University of Pretoria etd Ferreira M 2006

CHAPTER 5

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

Abstract

I investigated geographical variation in the calling song and morphometrics of ten wild-caught and six captive-reared F_1 African and European populations of the field cricket, *Gryllus bimaculatus*. Inter-continental and regional variation in the calling song and morphometrics of *G. bimaculatus* is probably not explained by an isolation-by-distance effect. The call and morphological traits differ more within-regions than between-continents, reflecting the large amount of gene flow that occurs among the continents. A significant part of the geographical variation is probably environmentally induced, based on the following findings: (a) except for the Queenstown population, between-population differences observed in wild-caught populations are not evident in captive-reared F_1 populations, (b) captive-reared F_1 populations have smaller degrees of variances in their phenotypic traits and (c) there is no correlation of traits from wild-caught populations with traits from their captive-reared F_1 offspring. The large degree of between-population differences in the calling song of *G. bimaculatus* can compromise the communication system in this cricket. It is therefore suggested that female preference studies be conducted to investigate the evolutionary implications of between-population differences of the male calling song of *G. bimaculatus*.

Keywords: *Gryllus bimaculatus*, Calling song, Morphology, Inter-continental variation, Between-population variation, Environmental effects, Communication system

Introduction

Calling songs, emitted by male crickets, are species-specific, under neurological control (Bennet-Clark, 1989; Huber, 1962; Huber & Thorson, 1985; Schildberger *et al*., 1989) and elicit phonotaxis by females (Alexander, 1962). Despite the importance of the calling song in mate recognition, there is significant within-male, within-population and between-population variation in the calling song of the field cricket, *Gryllus bimaculatus* (Ferreira, 2006a, b, Chapters 2 & 3; Ferreira & Ferguson, 2002). Several authors have suggested that comparative information about the between-individual variability and between-population variability in calls across a species range is needed (Asquith *et al*., 1988; Zuk *et al*., 2001) and Zuk *et al*. (2001) emphasized that geographical variation is one of the most important sources of variation. The calling song is regarded as one of the most important premating barriers in crickets (Otte, 1989), and it is therefore important to have an estimate of between-population variability in this song across a species range. If differences in the sexual signaling system is due to between-population genetic differences, it could give rise to prezygotic isolation and one needs to understand the mechanisms underlying geographical differentiation in this system (Butlin, 1996). In addition, evolutionary forces act on communication systems (Ryan & Wilczynski, 1988) and Ryan & Rand (1995) suggested that it is important to have an understanding of signal variation and its influence on the behavioural response of the receiver. According to Endler & Houde (1995), geographical variation in the calling song could give rise to correlated female preferences, but Zuk *et al*. (2001) suggested that rapidly evolving populations with different signals can resist differentiation if gene flow among populations is maintained. However, this was not the case in the planthopper, *Nilaparvata lugens*, where genetic drift was suggested to be the most probable cause for male signal-female preference divergence (Butlin, 1996). According to Paterson (1985), there should be stabilizing selection on call traits involved in mate recognition. Geographical variation in these traits should be environmentally induced and not genetically induced (Butlin, 1995), since these traits are under stabilizing selection while a species occupies its normal habitat (Paterson, 1985).

Geographical variation in advertisement calls of anurans and crickets could be explained by isolation by distance effects, different environmental conditions as

well as between-population genetic differences. An isolation by distance effect was the main cause of geographical variation in the advertisement song of the túngara frog, *Physalaemus pustulosus*, (Ryan *et al*., 1996) and the mating call of the green treefrog, *Hyla cinerea* (Asquith *et al*., 1988). Different environmental conditions (open vs. forest habitat) experienced by populations of the cricket frog, *Acris crepitans*, explained between-population differences in call traits (Ryan & Wilczynski, 1991). Between-population genetic differences were the main cause of clinal variation in the advertisement call of *A*. *crepitans* (Ryan & Wilczynski, 1991) and geographical variation in the calling song of the field cricket, *Teleogryllus oceanicus* (Zuk *et al*., 2001). However, some studies could not explain geographical variation found in some frog and cricket advertisement calls. Geographical variation in the advertisement call of the frog *Leptodactylus fuscus* did not coincide with genetic variation or geographic distance (Heyer & Reid, 2003) and it is unclear whether the large degree of geographical variation found in the syllable number of the calling song of the bushcricket, *Ephippiger ephippiger*, is environmentally or genetically induced (Ritchie, 1992).

Ferreira & Ferguson's study (2002) on geographical variation in the calling song of *G. bimaculatus* mainly focused on the influence of geographical variation on species recognition and sexual selection call traits, and did not provide any explanation for between-population differences in the call traits or morphometrics. Their study only included four populations from South Africa. This study, on the contrary, investigates geographical variation in the calling song and morphometrics of *G*. *bimaculatus* on an inter-continental and regional scale by investigating wild-caught and captive-reared crickets from two European regions and eight South African localities.

The aims of this study are:

1. To relate geographical variation in the calling song and morphometrics of *G. bimaculatus* to geographic distance between populations as well as between-population genetic differences and gene flow estimates, following the results of Ferreira (2006c, Chapter 4).
2. To infer to what degree the between-population differences in the calling song and morphometrics of field crickets could be environmentally induced by comparing the variances in the calling song characteristics and morphometrics

of wild-caught populations from South Africa and Europe with their captive-reared F_1 offspring.

Materials and methods

Geographical variation in calling song characteristics and morphometric measurements of ten wild-caught populations

Sampling localities

During 2001 – 2003, wild field crickets, *G. bimaculatus*, were captured in South Africa (Figure 5.1a) at Dullstroom, Dundee, Hotazel, Makhado, Paarl, Pretoria, Queenstown and Wolmaransstad (27°23'S, 26°00'E). During 2003, wild field crickets were captured in Italy at Genova, Modigliana, Peccioli and Russi (Figure 5.1b) and in Spain at Sevilla (Figure 5.1b). Longitudes and latitudes of these localities are provided in Ferreira (2006c, Chapter 4). Due to the small sample sizes at each of the Italian localities, the four Italian populations were combined to form one Italian sample.

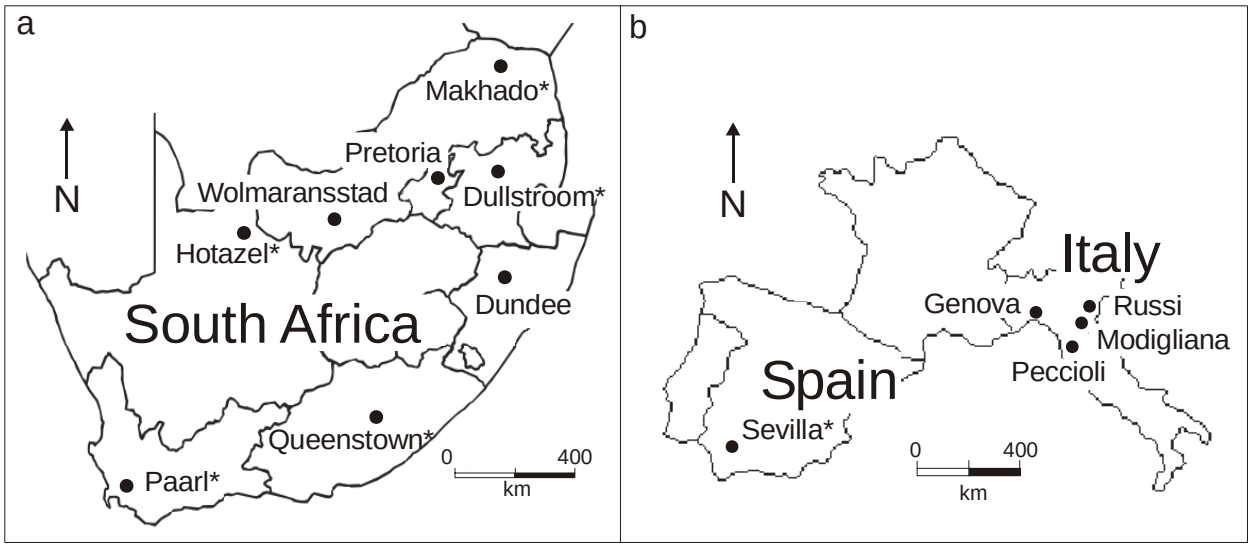


Figure 5.1. Sampling localities of wild-caught *G. bimaculatus* in (a) South Africa and (b) Europe. '*' indicates the localities of wild-caught crickets for which captive-reared F_1 offspring were reared.

Calling song recordings

Males were placed in sound damped recording chambers where the calling song of each male was recorded. 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 and morphometric measurements

Canary V1.2.4 (Cornell Laboratory of Ornithology, Ithaca, New York) was used on an Apple Macintosh computer 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 traits see Ferreira (2006a, Chapter 2). Three consecutive chirps were measured from each recording. A mean was calculated for the six recordings for each of the call traits. Each chirp comprised three to six syllables (Desutter-Grandcolas & Robillard, 2003). Calling songs of 314 males were recorded as described above (Dullstroom: *n* = 24, Dundee: *n* = 17, Hotazel: *n* = 16, Italy: *n* = 9, Makhado: *n* = 49, Paarl: *n* = 30, Pretoria: *n* = 102, Queenstown: *n* = 28, Wolmaransstad: *n* = 19 and Sevilla: *n* = 20). After killing a male with ethyl acetate its head length, head width, thorax length, thorax width, thorax area (thorax length x thorax width), length of the right hind femur (Fem-R) and length of

the right hind tibia (Tib-R) were measured using the VideoBlaster FS200 video kit (Creative Laboratories, Singapore; resolution = 16 microns). After the body measurements were taken, the wings of each male were removed and three mirror measurements (M4_M8, M6_M8 and H1_M9) and five harp measurements (H1_H2, H2_H3, H1_H3, H10_H11 and harp size) of each right wing were measured following Ferreira & Ferguson (2002) and Ferreira (2006a, Chapter 2).

Geographical variation in calling song characteristics and morphometric measurements of six captive-reared F_1 populations

Separate breeding colonies of six wild caught populations (Dullstroom, Hotazel, Makhado, Paarl, Queenstown and Sevilla) were established in a climate room at 27°C ± 2°C with a 12h:12h L:D light regime. Each breeding colony was maintained in a 100-litre plastic container and were provided with egg cartons for shelter, petri dishes filled with cotton wool serving as egg laying trays, high protein food (Pronutro® and fish flakes) and water *ad libitum*. The egg laying trays of each colony were collected on a weekly basis and placed separately in labeled 2-litre plastic containers. Each breeding colony's F_1 first instar nymphs were transferred to a 100-litre plastic container and reared until final instar was reached. Twenty-five of these penultimate instar males were randomly chosen from each of the F_1 colonies and placed individually in labeled 2-litre plastic containers. Males were checked daily and their calling songs were recorded ten days after final ecdysis.

Calling songs of 118 males were recorded as described above (Dullstroom: n = 22, Hotazel: n = 14, Makhado: n = 18, Paarl: n = 23, Queenstown: n = 21 and Sevilla: n = 20). After obtaining the recordings, call traits and morphometric measurements were measured as described above.

Statistical analyses

Analyses were performed on two separate data sets. The first data set comprised wild-caught crickets and the second comprised captive-reared F_1 crickets. Unless indicated otherwise, the wild-caught data set comprised ten populations and the captive-reared F_1 data set comprised six populations.

SAS V8.02 (Proc GLM; SAS Institute Inc., Cary, NC, USA) was used to perform a univariate analysis of variance (ANOVA) to compare the means of the

call traits as well as the means of five morphometric measurements (thorax area, thorax length, thorax width, Fem-R and harp size) between the populations. Tukey HSD *post-hoc* analyses were performed to determine which populations differed significantly from one another. A Bonferroni adjustment (Rice, 1989) was applied to the Tukey HSD *post-hoc* results of both data sets. A random effects ANOVA (Proc Varcomp; SAS) was performed on the wild-caught data set to determine the between-continent, within-continent and error variance components for the call and morphological traits used in the univariate ANOVA. Principal components analysis (PCA: varimax rotation on normalised data) was used, with Statistica V5.5 (StatSoft, Inc. (1999), Tulsa, USA), to determine which call traits and morphometric measurements (excluding thorax length and thorax width, due to the inclusion of thorax area) contributed most to the total variation of each data set. Forward stepwise discriminant function analysis (DFA), with Statistica, was used to determine which call traits could be used to discriminate between populations.

A cluster analysis was performed in Statistica to create Euclidian distance matrixes (Sokal & Sneath, 1963) for both data sets utilizing either ten call traits and five morphometric measurements (thorax area, thorax width, thorax length, Fem-R and harp size) or only ten call traits. Distance matrixes of the ten wild-caught and six F_1 populations were then compared with geographic distances between populations (in km) using a Mantel test (Mantel, 1967), within Mantel V2.0 (Liedloff, 1999) with 1000 permutations. These Mantel tests were performed to determine whether crickets from closely located populations have more similar call structures and morphometrics than crickets that are located further apart. A Euclidean distance matrix was then created for nine wild-caught populations (Dullstroom, Dundee, Hotazel, Makhado, Pretoria, Paarl, Queenstown, Italy and Sevilla). The Wolmaransstad population was excluded from this specific data set, since this population was not included in the genetic study of Ferreira (2006c, Chapter 4). The distance matrixes and F_{ST} statistics (Tamura-Nei distance parameter: Ferreira, 2006c, Chapter 4) of nine wild-caught and six F_1 populations were compared respectively, using Mantel with 1000 permutations, to determine whether populations with large amounts of between-population gene flow have crickets more similar in call structure and morphometrics than populations with smaller amounts of between-population gene flow.

Following the results of the univariate ANOVA, the overall variance and within-population variance were calculated. Overall variance was calculated for all individuals across all populations. A random effects ANOVA was performed to determine the between-population variance and within-population variance component for the call traits (Proc Varcomp; SAS).

A two-way fixed effects ANOVA was performed on each of the ten call traits and five morphometric measurements, with Statistica, of the six wild-caught populations and their six captive-reared F_1 populations, to determine the effect of locality and captivity on these traits. This was followed by Tukey HSD *post-hoc* comparisons. A Bonferroni adjustment (Rice, 1989) was applied to the significant ($P < 0.05$) *post-hoc* comparisons.

A Pearson product moment correlation was performed, with Statistica, to determine any correlation of the call traits and five morphometric measurements of six wild-caught populations (Dullstroom, Hotazel, Makhado, Paarl, Queenstown and Sevilla) with their captive-reared F_1 offspring.

Results

Ten wild-caught populations

Between-population differences in call traits and morphology of wild-caught males

Univariate ANOVA showed that there were significant between-population differences for all the morphometric measurements and the majority of the call traits (Table 5.1). Tukey *post hoc* comparison, after a Bonferroni adjustment, revealed that the call traits differed significantly among several South African populations, while the morphometric measurements mainly differed significantly between the South African and Italian populations (Table 5.2). Wild-caught populations from the northeastern part of South Africa differed significantly from those in the southwestern parts (Table 5.2). These patterns are consistent with gene flow estimates (Ferreira, 2006c, Chapter 4). For the chirp traits, the Queenstown and Paarl populations differed significantly from the other South African populations. The Queenstown population had a significantly shorter chirp duration and the significantly longer inter-chirp interval of the Paarl population gave rise to the significantly longer chirp period of this population (Figure 5.2). For the syllable and spectral call traits, the Hotazel population differed significantly from most of the other populations, while the Queenstown population differed

significantly from all of the other populations. The Hotazel population produced a significantly longer syllable duration that gave rise to the significantly longer syllable period compared with the shorter syllable periods of the other populations (Figure 5.2). On the other hand, the Queenstown population produced a significantly shorter syllable duration that gave rise to the significantly shorter syllable period (Figure 5.2). While the Queenstown population produced a significantly higher calling song frequency than the other populations, the Hotazel population produced a significantly lower calling song frequency (Figure 5.2). For the morphometric measurements, the Italian population had a significantly larger body size than the other populations, while the Queenstown and Paarl populations had significantly smaller harp sizes compared with the other populations (Figure 5.2).

Between-continent variation and within-continent variation in call traits and morphology of wild-caught males

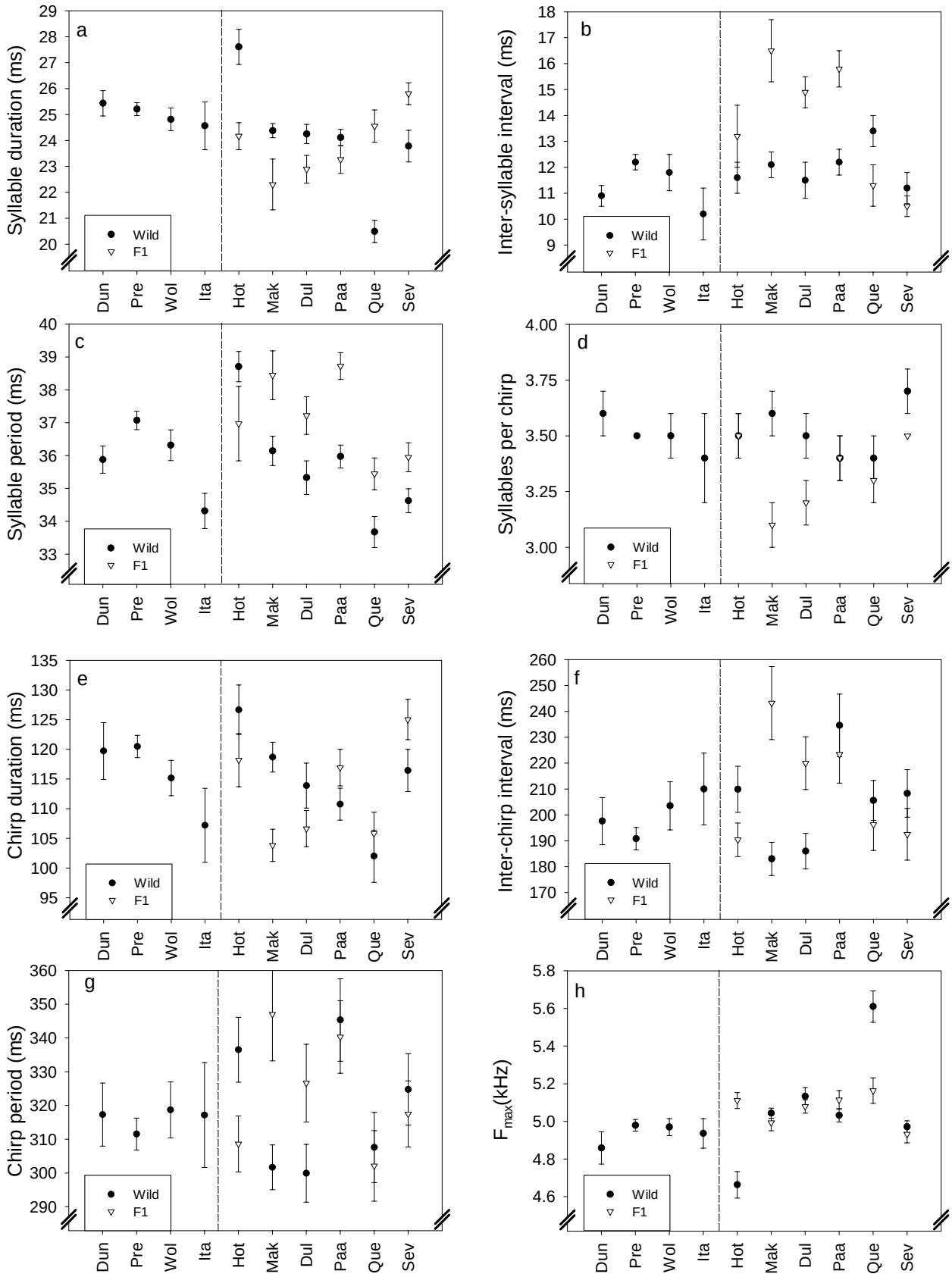
Only bandwidth and inter-syllable interval had a larger between-continent than within-continent variance component (Table 5.1). The within-population variance component (i.e. error variance %) contributed the most to the variation in the call and morphological traits (Table 5.1).

Table 5.1. Results of a univariate ANOVA and a random effects ANOVA of call traits and morphological measurements of *G. bimaculatus* from South Africa and Europe. The univariate ANOVA was performed on two data sets, the first comprised ten wild-caught populations (10 Wild) and the second comprised six captive-reared F_1 populations (6 Captive). Significance values are indicated by asterisks: $P < 0.05$ *, $P < 0.01$ ** and $P < 0.001$ ***. The random effects ANOVA was performed on ten wild-caught populations

	Univariate ANOVA		Random effects ANOVA		
	F statistic		Variance components (10 Wild)		
	10 Wild	6 Captive	Between continents %	Within continents %	Error %
Variable	$F_{9,311}$	$F_{5,112}$			
S_C	1.03	4.03**	0.00	0.28	99.72
CD (s)	3.91***	6.41***	0.00	8.54	91.46
ICI (s)	3.82***	3.575**	0.00	8.77	91.23
CP (s)	2.55**	2.47*	0.00	4.13	95.87
SD (s)	14.91***	4.29**	0.00	24.13	75.87
SP (s)	7.99***	4.85***	0.66	17.21	82.13
ISI (s)	1.65	9.22***	5.85	0.28	93.87
F_{max} (kHz)	18.07***	3.056*	0.00	24.13	75.87
Fwidth-A (kHz)	2.72**	1.322	7.72	2.79	89.49
Fwidth-B (kHz)	2.37*	1.053	9.41	1.66	88.94
Thorax area (mm^2)	5.88***	9.74***	0.00	8.45	91.55
Thorax length (mm)	3.42***	9.22***	0.60	4.68	94.72
Thorax width (mm)	5.69***	7.22***	0.00	7.68	92.32
Femur-R (mm)	6.08***	$F_{5,109}$: 6.56***	5.46	17.00	77.53
Harp (mm^2)	6.87***	$F_{5,110}$: 3.52**	0.00	13.69	86.31

Table 5.2. Results of Tukey *post-hoc* comparison tests, after Bonferroni adjustments, showing populations that differed significantly ($P < 0.05$) for each of the variables subjected to the univariate ANOVA in Table 5.1. To save space the following abbreviations are used and only apply here: traits are referred to as being related to chirps (prefix C), frequency (prefix F), syllables (prefix S) or morphology (prefix M): chirp traits = C_1: S_C, C_2: CD, C_3: ICI, C_4: CP; syllable traits = S_1: SD, S_2: SP, S_3: ISI; frequency traits = F_1: F_{max}; morphometric traits = M_1: thorax area, M_2: thorax length, M3: thorax width, M_4: Fem-R, M_5: harp size. Comparisons above the diagonal represent comparisons between ten wild-caught populations, while comparisons below the diagonal represent comparisons between six captive-reared F_1 populations of *G. bimaculatus*. 'NS' indicates non-significant comparisons, $P > 0.05$. '-' indicates comparisons that could not be made due to the absence of captive-reared F_1 crickets of these populations

	Dullstroom	Hotazel	Makhado	Paarl	Queenstown	Sevilla	Dundee	Pretoria	Wolmaransstad	Italy
Dullstroom		S_1, S_2, F_1	M_4	C_3, M_5	S_1, F_1, M_5	NS	NS	NS	NS	M_1, M_2, M_4
Hotazel	NS		S_1, F_1	S_1, F_1	C_2, S_1, S_2, F_1	S_1, S_2	NS	F_1	NS	S_2
Makhado	NS	C_1, C_3		C_3, C_4, M_1, M_3, M_5	C_2, S_1, S_2, F_1, M_1, M_5	NS	NS	M_4	NS	NS
Paarl	NS	NS	NS		S_1, F_1	NS	NS	C_3, M_5	M_1	M_1, M_3, M_5
Queenstown	NS	M_1, M_2, M_3	C_3, S_2, S_3, M_1, M_2, M_3, M_4	S_2, S_3, M_1, M_2, M_3, M_4		S_1, F_1	S_1, F_1	C_2, S_1, S_2, F_1, M_5	S_1, F_1, M_1, M_5	S_1, F_1, M_1, M_2, M_3, M_5
Sevilla	C_2, S_1, S_3	S_3, M_1, M_3	C_1, C_2, C_3, S_1, S_3, M_2	S_2, S_3, M_1, M_2, M_3, M_5	C_2, F_1		NS	S_2	NS	M_1, M_3
Dundee	-	-	-	-	-	-		NS	NS	M_1, M_3
Pretoria	-	-	-	-	-	-	-		NS	M_1, M_4
Wolmaransstad	-	-	-	-	-	-	-	-		NS
Italy	-	-	-	-	-	-	-	-	-	


a
Syllable duration (ms)
b
Inter-syllable interval (ms)
c
Syllable period (ms)
d
Syllables per chirp
e
Chirp duration (ms)
f
Inter-chirp interval (ms)
g
Chirp period (ms)
h
F_{max}(kHz)
Wild
F1
Dun
Pre
Wol
Ita
Hot
Mak
Dul
Paa
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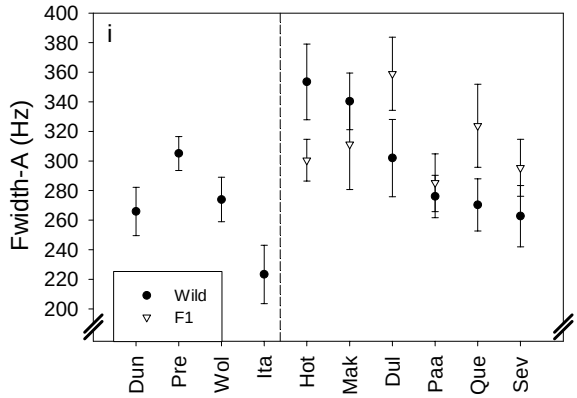
Sev


i
Fwidth-A (Hz)
400
380
360
340
320
300
280
260
240
220
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Wild
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Wol
Ita
Hot
Mak
Dul
Paa
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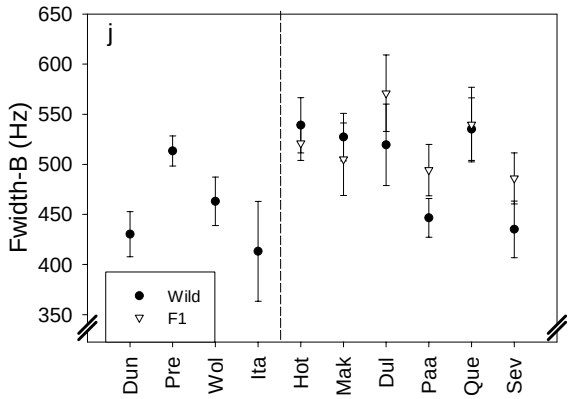
Sev


j
Fwidth-B (Hz)
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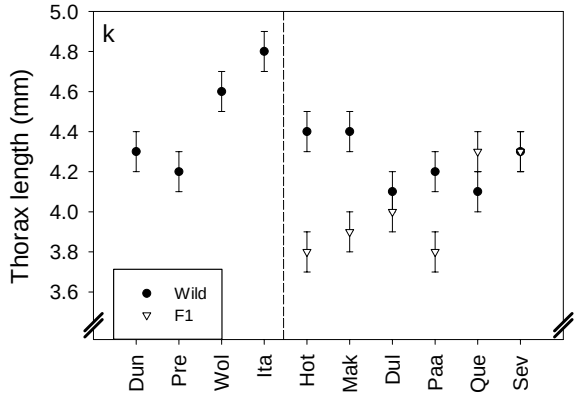
k
Thorax length (mm)
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
Wild
F1
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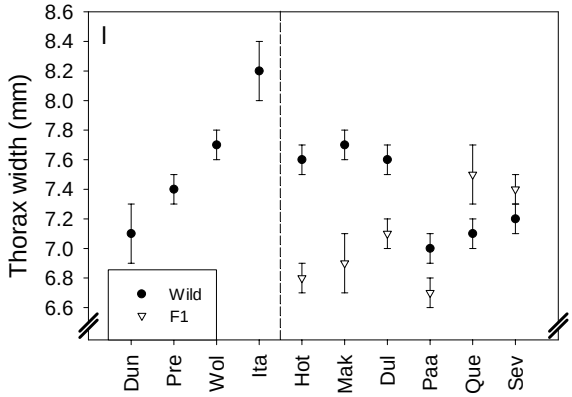
l
Thorax width (mm)
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
Wild
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Wol
Ita
Hot
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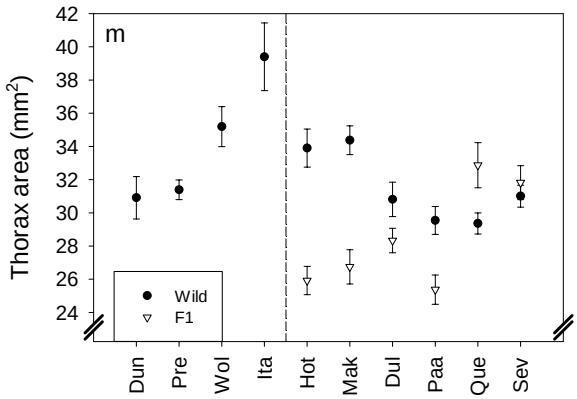
m
Thorax area (mm2)
42
40
38
36
34
32
30
28
26
24
Wild
F1
Dun
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Wol
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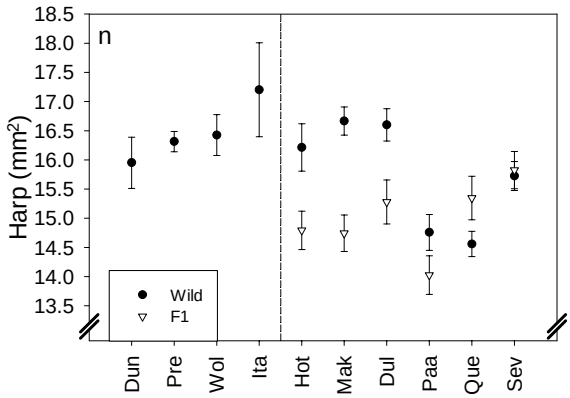
Hot
Mak
Dul
Paa
Que
Sev


n
Harp (mm2)
18.5
18.0
17.5
17.0
16.5
16.0
15.5
15.0
14.5
14.0
13.5
Wild
F1
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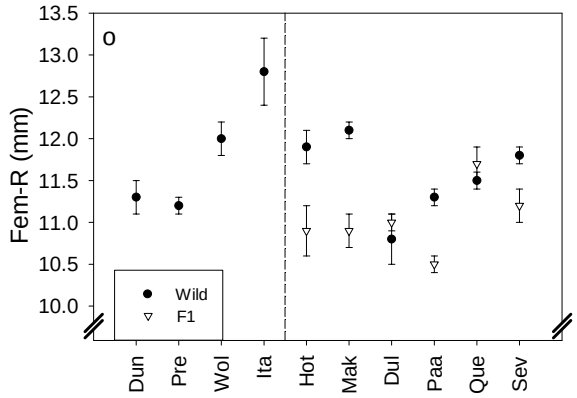
o
Fem-R (mm)
13.5
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12.0
11.5
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Wild
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Sev

Figure 5.2a-o. Mean values with associated standard errors (error bars) for the calling song characteristics and morphometric traits of ten wild-caught and six captive-reared F_1 populations of *G. bimaculatus*. Populations left of the vertical broken line lack captive-reared F_1 offspring. Locality names are abbreviated as follows: Dun = Dundee; Pre = Pretoria; Wol = Wolmaransstad; Ita = Italy; Hot = Hotazel; Mak = Makhado; Dul = Dullstroom; Paa = Paarl; Que = Queenstown; Sev = Sevilla.

Variables contributing to the variation in the wild-caught populations

Principal components analysis showed that six principal components (eigenvalues > 1) explained more than 80% of the total variation in the wild-caught data set (Table 5.3). The wing measurements contributed significantly to principal component 1, representing 40% of the total variation. Bandwidth contributed significantly to principal component 2, representing some 14% of the total variation and F_{max} did not contribute in a measurable way to the total variation. The chirp traits contributed significantly to the third and fourth principal components, representing 16% of the total variation. Syllable duration was the only syllable trait that contributed significantly to any of the principal components, principal component 5, representing 6% of the total variation. The body measurements contributed significantly to principal component 6, representing only 5% of the total variation. Although there was a tendency for same-population individuals to cluster together in R-rotated PCA plots, there was no distinct clustering of the individuals into their respective populations (Figure 5.3a).

Table 5.3. Principal components (PC) showing to what degree the calling song characteristics and morphometric measurements contributed to the variation in ten wild-caught populations (W) and six captive-reared F_1 populations (F_1) from South Africa and Europe. Loadings indicated by W and F_1 are > 0.700. The following variables did not contribute in a measurable way to the variation in either of the data sets: F_{max}, M4_M8 and H10_H11

Variable	PC1	PC2	PC3	PC4	PC5	PC6
S_C			W	F_1		
CD			W	F_1		
ICI		F_1		W		
CP		F_1		W		
SD					W	
ISI		F_1				
SP		F_1				
Fwidth-A		W	F_1			
Fwidth-B		W	F_1			
Head length					F_1	W
Head width					F_1	
Thorax area					F_1	
Fem-R					F_1	W
Tib-R					F_1	W
H1_H2	W, F_1					
H1_H3	W, F_1					
H2_H3	W, F_1					
H1_M9	W, F_1					
M6_M8	W					
Harp	W, F_1					
Total variance explained % (F_1)	39.81	14.22	9.15	6.64	5.86	5.37
Total variance explained % (W)	36.64	13.46	9.19	8.62	7.19	

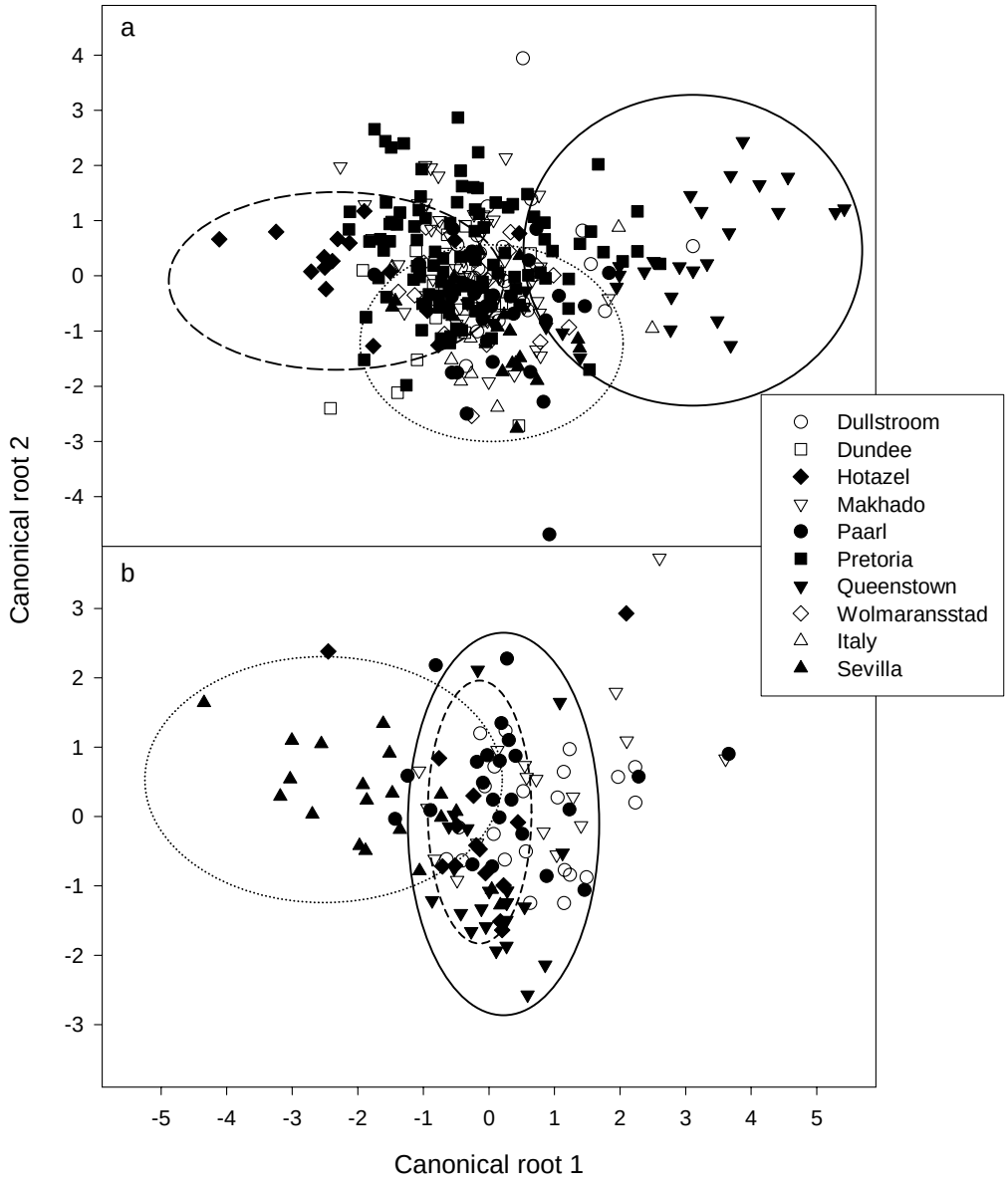

Figure 5.3. Clustering of (a) ten wild-caught populations and (b) six captive-reared F_1 populations from South Africa and Europe following discriminant function analyses that utilized ten calling song characteristics of *G. bimaculatus*. The Hotazel, Queenstown and Sevilla populations (circled) have distinct clusters in at least one of the analyses.

Call traits useful in discriminating between wild-caught populations

Following the DFA, calling song frequency, syllable duration, inter-chirp interval, syllable period, Fwidth-A and Fwidth-B (F_{max} = most important and Fwidth-B = least important) can be used to discriminate between populations (Wilks' Lambda = 0.34, $F_{54, 1529} = 6.66$, $P < 0.001$, $n = 314$). The significantly different values of syllable duration and calling song frequency of the Queenstown and Hotazel populations (Figure 5.2a, h), enhanced the effectiveness with which these two call traits can be used to discriminate between populations. A large degree of similarity was observed between the Dullstroom, Makhado, Pretoria and Wolmaransstad populations as well as between the Dundee, Italian, Paarl and Sevilla populations (Figure 5.3a). The Queenstown and Hotazel populations clustered separately from each other and the other populations. However, only 44% of the individuals were classified correctly into their respective populations. No individuals from Hotazel were assigned to Queenstown or *vice versa*. Four of the South African populations (Makhado, Paarl, Queenstown and Wolmaransstad) had at least one individual classified mistakenly into the Sevilla population. The Wolmaransstad and Italian populations did not have a single individual classified into them. The majority of the Wolmaransstad individuals were mistakenly classified into the Pretoria population while the Italian individuals were mistakenly classified into the Sevilla, Pretoria, Queenstown and Dundee populations. Sixteen of the 20 Sevilla individuals were mistakenly classified into the Pretoria, Paarl and Makhado populations.

Correlation with geographic distances, call traits and morphology in ten wild-caught populations

There was no significant association between geographic distances among localities and any of the call traits or morphometric measurements (Mantel test: g = 1.55, r = 0.263, $P > 0.05$, $n = 314$), suggesting that populations that are located close to each other do not have more similar crickets (based on call traits and morphological measurements) than those populations located further apart. A Mantel test performed only on call traits did not reveal any significant association between geographic distances and call traits (g = 1.55, r = 0.262, $P > 0.05$, $n = 314$), suggesting that cricket populations that are located close to each other do not have more similar calling songs, than those populations located further apart.

Correlation with gene flow, call traits and morphology in nine wild-caught populations

There was no significant association between the gene flow estimates (F_{ST} statistics) from Ferreira (2006c, Chapter 4) and any of the call traits or body measurements (Mantel test: g = 0.396, r = 1330.140, $P > 0.05$, $n = 295$). This suggests that populations with large degrees of between-population gene flow do not have crickets that are more similar in body size or call traits than populations with smaller degrees of between-population gene flow. The Mantel test also did not reveal any significant association between the F_{ST} statistics and call traits (g = 0.391, r = 1327.140, $P > 0.05$, $n = 295$), indicating that cricket populations with large degrees of between-population gene flow do not have more similar calling songs than populations with smaller degrees of between-population gene flow.

Six captive-reared F_1 populations

Between-population differences in call traits and morphology of captive-reared F_1 males

As for the wild-caught populations, the univariate ANOVA also revealed extensive between-population differences in the call traits and morphometric measurements of the captive-reared F_1 populations (Table 5.1). The Tukey *post-hoc* comparison, after a Bonferroni adjustment, showed that the Queenstown population differed significantly from Makhado and Paarl, while Sevilla differed significantly from most of the South African populations (Table 5.2; Figure 5.2). For the chirp traits, the Sevilla population had a significantly longer chirp duration and a significantly shorter inter-chirp interval compared with the South African populations. The Queenstown and Sevilla populations had significantly longer syllable durations and significantly shorter inter-syllable intervals than the other populations, which gave rise to the significantly shorter syllable period produced by these two populations. Calling song frequency was the only spectral trait that differed significantly between populations and it only differed between the Sevilla and Queenstown populations. For the morphometric measurements, the Queenstown population was significantly larger than all of the South African populations except Dullstroom. The Paarl population was significantly smaller than the Sevilla and Queenstown populations. For the wing measurements, harp size only differed

significantly between two populations, with the Sevilla population having a larger harp size than the Paarl population.

Variables contributing to the variation in the captive-reared F_1 populations

Principal components analysis showed that five principal components (eigenvalues > 1) explained 75% of the total variation in the captive-reared F_1 data set (Table 5.3). The wing measurements contributed significantly to principal component 1, representing 37% of the total variation. Two chirp (CP and ICI) and two syllable (SP and ISI) traits contributed significantly to principal component 2, representing 14% of the total variation. Calling song frequency did not contribute in a measurable way to the total variation, while bandwidth contributed significantly to principal component 3, representing 9% of the total variation. The remaining two chirp traits (S_C and CD) contributed to principal component 4, representing only 9% of the total variation. The morphometric measurements contributed the least to the total variation, only 6%, and contributed significantly to principal component 5. Same-population individuals clustered together in the R-rotated data, although there was no complete clustering of the individuals of different populations (Figure 5.3b).

Call traits useful in discriminating between captive-reared F_1 populations

Only three of the ten call traits (inter-syllable interval, chirp duration and calling song frequency: ISI = most important and F_{max} = least important) can be used to discriminate between populations (Wilks' Lambda = 0.39, $F_{25, 402} = 4.645$, $P < 0.001$, $n = 118$). The significantly different attributes of inter-syllable interval of the Sevilla and Queenstown populations (Figure 5.2) contributed to the effectiveness with which there can be discriminated between populations. Although the Sevilla and Queenstown populations clustered separately from each other and other populations, there was overlap between the Queenstown and Hotazel populations (Figure 5.3b). There was a large degree of similarity between the Dullstroom, Makhado and Paarl populations. Fifty five percent of the individuals were classified correctly into their respective populations. The Sevilla population had the highest percentage (75%) of correctly classified individuals, followed by the Queenstown population (71%). The remaining populations had less than 53% correct classifications. Five of the 20 Sevilla individuals were mistakenly classified

into three South African populations (Hotazel, Paarl and Queenstown), while three individuals from Paarl and Hotazel were mistakenly classified under the Sevilla population.

Correlation with geographic distances, call traits and morphology in six captive-reared F_1 populations

The Mantel test found no significant association between geographic distance and any of the call traits or body measurements (g = -0.259, r = -0.083, $P > 0.05$, n = 118). There was also no significant association between geographic distance and any of the call traits (g = -0.263, r = -0.084, $P > 0.05$, n = 118), reflecting the results found in the wild-caught populations.

Correlation with gene flow, call traits and morphology in six captive-reared F_1 populations

There was no significant association between the gene flow estimates (F_{ST} statistics) from Ferreira (2006c, Chapter 4) and any of the call traits or body measurements (Mantel test: g = -0.453, r = -0.134, $P > 0.05$, n = 118) or between the gene flow estimates and call traits (Mantel test: g = -0.471, r = -0.139, $P > 0.05$, n = 118) for the six captive-reared F_1 populations. This indicates that populations with large degrees of between-population gene flow do not have crickets that are more similar in call structure or morphometrics than populations with small degrees of between-population gene flow.

Comparison of wild-caught populations with captive-reared F_1 populations

Between-population variation in the calling song characteristics and body measurements

There was a significant difference in the overall and within-population variances of the majority of the call traits and morphometric measurements for both the wild-caught and captive-reared F_1 populations (Table 5.4). The overall and within-population variance of all the syllable traits was higher in the captive-reared F_1 populations than in the six wild-caught populations, while this was only true for one of the chirp traits and two of the body measurements. In contrast, the overall and within-population variances of the spectral traits of the six wild-caught populations were higher than those of captive-reared F_1 crickets.

On a finer scale, the random effects ANOVA revealed that the chirp traits and inter-syllable interval had a larger degree of between-population variation in the six captive-reared F_1 populations than in the ten wild-caught populations (Figure 5.4a). However, when comparing the six captive-reared F_1 populations with the six wild-caught populations only three call traits (syllables per chirp, chirp duration and inter-syllable interval) had a larger degree of between-population variation in the captive-reared F_1 crickets than the wild-caught crickets (Figure 5.4b). The between-individual variance component contributed the most to the variation in all the call traits in the six wild-caught populations (SD and F_{max} < 65%; other call traits > 80%) as well as in the six captive-reared F_1 populations (spectral traits and CP: > 90%; other call traits less than 90%).

The results of the two-way fixed effects ANOVA revealed a strong effect of locality on all but four traits (Table 5.5). Although the effect of captivity was mostly evident in the syllable traits and morphometric measurements, there was a significantly strong interaction between locality and captive state on the majority of traits (Table 5.5). The Sevilla population did not differ markedly from the South African populations. The Hotazel population differed from the other populations in syllable duration, while the Queenstown population differed significantly from the other populations in calling song frequency. The Paarl population differed significantly from the other populations in body size. Three call traits (syllables per chirp, inter-syllable interval and syllable period) differed significantly between the wild-caught and captive-reared F_1 populations. The morphometric measurements of the wild-caught populations differed significantly from the captive-reared F_1 populations.

Table 5.4. Overall variance and within-population variance of the call traits and body measurements for ten wild-caught populations (10 Wild), six wild-caught populations that have captive-reared F_1 offspring (6 Wild) as well as their six captive-reared F_1 populations (6 Captive). The *F* statistic was calculated for each variable, using the six wild-caught and six captive-reared F_1 populations. '*' indicates significance for $P < 0.05$

	Total variance				Within-population variance			
Variable	10 Wild	6 Wild	6 Captive	*F* statistic	10 Wild	6 Wild	6 Captive	*F* statistic
S_C	0.197852	0.203719	0.141261	$F_{166, 117} = 1.442$*	0.191979	0.194984	0.119736	$F_{166, 117} = 1.628$*
CD	0.000360	0.000368	0.000272	$F_{166, 117} = 1.354$*	0.000322	0.000319	0.000211	$F_{166, 117} = 1.51$*
ICI	0.002122	0.002421	0.002565	$F_{117, 166} = 1.059$*	0.001906	0.002073	0.002212	$F_{117, 166} = 1.067$*
CP	0.002459	0.002848	0.002592	$F_{166, 117} = 1.099$	0.002286	0.002534	0.002335	$F_{166, 117} = 1.085$
SD	0.000007	0.000008	0.000009	$F_{117, 166} = 1.099$*	0.000005	0.000004	0.000007	$F_{117, 166} = 1.648$*
SP	0.000008	0.000008	0.000008	$F_{117, 166} = 1.096$*	0.000006	0.000006	0.000007	$F_{117, 166} = 1.179$*
ISI	0.000009	0.000009	0.000018	$F_{117, 166} = 2.058$*	0.000008	0.000009	0.000013	$F_{117, 166} = 1.532$*
F_{max}	0.117454	0.131717	0.052854	$F_{166, 117} = 2.492$*	0.076520	0.065438	0.046508	$F_{166, 117} = 1.407$*
Fwidth-A	0.012097	0.013075	0.011499	$F_{166, 117} = 1.137$	0.011195	0.011909	0.010858	$F_{166, 117} = 1.1$
Fwidth-B	0.022739	0.024436	0.020461	$F_{166, 117} = 1.194$	0.021249	0.022796	0.019543	$F_{166, 117} = 1.166$
Thorax length	0.264145	0.179543	0.175609	$F_{166, 117} = 1.022$	0.239888	0.156134	0.124395	$F_{166, 117} = 1.255$*
Thorax width	0.452909	0.323587	0.399356	$F_{117, 166} = 1.234$*	0.387573	0.253523	0.302016	$F_{117, 166} = 1.191$*
Thorax area	32.445717	26.998301	27.974020	$F_{117, 166} = 1.036$*	27.633437	22.490586	19.497508	$F_{166, 117} = 1.153$
Fem-R	1.266426	0.947301	0.655581	$F_{147, 114} = 1.445$*	1.053002	0.740582	0.503853	$F_{147, 114} = 1.47$*
Harp	0.008486	2.941981	2.606636	$F_{165, 115} = 1.129$	0.058300	2.154238	2.247462	$F_{165, 115} = 1.043$*

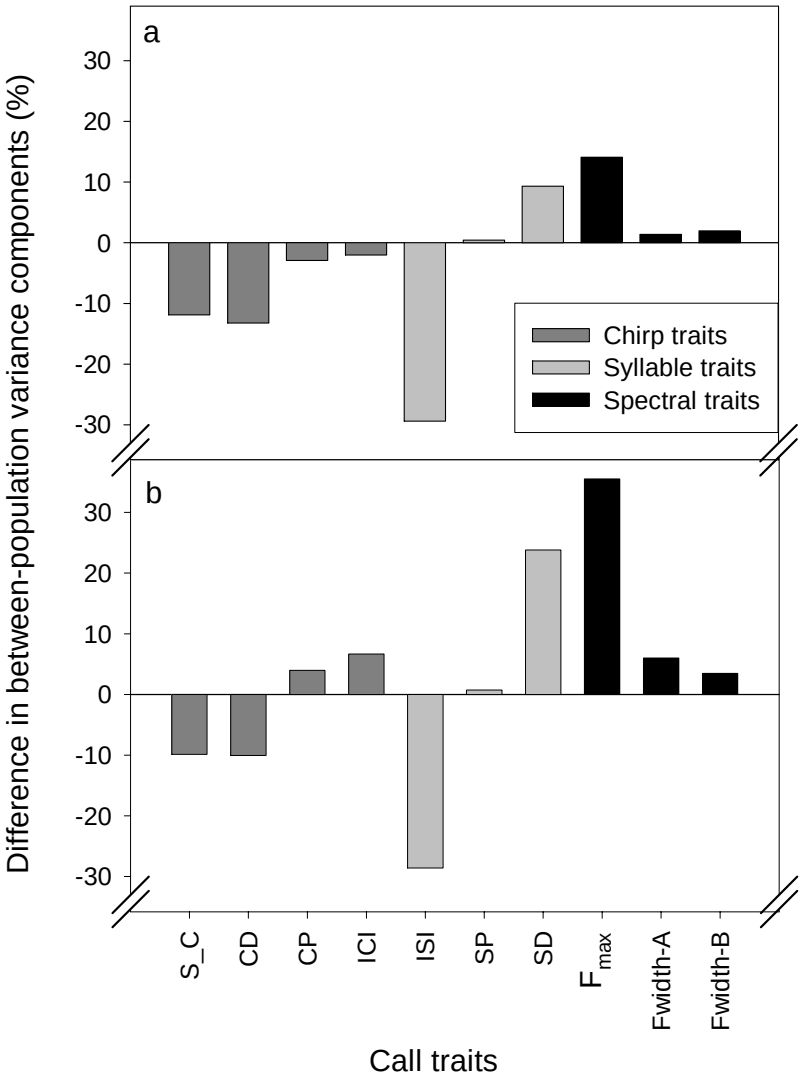

Figure 5.4. The difference between the between-population variance components of the calling song characteristics of *G. bimaculatus* for (a) ten wild-caught and six captive-reared F_1 populations and (b) six wild-caught and six captive-reared F_1 populations.

Table 5.5. Results for a two-way fixed effects ANOVA performed on the six wild-caught populations and their captive-reared F_1 offspring for two treatments (locality and wild/captive) and the interaction between them (interaction). The degrees of freedom of the *F* statistic for Fem-R are given directly after the value of its *F* statistic. Results for Tukey *post-hoc* comparisons, after Bonferroni adjustments, are indicated with asterisks. Empty cells indicate non-significant comparisons ($P > 0.05$). Asterisks indicate significance values as follows: $P < 0.05$ *, $P < 0.01$ ** and $P < 0.001$ ***

	Two-Way ANOVA			*Post-hoc* analyses														
	Locality	Wild/Captive	Interaction	Locality														
	F statistic	*F* statistic	*F* statistic	Hot-Mak	Hot-Dul	Hot-Paa	Hot-Que	Hot-Sev	Mak-Dul	Mak-Paa	Mak-Que	Mak-Sev	Dul-Paa	Dul-Que	Dul-Sev	Paa-Que	Paa-Sev	Que-Sev
Call trait	$F_{5, 273}$	$F_{1, 273}$	$F_{5, 273}$															
S_C	2.498*	11.162***	2.435*															
SD	9.069***	0.829	14.256***	***	***	***	***											***
ISI	6.752***	17.467***	7.538***									***			***		***	
SP	11.079***	18.851***	3.32**				***	***			***	**				***	**	
CD	6.733***	0.902	3.665**				***											***
ICI	2.878*	1.2	5.733***															
CP	3.193**	0.482	3.051*													**		
F_{max}	21.848***	0.11	13.275***		**		***				***			***		***		***
Fwidth-A	2.15	0.753	1.71															
Fwidth-B	2.575*	1.063	0.612															
Body measurements	$F_{5, 274}$	$F_{1, 274}$	$F_{5, 274}$															
Thorax length	4.19**	24.669***	8.38***															**
Thorax width	5.667***	22.929***	9.386***							***			***			***	**	
Thorax area	4.45***	25.53***	10.084***							**						**	***	
Fem-R	6.561***; $F_{5, 252}$	25.09***; $F_{1, 252}$	6.275***; $F_{5, 252}$						**	**				**		***	**	
Wing measurement	$F_{5, 271}$	$F_{1, 271}$	$F_{5, 271}$															
Harp	7.491***	15.62***	5.2***							***			***				***	

Correlations between wild-caught and captive-reared F_1 crickets' call traits and morphometric measurements

No significant correlations between the call traits and morphometric measurements of the six wild-caught populations and six captive-reared F_1 populations were found (S_C: r = 0.107, CD: r = 0.335, CP: r = -0.06, ICI: r = -0.344, SD: r = -0.215, SP: r = 0.411, ISI: r = 0.162, F_{max}: r = 0.369, Fwidth-A: r = 0.063, Fwidth-B: r = 0.712, thorax area: r = -0.472, thorax length: r = -0.332, thorax width: r = -0.315, Fem-R: r = 0.015, harp: r = 0.157; $P > 0.11$ in all cases, $n = 6$).

Discussion

Inter-continental and regional differences in male calling song and morphometrics

This study found only a small degree of differentiation among European and South African populations in the calling song and morphometrics of wild-caught *G. bimaculatus* (Tables 5.1 & 5.2; Figure 5.3). The large amounts of gene flow that occur between Europe and South Africa (Ferreira, 2006c, Chapter 4) should increase the degree of similarity in the calling songs and morphometrics between these populations, especially since this study could not find any evidence of clinal variation. The between-population differences within South Africa were more evident than in Ferreira & Ferguson's (2002) study. This is probably explained by the increase in sampling localities and sample sizes at each locality. In addition, the present sampling localities represent various climatic regions of South Africa (Preston-Whyte & Tyson, 1988; Schulze, 1994) and are therefore more representative of the range of environmental conditions that these crickets may experience in the wild. There was a larger degree of regional variation and between-population differences in the call traits and morphometrics of *G. bimaculatus* (Figure 5.2; Tables 5.1 & 5.2) compared with inter-continental variation and inter-continental differences (i.e. between South Africa and Europe). Zuk *et al*. (2001) found similar results: there were larger degrees of between-region (i.e. within-continents) than between-continent variation in the calling song of the field cricket, *Teleogryllus oceanicus*. The mate recognition system of *Drosophila melanogaster* is also very stable among populations worldwide (Henderson & Lambert, 1982). My findings suggest that there is large-scale stability in the communication system of this cricket, consistent with Paterson's (1985) prediction of stabilizing selection in mate recognition systems among populations.

Although there was variation in the song traits within South Africa, the call traits important for mate recognition (calling song frequency and syllable period; Bennet-Clark, 1989; Schildberger *et al*., 1989) had less between-population differences (Table 5.2) and contributed less to the total variation in the data set (Table 5.3) compared with other call traits, supporting the findings of Ferreira & Ferguson (2002). Ryan *et al*. (1996) also found that although there was significant between-population variation in the advertisement call of *P*. *pustulosus*, the traits important for mate recognition had smaller degrees of variation than other call traits.

Environmentally induced variation in the male calling song and morphometrics

I did not conduct a quantitative study (e.g. calling song of *G*. *integer*: Gray & Cade, 1999; morphology of *G. bimaculatus*: Simmons, 1987; morphology of *G. pennsylvanicus*: Simons & Roff, 1994) and my results can therefore not provide a precise estimate of the amount of environmental variance that contributes to variation in the calling song and morphometrics of *G. bimaculatus*. However, my conclusions based on the comparative analyses of the wild-caught and captive-reared F_1 populations, estimates part of the environmental component of geographical variation in the call and morphological traits. When Mousseau & Howard (1998) compared wild-caught and laboratory-reared populations of two species of ground crickets, *Allonemobius fasciatus* and *A. socius*, as well as a hybrid population, they found a significant effect of the environment on call traits. A significant part of the geographical variation in the call traits and morphometric measurements of *G. bimaculatus* is probably environmentally induced based on the following results. Firstly, overall and within-population variances were larger in the wild-caught than the captive-reared F_1 populations for six of the ten call traits and two of the morphometric measurements (Table 5.4). Secondly, there was a decrease in the between-population variances of seven call traits in six captive-reared F_1 populations compared with six wild-caught populations (Figure 5.4b). Thirdly, no significant correlation of the call traits and morphometric measurements of the captive-reared F_1 populations with the wild-caught populations could be found. Fourthly, there was a strong interaction effect of locality and captivity on eight call traits and all of the morphometric measurements (Table 5.5). Lastly, with the exception of the Queenstown population, significant between-population differences in the call traits

and morphometric measurements of the wild-caught populations were not evident in the captive-reared F_1 populations (Table 5.2). The wild-caught and captive-reared F_1 Queenstown populations differed significantly from the other populations in call structure and body size (Tables 5.2 & 5.5; Figure 5.2). The wild-caught Queenstown population also clustered separately from the other populations in the discriminant function analysis (Figure 5.3a). The phylogeographical study of Ferreira (2006c, Chapter 4) on *G. bimaculatus* revealed (a) that only small amounts of gene flow occurred among the Queenstown population and other South African populations, (b) that the Queenstown population has a small degree of within-population genetic differentiation and (c) that it differs significantly from the other populations. This could imply that part of the distinctive genetic characteristics of the Queenstown population are reflected in this population's phenotypic traits.

Correlation of calling song and morphometric differences with geographical distance and gene flow

This study could not find any evidence of an isolation-by-distance effect in the call traits and morphometric measurements (see results) as were found in other studies (Asquith *et al*., 1988; Ryan & Wilczynski, 1991; Ryan *et al*., 1996; Zuk *et al*., 2001). There was also no significant association between the call and morphometric traits with gene flow (see results), suggesting that populations with large amounts of gene flow among them are not necessarily more similar in call structure or morphology. Indeed, there were fewer differences in the song and morphometrics of the individuals from the Queenstown and Sevilla populations compared with Queenstown and other South African populations (Table 5.2). More individuals of these two populations share a specific haplotype (h2; Ferreira, 2006c, Chapter 4) compared with the other populations, which could explain why the call and morphological traits of the Queenstown and Sevilla populations do not differ to such a great extent from each other. Call traits and morphometric measurements of wild-caught populations in the northeastern parts of South Africa differed significantly from those of the wild-caught populations in the southwestern parts of the country (Table 5.2), thereby reflecting the gene flow patterns observed in Ferreira (2006c, Chapter 4). However, this pattern is not observed in the captive-reared F_1 populations, suggesting that geographical variation in the call traits and morphometric measurements of this

cricket is not solely explained by between-population genetic differentiation, but rather by different environmental conditions.

Estimates of the magnitude of between-population variances

Between-individual variances were larger than between-population variances in the call traits of the six wild-caught and six captive-reared F_1 populations (see results). The within-population variance component was also larger than the inter-continental and between-population variance components in the call and morphological traits of the ten wild-caught populations (Table 5.1). These results are consistent with the findings of Ferreira (2006b, Chapter 3) who showed that between-individual and within-individual variances contribute largely to the variation in the call traits of two populations of *G. bimaculatus*. These findings suggest that there are not large degrees of between-population differences in the call structure and body sizes of *G. bimaculatus* crickets among the European and South African populations.

Implications for the communication system: efficiency of communication

This study showed that variation in the calling song and morphometrics of *G. bimaculatus* crickets is more evident within-populations than among European and South African populations of wild-caught and captive-reared F_1 crickets. Diverse environmental effects experienced by populations could act as noise affecting the communication system of *G. bimaculatus*. This could, in turn, affect the reliability with which males signal their qualities (e.g. body size and health; Simmons, 1988). However, these effects on the sexual selection process need to be investigated by in-depth studies on the communication system of these crickets.

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