apparently males are judged based on their functional or biologically useful mass; females therefore consider the less-useful mass of the tegmen when assessing a male’s overall mass. Females appear to be selecting mates based on a combination of these biometric features, which are reflective of his size and possibly his energy efficiency, and thus his fitness. Energy that is spent on
production of a particularly long tegmen in this species very well could be energy that might have been better used for reproduction or spermatophore production.

In order to assess the mechanism behind female mate choice in *P. brachyptera*, the successful and unsuccessful males were considered on a daily basis. As mass was found to be important in male mating success and is indicative of male fitness in many insect species (Gwynne 1982, Thornhill and Alcock 1983, English 2001), masses of mated and unmated males were compared for each day of mate-choice experiment. When no significant results were found, masses of daily groups of males were divided into an upper and lower mass half for comparisons. There was a significant difference between the upper and lower halves for each day, implying that there was enough variation within each day’s group to detect significant differences in mass should they exist. Successfully mated males were generally found distributed in both the upper and lower mass groups for each day, illustrating that there was no difference between these mass groups in terms of mating success. This was supported by the absence of a significant difference between masses of mated and unmated for each day’s experimental groups. Therefore, it seems that females are not selecting mates using a best-of-n strategy; if they were, each day’s upper mass group would contain all the successfully mated males, as females select the best males with the greatest mass in whatever group she is presented. Since this is not the case, it seems that females were choosing mates based on a fixed threshold decision-making strategy. That successful males were found in a relatively wide distribution of masses within the daily experimental groups suggests that most of the males tested over four days of mate choice experimentation were above the mass threshold. From the results of the overall mated vs. unmated male mass comparisons, it appears that mass threshold is around 100mg (Figure 6). Above this threshold, mating
appears to be “randomly distributed among males meeting the acceptance criteria” (Cooley and Marshall 2004) at least with regards to mass, consistent with fixed-threshold selection strategies (Janetos 1980).

In *P. brachyptera*, it appears that females are using bioacoustic calls only to determine species, relative location, and general health of males, which must be good enough to expend the energy to call and increase his risk of predation. Instead, male mating success corresponds to female preference for males of greater mass with smaller tegmen, favoring heavier males, without excess energy spent on tegmen production beyond what was necessary to produce calls. The balancing selection at work in the sexual selection of the *P. brachyptera* mating system involves these two biometric measurements, both of which are apparently unrelated to the displayed acoustic calls. However it is unclear how these biometrics might operate on a larger evolutionary scale. Sexual selection based on displayed bioacoustic characteristics of males is more coherent with ideas of divergence and speciation. Data from bioacoustic call recordings of both mated and unmated males could clarify what — if any — role bioacoustics actually play in mate selection, as well as mate attraction, in *P. brachyptera*.

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APPENDIX

TABLES

Table 1. Relationships among biometric measurements of the male population (n = 97). Bolded p-values and \(R^2\)-values indicate statistical significance of a positive relationship. Asterisks indicate biologically relevant relationships discussed in the text.

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<th>tegmen</th>
<th>mass</th>
<th>volume</th>
<th>femur</th>
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<tr>
<td>femur</td>
<td>(p = 0.0020)</td>
<td>(p = 0.0002^*)</td>
<td>(p = 0.4040)</td>
<td>(p &lt; 0.0001)</td>
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<td>(p = 0.029)</td>
<td>(p = 0.4401)</td>
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Table 2. Relationships among call parameters. Bolded p-values and \(R^2\)-values indicate statistical significance. Positive and negative relationships of significance are indicated with a (+) or (-). (n = 28)

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<th>low frequency</th>
<th>delta frequency</th>
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Table 3. Relationships between call parameters and biometric measurements. Only syllable length and femur length were found to be significantly related. (n = 28)

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<td>R² = 0.09</td>
<td>R² = 0.10</td>
<td>R² = 0.007</td>
<td>R² = 0.003</td>
<td>R² = 0.008</td>
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<td>mass</td>
<td>p = 0.86</td>
<td>p = 0.43</td>
<td>p = 0.98</td>
<td>p = 0.93</td>
<td>p = 0.94</td>
<td>p = 0.88</td>
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<tr>
<td></td>
<td>R² = 0.001</td>
<td>R² = 0.02</td>
<td>R² = 0.0004</td>
<td>R² = 0.002</td>
<td>R² = 0.0002</td>
<td>R² = 0.0009</td>
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<tr>
<td>tegmen</td>
<td>p = 0.11</td>
<td>p = 0.34</td>
<td>p = 0.35</td>
<td>p = 0.91</td>
<td>p = 0.93</td>
<td>p = 0.90</td>
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<tr>
<td></td>
<td>R² = 0.09</td>
<td>R² = 0.04</td>
<td>R² = 0.03</td>
<td>R² = 0.0005</td>
<td>R² = 0.0003</td>
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<tr>
<td>pronotum</td>
<td>p = 0.42</td>
<td>p = 0.74</td>
<td>p = 0.87</td>
<td>p = 0.17</td>
<td>p = 0.93</td>
<td>p = 0.27</td>
</tr>
<tr>
<td></td>
<td>R² = 0.02</td>
<td>R² = 0.004</td>
<td>R² = 0.001</td>
<td>R² = 0.07</td>
<td>R² = 0.0003</td>
<td>R² = 0.05</td>
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</tbody>
</table>

Table 4. ANOVA results from comparison of biometrics of the experimental group and the entire collected male population. Males of the experimental group had significantly higher masses than males of the overall population. (df = 141)

<table>
<thead>
<tr>
<th></th>
<th>pronotum</th>
<th>tegmen</th>
<th>mass</th>
<th>volume</th>
<th>femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>experimental group vs. population</td>
<td>p = 0.30</td>
<td>p = 0.33</td>
<td>p = 0.002</td>
<td>p = 0.50</td>
<td>p = 0.18</td>
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<tr>
<td></td>
<td>t = 1.05</td>
<td>t = 0.97</td>
<td>t = 3.14</td>
<td>t = 0.68</td>
<td>t = 1.32</td>
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</table>
Figure 1. Song of *Pseudopoma brachyptera*. Two syllables are shown; high, low and delta frequency measures are indicated. The second syllable has a total length of 0.465s and is comprised of 9 syllables divided by 8 pauses. Call parameters analyzed included high, low, and delta frequencies, syllable length, number of pulses/syllable, and syllable: pause duration ratio.
Figure 2. Femur length (mm) and tegmen length (mm). A significant positive relationship existed between femur and tegmen length in the entire male population ($R^2 = 0.14$, $n = 97$, $P = 0.0002$).
Figure 3. Mass (mg) and tegmen length (mm). A significant relationship was found between mass and tegmen length in the entire male population ($R^2 = 0.12$, $n = 97$, $P = 0.0006$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Pulses/syllable</td>
<td>3.7657</td>
<td>± 2.0336</td>
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<tr>
<td>Syllable length (s)</td>
<td>0.2151</td>
<td>± 0.1105</td>
</tr>
<tr>
<td>High frequency (Hz)</td>
<td>19680.3</td>
<td>± 1517.3191</td>
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<tr>
<td>Low frequency (Hz)</td>
<td>4574.975</td>
<td>± 703.8454</td>
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<tr>
<td>Delta frequency (Hz)</td>
<td>15305.339</td>
<td>± 1817.2059</td>
</tr>
<tr>
<td>Syllable/pause duration ratio</td>
<td>3.1</td>
<td>± 0.8349</td>
</tr>
</tbody>
</table>

Figure 4. Distributions of bioacoustic call parameters.
Figure 5. Femur length (mm) and syllable length (s). Syllable length and femur length exhibited a significant positive relationship ($R^2 = 0.16$, $n = 28$, $P = 0.04$).
Figure 6. Mass (mg) comparisons of mated and unmated males in male mating success experiment. Males that acquired mates had significantly greater masses than those who did not (t = 2.9, df = 44, P = 0.0052). Mated males all had mass above 100 mg.

![Graphs showing mass comparisons](image)

Figure 7. Daily comparisons of upper and lower mass (mg) groups in the male mating success experiment. Significant differences were found between upper and lower mass groups over the four days of experimentation: a. July 20 (Student's t-test: t = 3.86, df = 9, P = 0.004), b. July 21 (Student's t-test: t = 4.74, df = 8, P = 0.002), c. July 23 (Student's t-test: t = 4.9, df = 12, P = 0.0004), and d. July 24 (Student's t-test: t = 4.36 df = 6, P = 0.005). Successfully
mated males are designated with diamond, while unmated males are represented by a rectangle.