CHAPTER 3

High levels of environmentally-induced variation in the calling song of the field cricket, *Gryllus bimaculatus* **De Geer (Orthoptera: Gryllidae)**

Abstract

The calling song of the field cricket is involved in both sexual selection and species recognition. Several environmental parameters could influence the calling song traits and, in turn, the reliability with which a male signal reflects its quality. This study investigates variation in the calling songs of two wild cricket populations in South Africa, recorded under standardised conditions. Depending on the particular call trait measured, up to 62% of the total within-population variation was explained by within-individual variation in call traits, suggesting considerable flexibility in the communication system of these crickets. There were differences in the partitioning of variation in the two populations. However, the call traits suggested to be involved in species recognition had a smaller degree of within-individual variance. In addition, male size and call traits differed between seasons: males were smaller, produced higher calling song frequencies and called at a faster syllable rate during summer, compared with winter. An experiment was performed to test whether these seasonal differences are attributable to temperature-related body size differences. Males raised at 27°C were significantly heavier than those raised at 22°C. We conclude that other environmental factors such as food availability and brood size result in seasonal differences in body size. In addition, this study found only weak correlations between male body size and call traits and suggests that females probably cannot use male calling song as an indicator of male body size. Call traits at a particular site were largely consistent between years.

Keywords: *Gryllus bimaculatus*, Calling song, Within-population variation, Within-individual variation, Seasonal variation

Introduction

Male field crickets, *Gryllus bimaculatus*, produce calling songs that attract females (Alexander, 1962). Several calling song characteristics are suggested to reflect male phenotypic attributes and to be sexually selected (number of syllables per chirp: *Acheta domesticus*: Gray, 1997; syllable rate, chirp rate, chirp duration: *G. bimaculatus*: Simmons, 1988; calling song frequency: *G. campestris*: Simmons & Ritchie, 1996; chirp duration: *Teleogryllus oceanicus*: Simmons *et al*., 2001). As with most quantitative traits, phenotypic variation in the calling song of crickets comprises both genetic and environmental components (Falconer & Mackay, 1996). Since additive genetic variance determines the predictable genetic properties of individual males (Lynch & Walsh, 1998) it forms the basis on which selection, e.g. sexual selection, can act. After strong sexual selection one would expect environmental variation to constitute the bulk of the remaining phenotypic variation. However, in certain studies this was not the case and the maintenance of additive variation in sexually selected characteristics has been explained as the result of changing selective pressures on these communication systems (Iwasa & Pomiankowski, 1995; Pomiankowski & Møller, 1995). On the other hand, substantial remaining environmental variation could be thought of as a noise factor that obscures efficient communication during courtship and that slows down the rate at which selection removes additive genetic variation. This could decrease the selection pressure on sexually selected characteristics as envisaged by the above authors. An aspect of cricket communication that has received virtually no attention is the degree of stability in the calls of an individual male. This would determine the reliability with which a female could judge the call and, consequently, the quality of a male before mating (e.g. Simmons, 1988; Simmons & Ritchie, 1996). An understanding of the degree of environmentally-induced variation is therefore important for understanding sexual selection in crickets since it complements estimates of heritability on call traits (Gray & Cade, 1999). Direct measurements of environmental variation could be derived from multiple measurements on the same individuals (the 'special environmental component'; Falconer & Mackay, 1996). Furthermore, variation in communication characteristics over short time periods (e.g. between seasons of the same year) within the same population is also likely to comprise mostly an environmental component since the genetic composition of a population is expected to remain nearly constant over such short periods. Environmentally-induced variation in the

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cricket communication system could also be brought about by a correlation between signaling characteristics and environmentally affected morphological characteristics such as body size. Variation in cricket song could result from three causes: (a) variation in motivation for calling, (b) immediate environmental effects on the physical and physiological processes controlling calling behaviour and (c) developmental effects brought about by the environment.

Body size in many invertebrates is significantly affected by rearing environment (Sibly & Atkinson, 1994). Environmentally induced developmental variation can also cause variation in adult behaviour and the interaction between developmental environment and adult environment can cause variation in call traits of crickets (Grace & Shaw, 2004). For instance, calling temperature, rearing temperature and the interaction between them had a significant effect on call traits in the striped ground cricket, *Allonemobius fasciatus* (Olvido & Mousseau, 1995). Conversely, Grace & Shaw (2004) found that the effect of the rearing environment on the calling song in the Hawaiian cricket, *Laupala cerasina*, was not permanent. The quality of the rearing environment, i.e. food availability and food quality, could influence a male's communicatory system. *Gryllus campestris* males reared on a poor diet produced a high carrier frequency due to disproportionate reduction in the harp size (Scheuber *et al.*, 2003). However, this effect is not universal, as none of the courtship song characteristics measured for *G. texensis* and *G. lineaticeps* were affected by food treatment in nymphs (Gray & Eckhardt, 2001) and in adults (Wagner & Reiser, 2000).

Two studies on female preferences in *G. bimaculatus* showed that females not only preferred large males, but that they also received more sperm from them (Bateman *et al.*, 2001; Simmons, 1986). Several studies have demonstrated a relationship between male body size and calling song characteristics (Brown *et al.*, 1996; Gray, 1997; Simmons, 1988). Small-scale local differences in the environment could affect male body size and could, in turn, influence the call traits. *Gryllus campestris* females preferred low calling song frequencies (Simmons & Ritchie, 1996) that, in turn, resulted in the selection of larger males (Scheuber *et al.*, 2003). Large male black-horned tree crickets, *Oecanthus nigricornis*, produced calls with a lower frequency that could potentially be used by females as a pre-copulatory mechanism to choose larger males (Brown *et al.*, 1996). Gray (1997) found that large male house crickets, *A. domesticus*, produced more syllables per chirp and that females preferred the calling song of these

males. Since *G. bimaculatus* females respond to male calls through phonotaxis, it could be to a female's advantage to judge the size of a calling male from its calling song. Chirp rate and calling rate could potentially be used in certain cricket species as reliable indicators of the current condition of males. *Gryllus campestris* males that received more food had a significant increase in body mass and called for significant longer periods at a higher chirp rate than males that did not (Holzer *et al.*, 2003; Scheuber *et al.*, 2003). Although *G. lineaticeps* males did not show a significant increase in body mass when fed high nutrient food, they did call for significant longer periods than males that received low nutrient food (Wagner & Hoback, 1999). Conversely, Gray & Eckhardt (2001) found that, although *G. texensis* males fed on a high quality diet gained significantly more weight than males on a low quality diet, none of their courtship song characteristics were affected by residual body mass. The investment of energy appears to be species-specific where some taxa invest energy into body reserves as well as calling rate with others investing it into either.

The aims of this study are:

1. To quantify the within-individual variation in male calling song in order to assess the degree to which (within a framework of sexual selection) a male's signal, at a particular moment, is a reliable indicator of longer term signaling characteristics.

2. To determine whether the signaling characteristics within a population remain stable with time over seasons of the same year and over different years in order to test the hypothesis that the communication system has a large degree of temporal consistency at the same geographical site.

3. To determine whether sexually selected signaling characteristics differ in the above-mentioned respects from signaling characteristics not subject to sexual selection.

4. To determine whether, within a population, male size is correlated with call traits in order to test the hypothesis that the calling song can potentially be used by females to judge the size of a male.

5. To determine whether the rearing temperature affects body size in male and female crickets at adult eclosion. This tests the hypothesis that seasonal differences in body size are attributable to temperature differences.

Materials and methods

Within-individual and between-individual variation in calling songs of wild-caught males

Sampling localities

Wild male field crickets, *G. bimaculatus*, were captured at Pretoria (25°41'S, 28°13'E) and Makhado (23°05'S, 29°59'E) in South Africa, during 2001 – 2003. During 2000 – 2002, the annual rainfall was higher at Makhado than Pretoria (Makhado: mean = 1285 mm, range 414 – 2407 mm; Pretoria: mean = 765 mm, range = 426 – 1268 mm). In addition to being moister, Makhado, a montane site, had less extreme temperature fluctuations than Pretoria (Figure 3.1). Each population was analysed separately.
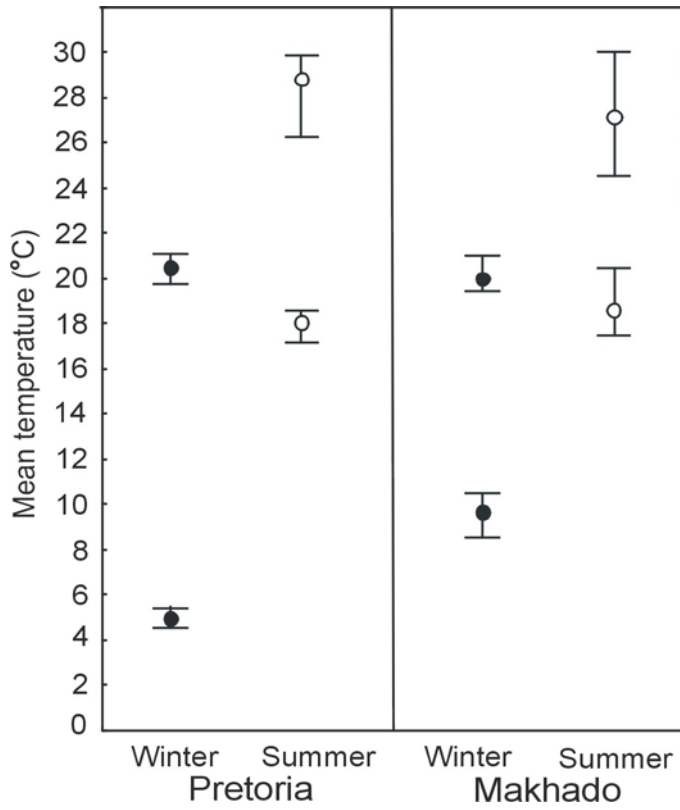
Figure 3.1. Mean minimum and mean maximum temperatures (with ranges) for winter and summer for Pretoria and Makhado during 1999 – 2003.

Calling song recordings

Males were placed in sound damped recording chambers where the calling song of each male was recorded. Sound-damped recording chambers (31 cm x 35 cm x 26 cm; acoustic isolation 50.13 ± 1.73 dB (mean ± SE) between neighbouring chambers) were maintained at 25°C ± 1°C using an electronically controlled heater. Each chamber was sound damped with a glass fibre mat (thickness 5 cm) and equipped with a Beringer XM200S dynamic microphone (50 Hz – 10 kHz ± 3 dB; Behringer Spezielle Studiotechnik GmbH, Germany). A Sony TC-D5M cassette recorder was used to record the calling songs. Each male was placed in a gauze container (11 cm x 11 cm x 12 cm) with a cardboard floor and a small piece of egg carton for shelter. High protein cereal (Pronutro®) and water were provided *ad libitum*. A 12h:12h Light:Dark (L:D) light regime was implemented using an 8000 mCd light-emitting diode above each gauze container. Three sound recordings of 30 seconds duration each were made per night for at least two nights (i.e. a total of six recordings) with between-recording intervals of at least five minutes.

Calling song characteristics

Canary V1.2.4 (Cornell Laboratory of Ornithology, Ithaca, New York) was used for spectrographic analysis of the calls. A power spectrum was generated to measure calling song frequency with maximum amplitude (F_{max}), bandwidth at 10 db below maximum amplitude (Fwidth-A) and bandwidth at 20 db below maximum amplitude (Fwidth-B) for each recording. An oscillogram was used to measure number of syllables per chirp (S_C), syllable period (SP), syllable duration (SD), inter-syllable interval (ISI), chirp period (CP), chirp duration (CD) and inter-chirp interval (ICI) for each recording. For formal definitions of these characteristics see Ferreira (2006, Chapter 2). To measure within-recording variation in call traits, six chirps were measured for the first recording of the first night with a 10 second interval between the first three and the last three chirps. Longer-term variation was measured by measuring two different recordings from the same night and between-night variation was measured using at least two recordings from each of two different nights. Three consecutive chirps from each of these recordings were measured. Each chirp comprises three to six syllables (Desutter-Grandcolas & Robillard, 2003).

Statistical analyses

For each population, SAS V8.02 (Proc Varcomp; SAS Institute Inc., Cary, NC, USA) was used to determine the within-individual and between-individual variance components for the call traits using restricted maximum-likelihood estimation (REML). Within-individual variation was broken down into four levels: (a) between nights within an individual, (b) between recordings within a night, (c) among repeats (six chirps were measured with a 10 second interval between the first three and last three chirps) within a recording and (d) among chirps within a repeat. The magnitude of the variance components of each of these sources of variation as well as the magnitude of their interactions were calculated.

Several of the analyses below reflect four subsets of data: (a) morphometric data, (b) chirp call traits (ICI, CD, CP and S_C), (c) syllable call traits (ISI, SD and SP) and (d) spectral call traits (F_{max}, Fwidth-A and Fwidth-B).

Seasonal and annual variation in calling songs and body size of wild-caught crickets

Data set

Calling song recordings of wild male crickets from Pretoria and Makhado were classified into a season, based on capturing dates, as follows: summer: 1 December – 28 February; autumn: 1 March – 31 May; winter: 1 June – 31 August; spring: 1 September – 30 November. Since cricket population densities fluctuate greatly in space and time, an even representation across all seasons was not possible. Data from Pretoria were available for six seasons: autumn 2001, spring 2001, summer 2002, autumn 2002, winter 2002 and autumn 2003. Data from Makhado were available for three seasons: winter 2001, autumn 2002 and summer 2003. Since the data from Makhado represent three different seasons from three different years, care needs to be taken with respect to the temporal interpretation of these analyses (see discussion). Between-year comparisons utilised the autumn data collected from Pretoria males during 2001 – 2003.

Calling song characteristics and morphometric measurements

Male calling songs were recorded and measured as described above. After killing a male with ethyl acetate, its thorax width, thorax length and the length of the right hind femur (Fem-R) were measured using the VideoBlaster FS200 video kit (Creative Laboratories, Singapore; resolution = 16 microns). Thorax area was calculated as: thorax width x thorax length (mm^2). The wings of each male were then removed and the harp size of each right wing was measured, following Ferreira (2006, Chapter 2).

Statistical analyses

For each separate population, SAS (Proc Varcomp) was used to determine the between-season variance component for the call traits of the wild-caught males. Statistica V5.5 (StatSoft, Inc. (1999), Tulsa, USA) was used to perform fixed effects analysis of variance (ANOVA) to compare the means of the call traits as well as the means of the morphometric measurements between seasons and between years. Tukey HSD *post-hoc* analysis was performed to determine which seasons or which years (autumn, Pretoria) differed significantly from one another. A Bonferroni adjustment (Rice, 1989) was applied to the Tukey HSD *post-hoc* data of both populations. Using Statistica, a principal components analysis (PCA; varimax rotation on normalised data) was performed to determine which call traits and morphometric measurements contributed the most to the total variation of the data set. Thorax width and thorax length were excluded from the PCA, due to the inclusion of thorax area.

Effect of rearing temperature on body size of laboratory-reared crickets

Setup of breeding colonies and morphometric measurements

Breeding colonies from three geographical sites from South Africa (wild-caught crickets from Queenstown (31°52'S, 26°52'E) and Wolmaransstad (27°23'S, 26°00'E) as well as a laboratory colony from Pretoria) were established in a climate room at 27°C ± 2°C with a 12h:12h L:D light regime. Egg-laying trays from each of these colonies were collected and placed separately in labelled 2-litre plastic containers. When the eggs hatched, 75 first instar nymphs were randomly chosen from each of the three colonies and divided into batches of 25 nymphs in three 9-litre plastic containers. One container per colony was placed in incubating chambers set at constant temperatures of 15°C, 22°C and 27°C. High protein

food (Pronutro® and fish flakes) and water were provided *ad libitum* and one egg carton was placed in each of the 9-litre containers to provide shelter. Crickets were killed with ethyl acetate after adult eclosion, sexed and weighed to the nearest milligram on a Mettler Toledo AG135 scale. The Creative Labs FS200 video kit was used to measure thorax width, thorax length, head width, head length, Fem-R, right hind tibia (Tib-R), left hind femur (Fem-L), left hind tibia (Tib-L) and harp size of the right male wing. Thorax area was calculated as: thorax width x thorax length (mm^2).

Statistical analyses

Due to the high mortality rate of nymphs in the temperature treatments at 15°C and 22°C, the offspring from all three different localities were combined for each of the temperature treatments and could not be analysed separately. Differences between the morphometric measurements of the temperature treatments were analysed using a two-way fixed effects ANOVA with treatment and sex as independent variables, using Statistica.

Body size as a factor explaining within-population variation in call structure of wild-caught crickets

Calling song characteristics and morphometric measurements

Call traits (described above) of Pretoria males were obtained for four seasons (summer, autumn, winter and spring). For each male, morphometric measurements comprised thorax length, thorax width, thorax area, head length, head width, Fem-R, Tib-R, Fem-L, Tib-L and harp size.

Statistical analyses

Using SAS (Proc GLM), an analysis of covariance (ANCOVA) on the data for each of the four seasons was performed on each of the call traits, using thorax area as a covariate, to determine the effect of size on the calling song during the four seasons. Following the ANCOVA, summer data differed significantly from the other seasons and were excluded from the following analyses (see results below). Linear least-square regression analysis was performed, using SAS (Proc Reg), on the morphometric measurements and call traits to determine any significant relationship between body size and calling song. Variables with significant relationships were subjected to forward stepwise multiple regression analysis,

using Statistica, to determine which morphometric measurements contributed mostly to the variation found in the call traits. Pearson product moment correlations between morphometric measurements were calculated using Statistica.

Results

Between-individual variation in calling songs of wild-caught males

For both Makhado and Pretoria, the between-individual variance component was larger than the within-individual variance component in the spectral traits (Table 3.1). This effect was pronounced in Pretoria comprising a larger sample size. However, the chirp traits mostly had a larger within-individual variance component than between-individual variance component, reflecting the relatively large variation in chirp traits at the individual level (Table 3.1). Syllable traits for Pretoria males had a somewhat larger between-individual than within-individual variance component, while the converse was found for the Makhado males (Table 3.1).

Table 3.1. Proportions (%) of the between-season, between-individual and within-individual effects contributing to the explainable variance in the calling song characteristics of wild *G. bimaculatus* from Makhado (n = 57) and Pretoria (n = 115)

Call traits	Makhado			Pretoria		
	Between season	Between individual	Within individual	Between season	Between individual	Within individual
S_C	11.84	54.52	33.63	0.00	48.14	51.86
ICI	0.00	35.32	64.68	1.35	34.97	63.69
CP	0.00	37.16	62.84	2.84	40.27	56.89
CD	28.76	41.29	29.95	7.12	49.29	43.59
ISI	24.15	20.31	55.54	12.05	66.13	21.82
SD	13.06	43.03	43.91	4.4	52.74	42.87
SP	42.86	19.14	38.01	32.38	54.4	13.22
F_{max}	15.05	68.12	16.83	30.5	54.74	14.76
Fwidth-A	13.01	56.83	30.17	16.91	47.71	35.38
Fwidth-B	19.25	45.09	35.66	15.39	47.2	37.41

Within-individual variation in calling songs of wild-caught males

The between-night variance component and the between-chirp variance component were mainly responsible for the variation found within male calls from Makhado (Figure 3.2a) and Pretoria (Figure 3.2b). The call traits of both populations showed a large degree of stability between recordings as well as between repeats within the same recording. Syllable period and calling song frequency showed a large degree of between-night stability in the Pretoria population, with a large degree of between-chirp variation (Figure 3.3). The large between-chirp variation in syllable period nullified between-individual variation (Figure 3.2). In contrast, a large degree of between-chirp stability was observed for Makhado males. This constituted an important difference in the distribution of sources of variation between the two study sites.
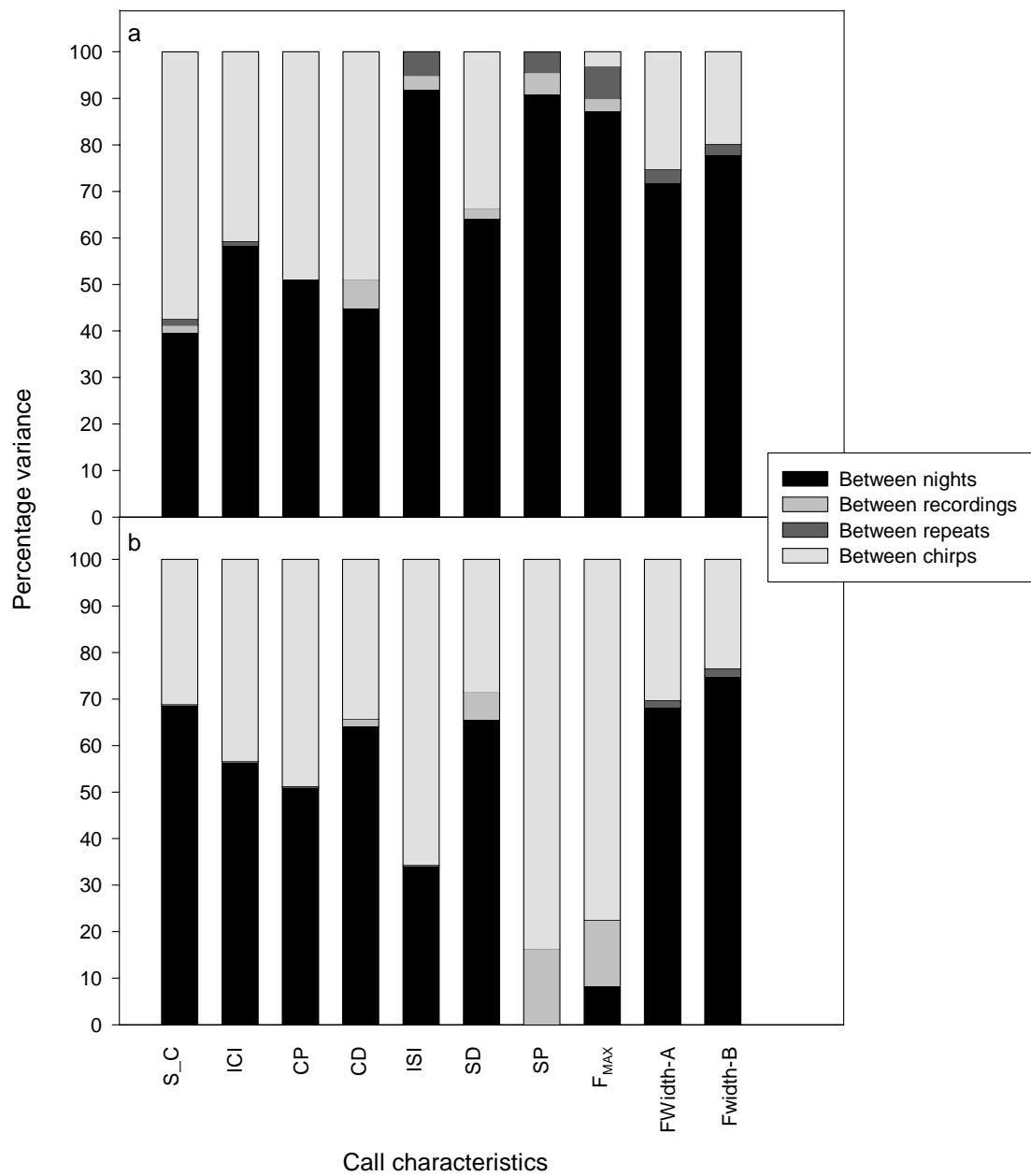
Figure 3.2. Results of a random-effects ANOVA, indicating sources of explainable variance contributing to within-individual variation in the calling song of the field cricket for (a) Makhado males ($n = 49$) and (b) Pretoria males ($n = 102$).
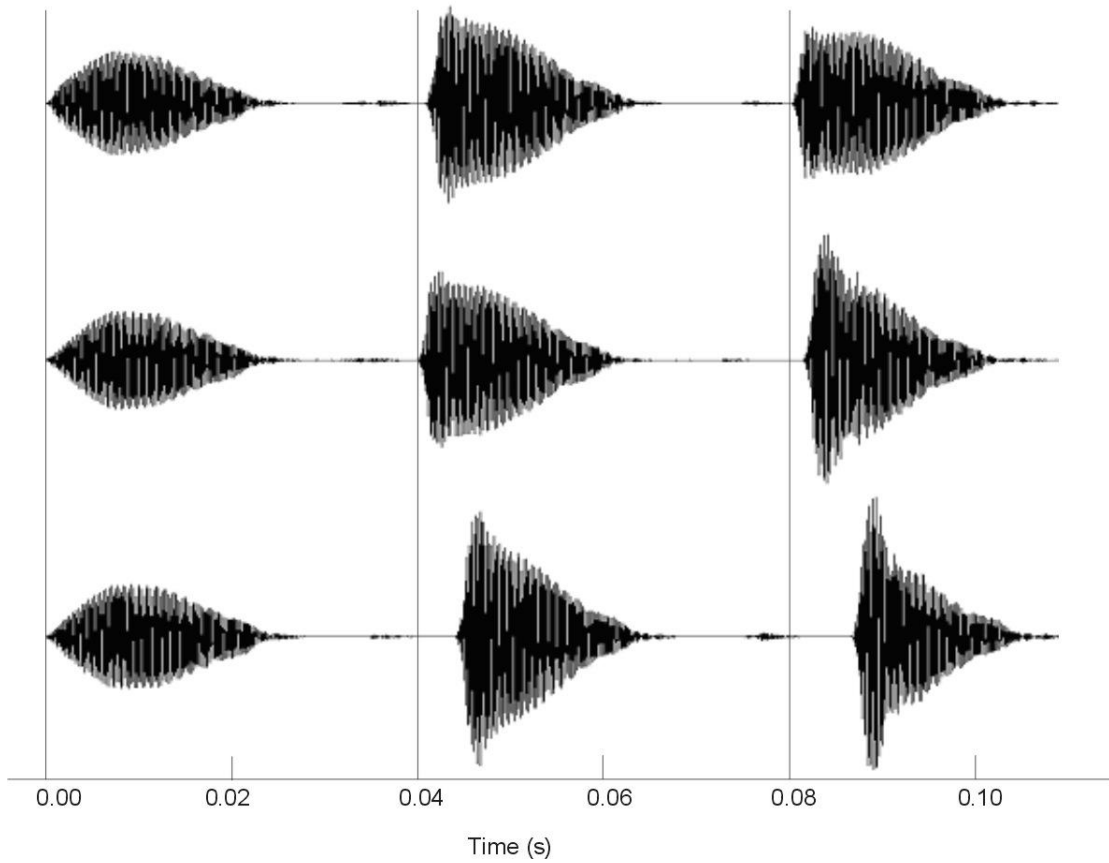
Figure 3.3. Oscillograms of three consecutive calling song chirps of male WPT 13, from Pretoria, indicating the between-chirp variation of syllable period.

Between-year comparisons of body size and call traits of wild-caught males

For the Pretoria data collected during autumn over three years, there was no clear systematic trend of between-year differences in body size (Table 3.2). Following ANOVA, after a Bonferroni adjustment, multiple comparisons revealed that male thorax length was significantly longer in 2001 compared with 2002, whereas Fem-R was significantly shorter in 2001 compared with 2003. There was no significant difference in harp size or any other body size measurement between years. Only two chirp-level measures showed significant between-year differences: males emitted slower calls during 2003 by producing significantly longer inter-chirp intervals and chirp periods in 2003 compared with 2002 (Table 3.2).

Table 3.2. Between-year analysis of the calling song characteristics and morphometric measurements of wild-caught Pretoria males (fixed effects ANOVA) as well as mean (±SE) values. A Tukey HSD *post-hoc* analysis, after a Bonferroni adjustment, indicates significant between-year differences. '*' indicates significance values for $P < 0.05$, while 'NS' indicates non-significant values for $P > 0.05$. '*n*' represents the number of crickets measured for each year

Variable	Mean (SE)			ANOVA	Tukey HSD test		
	2001	2002	2003	*F* statistic	2001-2002	2001-2003	2002-2003
Call characteristics							
n	8	20	15	$F_{2,40}$			
S_C	3.681 (0.159)	3.467 (0.097)	3.526 (0.116)	0.671	NS	NS	NS
CD (s)	0.128 (0.006)	0.118 (0.004)	0.119 (0.004)	0.886	NS	NS	NS
ICI (s)	0.193 (0.021)	0.179 (0.009)	0.22 (0.011)	3.855*	NS	NS	*
CP (s)	0.321 (0.018)	0.297 (0.01)	0.339 (0.012)	3.606*	NS	NS	*
SD (s)	0.027 (0.001)	0.025 (0.0)	0.025 (0.001)	1.837	NS	NS	NS
SP (s)	0.038 (0.001)	0.038 (0.001)	0.037 (0.001)	0.337	NS	NS	NS
ISI (s)	0.011 (0.001)	0.013 (0.0)	0.012 (0.001)	0.907	NS	NS	NS
F_{max} (kHz)	4.774 (0.028)	4.897 (0.033)	4.927 (0.057)	2.235	NS	NS	NS
Fwidth-A (kHz)	0.254 (0.02)	0.307 (0.024)	0.254 (0.021)	1.828	NS	NS	NS
Fwidth-B (kHz)	0.455 (0.033)	0.517 (0.031)	0.439 (0.032)	1.813	NS	NS	NS
Body measurements (mm)							
n	8	20	15	$F_{2,40}$			
Thorax length	4.767 (0.242)	4.175 (0.099)	4.446 (0.098)	4.669*	*	NS	NS
Thorax width	6.841 (0.347)	7.508 (0.204)	7.588 (0.191)	2.155	NS	NS	NS
Thorax area (mm^2)	32.263 (1.556)	31.669 (1.438)	33.878 (1.45)	0.625	NS	NS	NS
n	7	20	12	$F_{2,36}$			
Fem-R	10.975 (0.278)	11.721 (0.19)	12.028 (0.28)	3.273*	NS	*	NS
Wing measurement (mm^2)							
n	8	17	15	$F_{2,37}$			
Harp	16.735 (0.532)	16.594 (0.428)	16.776 (0.468)	0.047	NS	NS	NS

Between-season comparisons of body size and call traits of wild-caught males

Different trends in body size were observed between populations, reflected by results from fixed effects ANOVA. While Pretoria males were significantly smaller in summer (Table 3.3a), Makhado males were significantly smaller in autumn (Table 3.3b) compared with other seasons. Fixed effects ANOVA revealed significant between-season differences in the call traits of both populations. Several spectral and syllable related characteristics of the Pretoria males during summer differed significantly from those of other seasons (Table 3.3a). For Makhado, two syllable traits and one chirp trait differed significantly between the seasons (Table 3.3b). For both populations, the between-season variance component was less than the between-individual and within-individual variance components, affecting both spectral and chirp traits (Table 3.1). However, the between-season variance component contributed largely to the variation in the syllable traits of both populations.

Principal components analyses (PCA) showed that, for both populations, five principal components (eigenvalues > 1) explained more than 85% of the total variation (Table 3.4). The variables contributing most to the existing variation were similar for both the Makhado and Pretoria populations (Table 3.4). The large degree of variation in body size, encountered in the ANOVA's, were clearly reflected in the PCA, since body size contributed significantly to principal components 1 & 2 in both the Pretoria and Makhado populations, representing > 20% of the total variation. For Makhado, the chirp traits contributed largely to principal components 1 & 3, while for Pretoria they largely contributed to principal components 2 & 4, representing some 30% of the total variation for both populations. Syllable traits only contributed to the fifth principal component of both populations that comprised less than 16% of the total variation (Table 3.4). Calling song frequency did not contribute in a measurable way to the total variation. Although seasonal trends were detected in the PCA's there was no distinct seasonal clustering of the R-rotated data.

Table 3.3. Mean values (±SE; *n* if not the same as *n* in the first row) for the call, body and wing characteristic(s) of the respective seasons for male field crickets, *G. bimaculatus*, from (a) Pretoria and (b) Makhado. The *F* statistic and its significance for a fixed effects ANOVA, as well as the results for a Tukey HSD *post-hoc* comparison test, after a Bonferroni adjustment, are presented. Significance values are indicated with asterisks: $P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$, NS = Not significant ($P > 0.05$). '*n*' represents the number of crickets measured for each season. '–' indicates seasons not sampled for Makhado

	Mean (SE; *n* if not the same as *n* in the first row)				ANOVA	Tukey HSD test					
Variable	Summer (S)	Autumn (A)	Winter (W)	Spring (P)	*F* statistic	S-A	S-W	S-P	A-W	A-P	W-P
a											
n	22	43	11	26	$F_{3, 98}$						
S_C	3.557 (0.098)	3.527 (0.067)	3.75 (0.117)	3.497 (0.087)	0.929	NS	NS	NS	NS	NS	NS
CD (s)	0.111 (0.004)	0.12 (0.003)	0.135 (0.004)	0.123 (0.004)	4.661**	NS	NS	NS	NS	NS	NS
ICI (s)	0.178 (0.007)	0.196 (0.007)	0.164 (0.011)	0.205 (0.008)	3.458*	NS	NS	NS	NS	NS	NS
CP (s)	0.289 (0.01)	0.316 (0.008)	0.299 (0.011)	0.328 (0.009)	3.143*	NS	NS	NS	NS	NS	NS
SD (s)	0.024 (0.0)	0.025 (0.0)	0.025 (0.001)	0.026 (0.001)	4.243**	NS	NS	NS	NS	NS	NS
SP (s)	0.034 (0.001)	0.037 (0.0)	0.040 (0.001)	0.038 (0.0)	23.403***	***	***	***	NS	NS	NS
ISI (s)	0.01 (0.0)	0.012 (0.0)	0.014 (0.001)	0.012 (0.0)	5.7**	NS	NS	NS	NS	NS	NS
F_{max} (kHz)	5.378 (0.084)	4.884 (0.027)	4.78 (0.054)	4.883 (0.029)	27.893***	***	***	***	NS	NS	NS
Fwidth-A (kHz)	0.414 (0.029)	0.278 (0.014)	0.298 (0.027)	0.26 (0.016)	11.208***	***	NS	***	NS	NS	NS
Fwidth-B (kHz)	0.646 (0.035)	0.478 (0.02)	0.52 (0.04)	0.457 (0.024)	9.325***	***	NS	***	NS	NS	NS
Thorax length (mm)	3.648 (0.173)	4.379 (0.079)	4.064 (0.098)	4.551 (0.092)	11.794***	***	NS	***	NS	NS	NS
Thorax width (mm)	7.09 (0.176)	7.412 (0.136)	7.525 (0.124)	7.628 (0.123)	1.999	NS	NS	NS	NS	NS	NS
Thorax area (mm^2)	25.539 (1.015)	32.55 (0.882)	30.661 (1.136)	34.728 (0.918)	14.052***	***	*	***	NS	NS	NS
Femur-R (mm)	9.256 (0.203; 21)	11.681 (0.148; 39)	11.716 (0.148; 10)	11.834 (0.143; 22)	48.083***; $F_{3, 88}$	***	***	***	NS	NS	NS
Harp (mm^2)	14.611 (0.264)	16.691 (0.268; 40)	16.614 (0.35)	17.045 (0.272)	12.981***; $F_{3, 95}$	***	NS	***	NS	NS	NS
b											
n	18	21	10	-	$F_{2, 46}$						
S_C	3.741 (0.099)	3.417 (0.093)	3.7 (0.105)	-	3.48*	NS	NS	-	NS	-	-
CD (s)	0.124 (0.004)	0.108 (0.004)	0.132 (0.003)	-	11.39***	NS	NS	-	***	-	-
ICI (s)	0.185 (0.008)	0.187 (0.011)	0.172 (0.016)	-	0.42	NS	NS	-	NS	-	-
CP (s)	0.309 (0.008)	0.295 (0.012)	0.303 (0.016)	-	0.467	NS	NS	-	NS	-	-
SD (s)	0.025 (0.0)	0.024 (0.0)	0.025 (0.001)	-	2.03	NS	NS	-	NS	-	-
SP (s)	0.036 (0.001)	0.034 (0.0)	0.04 (0.001)	-	17.891***	NS	***	-	***	-	-
ISI (s)	0.011 (0.001)	0.011 (0.001)	0.015 (0.001)	-	9.331***	NS	NS	-	***	-	-
F_{max} (kHz)	5.079 (0.033)	5.077 (0.043)	4.908 (0.061)	-	3.644*	NS	NS	-	NS	-	-
Fwidth-A (kHz)	0.315 (0.022)	0.399 (0.034)	0.262 (0.031)	-	4.618*	NS	NS	-	NS	-	-
Fwidth-B (kHz)	0.491 (0.032)	0.607 (0.038)	0.425 (0.043)	-	5.688**	NS	NS	-	NS	-	-
Thorax length (mm)	4.527 (0.111; 19)	4.193 (0.079)	4.778 (0.125)	-	7.386**; $F_{2, 47}$	NS	NS	-	NS	-	-
Thorax width (mm)	7.859 (0.174; 19)	7.36 (0.083)	7.898 (0.139)	-	5.052*; $F_{2, 47}$	NS	NS	-	NS	-	-
Thorax area (mm^2)	35.88 (1.627; 19)	30.947 (0.838)	37.86 (1.548)	-	6.801**; $F_{2, 47}$	*	NS	-	**	-	-

Table 3.3b (continued)

Fem-R (mm)	12.45 (0.241; 19)	11.789 (0.214; 20)	12.147 (0.21)	-	2.384	NS	NS	-	NS	-	-
Harp (mm^2)	17.016 (0.447; 19)	15.849 (0.222)	17.674 (0.525; 9)	-	5.382**	NS	NS	-	NS	-	-

Table 3.4. Principal component analyses, with varimax normalised rotation, of seasonal variation in call traits and morphometric measurements of wild male crickets from Makhado (M) and Pretoria (P). Loadings indicated by 'M' and 'P' are > 0.700 for the respective geographical site. Calling song frequency did not contribute in a measurable way to the variation in either of the data sets

Variable	PC1	PC2	PC3	PC4	PC5
S_C		P	M		
CD		P	M		
ICI	M			P	
CP	M			P	
SD					P
SP					M
ISI					M, P
Fwidth-A			P	M	
Fwidth-B			P	M	
Thorax area	P	M			
Fem-R	P	M			
Harp	P	M			
Total variance explained % (M)	16.0	22.7	15.7	17.4	19.9
Total variance explained % (P)	24.90	16.40	18.10	16.70	12.00

Body size as a factor explaining variation in the call traits of wild-caught males

The ANCOVA revealed a significant seasonal effect ($P < 0.05$, $n = 102$) on all the call traits except for number of syllables per chirp. The only significant effect of body size was on calling song frequency ($F_{3,\ 100} = 126.65$, $P < 0.001$, $n = 102$). The ANOVA revealed that Pretoria males were smaller during summer than during the other seasons (Table 3.3a), while none of the remaining three seasons differed significantly with respect to any of the variables analysed below. For this reason the summer data were omitted from the correlation and regression analyses.

Although there was a highly significant correlation ($P < 0.01$ in all cases, $n = 56$) between harp size and several body size measurements (head length: r = 0.347, head width: r = 0.606, thorax width: r = 0.671, thorax length: r = 0.457, thorax area: r = 0.76, Fem-R: r = 0.575, Tib-R: r = 0.5, Fem-L: r = 0.501 and Tib-L: r = 0.398), the relationships between body size and call traits were much more tenuous. Both calling song frequency and inter-syllable interval showed a significant inverse relationship with harp size (F_{max}: $F_{1,\ 75} = 10.028$, $P = 0.002$, $n = 77$; ISI: $F_{1,\ 75} = 5.007$, $P = 0.028$, $n = 77$). Harp size explained 10.6% of the variation in calling song frequency and 5% of the variation in inter-syllable interval. There was a significant inverse correlation between thorax area and calling song frequency ($F_{1,\ 78} = 7.504$, $P = 0.008$, $n = 80$), with thorax area explaining 8% of the variation in calling song frequency (Figure 3.4). Forward stepwise regression analysis revealed that harp size contributed more to the variation in calling song frequency (partial correlation = -0.343, $P = 0.002$, $n = 77$) than did thorax width or thorax area.
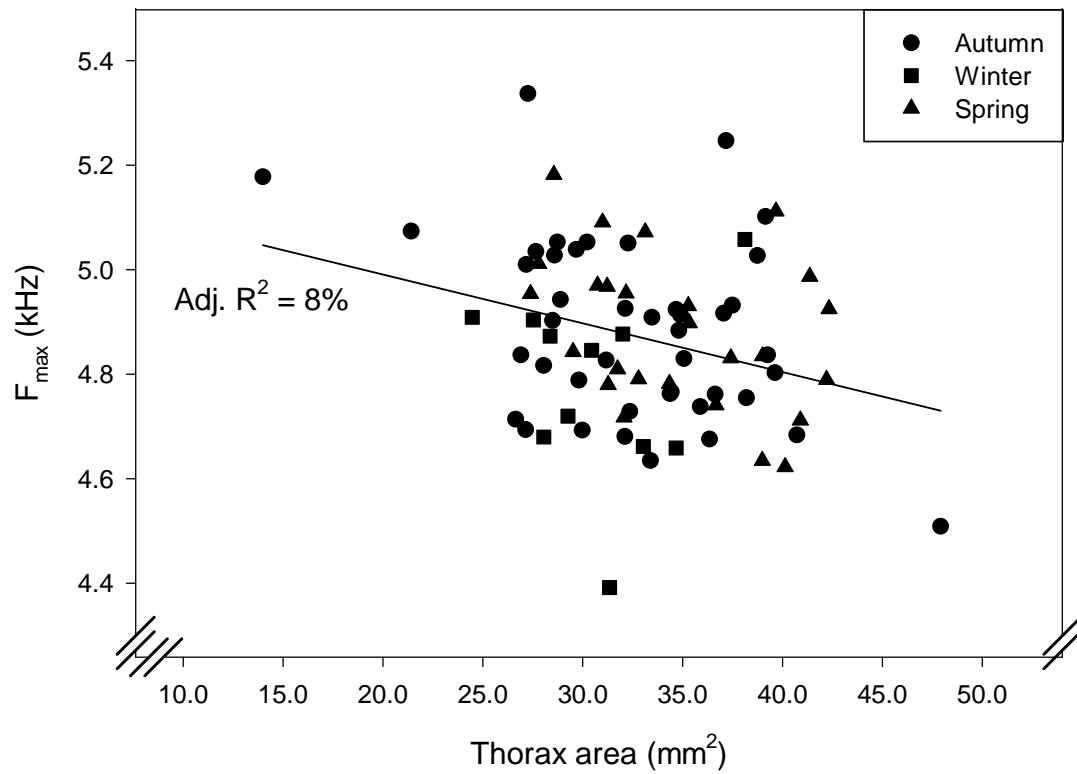
Figure 3.4. Scatter diagram indicating the relationship between calling song frequency (F_{max}) and thorax area for the Pretoria males for three different seasons of the year.

Effect of rearing temperature on body size of laboratory-reared crickets

There was a mortality of 100% at the 15°C treatment before the second instar was reached, therefore no useful data were obtained for this treatment. At 22°C the survival rate was 13%, with four males and six females reaching adulthood across the three breeding lines. At 27°C the survival rate was much higher at 47%, with 16 males and 19 females that reached adulthood across the three breeding lines.

Body size of crickets raised at 27°C was larger than for those raised at 22°C (significant effects for leg measurements and body mass; Table 3.5). Two-way fixed effects ANOVA revealed a strong treatment effect with respect to age at adult eclosion as well as a sex-specific treatment effect in body size (Table 3.5). Except for head length and right tibia at 22°C, females were always larger than males across both treatments. This effect was larger at 27°C, although only one of the size measurements showed a significant interaction effect, indicating a significantly stronger between-sex difference at the higher temperature. Females at 22°C were younger at adult eclosion than males at the same treatment (Table 3.5). Male harp size did not differ significantly between 22°C and 27°C (Table 3.5).

Table 3.5. Mean values (±SE) for the body and wing characteristic(s) of the males and females at adult eclosion for rearing environments at 22°C and 27°C. The *F* statistic and its significance for a two-way fixed effects ANOVA for the treatments (temperature (temp) and sex) and the interaction between them (temp-sex) are presented. Significance values are indicated as follows with asterisks: *P* < 0.05*, *P* < 0.01**, *P* < 0.001***. '*n*' represents the number of crickets measured

Variable	Mean (SE) 22°C Males	22°C Females	27°C Males	27°C Females	Two-Way ANOVA Temp *F* statistic	Sex *F* statistic	Temp-sex *F* statistic
Body measurements (mm)							
n	4	6	16	19	$F_{1, 41}$	$F_{1, 41}$	$F_{1, 41}$
Thorax length	3.672 (0.114)	4.036 (0.138)	3.699 (0.131)	4.407 (0.075)	1.805	13.132***	1.349
Thorax width	6.25 (0.048)	6.38 (0.174)	6.433 (0.229)	7.088 (0.105)	3.552	2.749	1.237
Thorax area (mm²)	22.954 (0.807)	25.856 (1.508)	24.215 (1.671)	31.362 (0.963)	3.321	7.322**	1.307
Head length	3.941 (0.157)	3.471 (0.09)	3.92 (0.146)	3.884 (0.105)	1.269	2.108	1.553
Head width	5.175 (0.062)	5.517 (0.237)	5.523 (0.196)	5.825 (0.079)	2.534	2.431	0.009
Mass (g)	0.5 (0.04)	0.613 (0.05)	0.592 (0.053)	0.852 (0.052)	5.046*	6.404*	0.994
n	4	6	16	18	$F_{1, 40}$	$F_{1, 40}$	$F_{1, 40}$
Fem-R	9.427 (0.163)	9.941 (0.277)	10.169 (0.268)	11.336 (0.162)	12.262**	7.589**	1.14
n	4	4	12	18	$F_{1, 34}$	$F_{1, 34}$	$F_{1, 34}$
Fem-L	9.441 (0.342)	9.564 (0.323)	10.225 (0.351)	11.359 (0.136)	14.425***	3.418	2.214
n	4	6	14	16	$F_{1, 36}$	$F_{1, 36}$	$F_{1, 36}$
Tibia-R	7.24 (0.172)	6.873 (0.163)	7.43 (0.225)	8.37 (0.188)	9.89**	1.139	5.941*
n	4	5	11	17	$F_{1, 33}$	$F_{1, 33}$	$F_{1, 33}$
Tibia-L	7.083 (0.243)	7.256 (0.141)	7.724 (0.78)	8.54 (0.143)	15.936***	4.249*	1.776
Wing measurement (mm²)							
n	4		14		$F_{1, 16}$		
Harp	10.255 (0.573)		11.714 (0.635)		1.37		
Age at adult eclosion (days)							
n	4	6	16	19	$F_{1, 41}$	$F_{1, 41}$	$F_{1, 41}$
Age	191.5 (10.137)	167.667 (9.912)	73.438 (3.673)	79.842 (2.366)	350.173***	2.509	7.553**

Discussion

For both populations, PCA indicated that the frequency and syllable call traits contributed little to the total variation in the normalised data set (Table 3.4). Conversely, the chirp call traits and morphological characteristics contributed largely to the observed variation. This is consistent with the conclusion of Ferreira & Ferguson (2002) that several call traits involved in mate recognition have relatively little variance and may be subject to stabilizing selection.

Between-individual differences

Taking into account that between-individual variance in the calling song of both populations results mostly from both general environmental and additive genetic causes, the between-individual differences in this study are suggestive of the maximum heritability of a trait (Falconer & Mackay, 1996). This implies that call traits with large between-individual variation and small within-individual variation (Table 3.1) could potentially be subject to sexual selection.

One of the obvious between-individual differences was body size. This gives rise to the question of how this factor contributed to between-individual differences in call traits. This is especially important since some workers inferred that female crickets can judge the size of a male through his calling song (Gray, 1997; Simmons, 1988). This study showed that large males have large harp sizes and produced shorter inter-syllable intervals and lower calling song frequencies (Figure 3.4). However, the proportion of variation in calling song frequency and inter-syllable interval explained by male size is extremely low. This study found no significant effect of body size on any chirp trait, opposing the findings of Ferreira (2006, Chapter 2). The low correlation between body size and calling song frequency could probably be explained by the indirect effect of body size on harp size that, in turn, correlates with calling song frequency. The utility of calling song frequency for indicating male size is thus limited and body size is not a major determinant of between-individual differences in calling song characteristics. This study could not repeat the conclusions of Simmons (1988) or Simmons & Zuk (1992) that syllable rate and chirp duration correlate with male size. These relationships are highly variable, evident from Brown *et al.* (1996) who found a significant inverse correlation in *O. nigricornis* between calling song frequency and body size, Gray (1997) who found a positive correlation between male size and

number of syllables per chirp in *A. domesticus* and Wagner & Hoback (1999) who did not find any correlations in *G. lineaticeps.*

Within-individual differences

Within-male variation, measured here as temporal variation in calling song characteristics, results from variation in either the environment or of the individual insect (Falconer & Mackay, 1996). Environmental variance in signals acts as noise within a system of selection on the communication system. The high levels of within-individual variation in the chirp traits (Table 3.1; Figure 3.3) limits the heritability and increases the error in intersexual communication using these traits within a framework of sexual selection.

For both populations, within-individual variation largely comprised between-night and between-chirp differences in the calling song, with less variation between repeats within a recording and between recordings. However, the main sources of variation differed between populations. For Pretoria, syllable period and calling song frequency varied less between nights than between chirps within a recording (Figure 3.2b). Conversely, for Makhado, the between-night component of variation contributed largely to within-individual variation (Figure 3.2a). Overall, calling song frequency and syllable period, important for species recognition (Huber & Thorson, 1985; Schildberger *et al.*, 1989), had a small degree of within-individual variance (Table 3.1).

Seasonal differences

There were significant between-season differences in male size and call traits (Table 3.3a). Male size and harp size were the smallest in the summer samples. For Pretoria, the between-season variance component explained a significant proportion of the variation in the syllable and spectral traits (Table 3.1). Calling song frequency was significantly higher during summer than during winter, consistent with the results of Van Wyk & Ferguson (1995), who found that males produced a lower calling song frequency during winter than autumn. In addition, syllable period during the present study was significantly shorter in summer, leading to rapid syllable rates. This could be explained by the temperature effect on the syllable and chirp characteristics. Doherty (1985) found that *G. bimaculatus* males produced shorter syllable and chirp periods at warmer temperatures. Males of three *Oecanthus* species produced faster chirp rates at warmer

temperatures than at cooler temperatures (Toms, 1992) and *Laupala cerasina* males produced significantly faster syllable rates at warmer temperatures than at cooler temperatures (Grace & Shaw, 2004).

Since cricket abundance varies temporally, reflecting environmental conditions such as rainfall and food availability, wild crickets could not be obtained for all consecutive seasons over the time period of this study. Therefore, some of the seasonal variation might be explained by medium-term temporal variation rather than fixed seasonal variation. This medium-term temporal variation could be caused by environmental conditions that the cricket experiences, such as food availability. Notwithstanding the distinction between fixed seasonal effects or less regular medium term effects, the temporal variation in calling song did not last from one year to the next.

If one extrapolates from the Pretoria crickets (which did not differ between years), the temporal calling song differences for Makhado (Table 3.3b) were probably due to seasonal differences. Both syllable and chirp traits of Makhado males differed significantly between seasons, with no significant between-season differences in body size (Table 3.3b). Significantly longer inter-syllable intervals during the winter sample caused longer syllable periods during winter. Overall, males from Makhado called at a slower chirp rate during the winter sample. This is consistent with the seasonal results from Pretoria. However, firm temporal interpretation of the data from Makhado is obscured by the irregular temporal structure of the samples.

Although the call characteristics important for species-specific communication differed between seasons, they contributed less to the variation than the chirp traits and the body measurements, evident from the PCA (Table 3.4). Seasonal variation is probably environmentally induced, since the genetic composition of a population remains more or less the same over short time spans. The between-season differences in the calling song were probably related to the local environment. Indeed, Pretoria males were the smallest during summer (Table 3.3a). If the environment (e.g. cricket density, food availability) affects body size, it may have an indirect effect on calling song characteristics. Since male size affects harp size which, in turn, correlates with calling song frequency, it is possible that the higher calling song frequency produced in summer was a consequence of the smaller body size in summer (Table 3.3a). However, it is unlikely that body size is singly important as it was only weakly correlated with the

call characteristics (Figure 3.4). For both populations syllable period was slower during winter than summer. This is probably explained by the colder temperatures experienced by these crickets during winter compared with summer. Indeed, *L. cerasina* males recorded at a constant temperature produced faster syllable rates when kept at warmer temperatures than at colder temperatures (Grace & Shaw, 2004). What remains is to have a better understanding of the relationship between body size and temperature to explain the relationship between body size and the calling song.

Body size effects due to temperature differences

Sibly & Atkinson (1994) predicted that ectotherms should develop faster and have a smaller body size at warmer temperatures under certain conditions. This study found that males and females developed faster at the warmer rearing temperature (Table 3.5). While females at the higher temperature had a larger body size, there was a smaller effect of rearing temperature on male body size. For males and females, development time from egg to adult was similar at each rearing temperature (Table 3.5). This suggests that between-season differences in male size are probably not temperature related but are rather affected by factors such as food availability, population density or other environmental factors. These results indicate that the seasonal differences in calling song characteristics of *G. bimaculatus* are not explained by a simple temperature-dependent effect of body size.

Annual differences

The Pretoria population was temporally stable in body size and call traits for autumn across three years. Only two chirp-level call traits differed between years (Table 3.2), whereas the syllable and spectral traits, important for communication, showed consistency over consecutive years. Harp size, an important correlate of calling song frequency, did not differ between years. Seasonal effects were more marked than between-year effects, indicating that across years the seasonal influence on the calling song probably remains the same.

Conclusion

Short-term variation in the sexually selected calling song characteristics possibly compromises the communication system. However, this effect could be negated,

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firstly, due to the parallel coupling of female preferences to the male signaling system at different temperatures (Doherty, 1985; Grace & Shaw, 2004) and secondly, due to wide preference ranges of females (L. Verburgt, personal communication). The absence of large differences in male size between two rearing temperatures suggests that rearing temperature is not an important determinant of male size. Due to the weak correlations between male size and the calling song characteristics and due to the large degree of between-night variation in the call traits within males, females probably cannot use the male calling song as a reliable indicator of male size-based quality.

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