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Variation in the Acoustic Properties of the Calling Songs of *Cicada barbara* and *C. orni* (Hemiptera: Cicadidae) at the Individual and Population Levels

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Sofia G. Seabra, Genage André, and José A. Quartau (2008) Variation in the acoustic properties of the calling songs of *Cicada barbara* and *C. orni* (Hemiptera: Cicadidae) at the individual and population levels. *Zoological Studies* **47**(1): 1-10. Variation in the time and frequency properties of the calling songs of 2 *Cicada* species, *C. barbara* and *C. orni*, was examined at the individual and population levels. Individual males were recorded in the field at different times of day, and within-individual coefficients of variation were generally lower than among-individual variation. The effect of temperature on calling song properties was also assessed, with time variables being more consistently related to temperature than the frequency variable. No significant differences among years within populations were detected in most populations. Studies of female preference in these species are necessary to assess the biological significance of the detected variation in the properties of these calling songs. http://zoolstud.sinica.edu.tw/Journals/47.1/1.pdf

Key words: *Cicada barbara*, *Cicada orni*, Cicadas, Insects, Song.

Studying natural variation in behavior provides opportunities to understand and elaborate hypotheses about the patterns and causes of behavioral evolution (Foster and Endler 1999). In particular, geographic variation in signals used to attract mates may provide insights into the evolution of differences among conspecific populations which may result in their divergence, and thus these may be important for understanding the speciation process (Verrell 1999). Temporal variation of behavioral traits used in communication is usually difficult to study in the wild. Studying within-individual variation is important for understanding the significance of variation among individuals as well as variation among years in the same population in order to better understand variation among populations. This constitutes an initial step in understanding which characteristics of a signal may have important communicative functions and which may be subjected to selection. This can then be related to patterns of preference by the females.

The 2 species of cicadas studied here, *Cicada barbara* and *C. orni*, presented an opportunity to examine within-individual and among-year variation in an important behavioral trait, the calling song of males, in each population. There are 2 main reasons for the suitability of these species for this research: (1) extensive field studies in consecutive years have been conducted on these species (which were mainly aimed at studying inter-population variation) (Pinto-Juma et al. 2005, Quartau and Simões 2006, Seabra et al. 2006) and (2) the males of these 2 cicadas usually sing for long periods on the same tree trunk or branch, which allows the recording of songs at different times of day and temperatures (Quartau et al. 2000).

Studies of within-individual acoustic variation in the wild have been conducted on several anu-

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rans and birds (e.g., Bee 2004, Kopuchian et al. 2004, Runciman et al. 2005, Friedl 2006), but insects are more difficult to study due to difficulties in marking and tracking individuals, and as such, are usually examined under controlled captive conditions (e.g., Butlin 1985, Fonseca and Revez 2002).

Cicada barbara (Stål) and *C. orni* L. are 2 closely related Mediterranean species, both of which occur on the Iberian Peninsula. Adults of both species appear in the beginning of the hot and dry season, after the nymphs have developed underground, and males sing on sunny days when ambient temperatures rise above 23°C or even at night if temperatures are well above 30°C. The calling song in cicadas is produced by tymbal membranes, located dorsolaterally on the 1st abdominal segment and activated by powerful muscles (Bennet-Clark and Young 1992). This song has a fundamental role in attracting conspecific females for mating and may act as an aggregation signal for other males in gregarious species (Boulard 2006) such as *C. barbara* and *C. orni*.

In this study, variation in the acoustic characters of the calling song of individual cicadas of *C. barbara* and *C. orni* was assessed in the field during the day. On another level of analysis, variation in the calling songs of cicada populations in the same locality was also studied among years.

MATERIALS AND METHODS

Sound recording and analysis

Calling songs of cicada males of *C. barbara* and *C. orni* were recorded in the field for up to 3 min each using a Sony (Japan) DAT recorder (TCD-D10 ProII and TCD-D8; at respective frequency ranges of 20-22,000 and 20-20,000 Hz, and a sampling frequency of 44.1 kHz) connected to a dynamic Sony F-780 microphone or a Telinga (Sweden) Pro4PiP microphone (with respective frequency responses of 50-18,000 and 40-18,000 Hz). Sound recordings were digitized using the software Avisoft-SASLab Pro (Specht 2002) at a sampling rate of 44.1 kHz and a resolution of 16 bits. Frequency spectra were computed with the same software using fast Fourier transformation with a resolution of 512 points and a Hamming window. Time and frequency variables were measured using the same software on fragments of about 60 s long. Gross-temporal variables analyzed in *C. orni* songs included the number and duration of acoustic elements (echemes), and the duration of the interval between them (inter-echeme interval) (Fig. 1). Echeme rates (number of echemes/s), echeme periods (duration between the beginning of 1 echeme and the beginning of the following echeme), and the ratio between the echeme duration and the inter-echeme duration were calculated. The peak frequency (the fre-

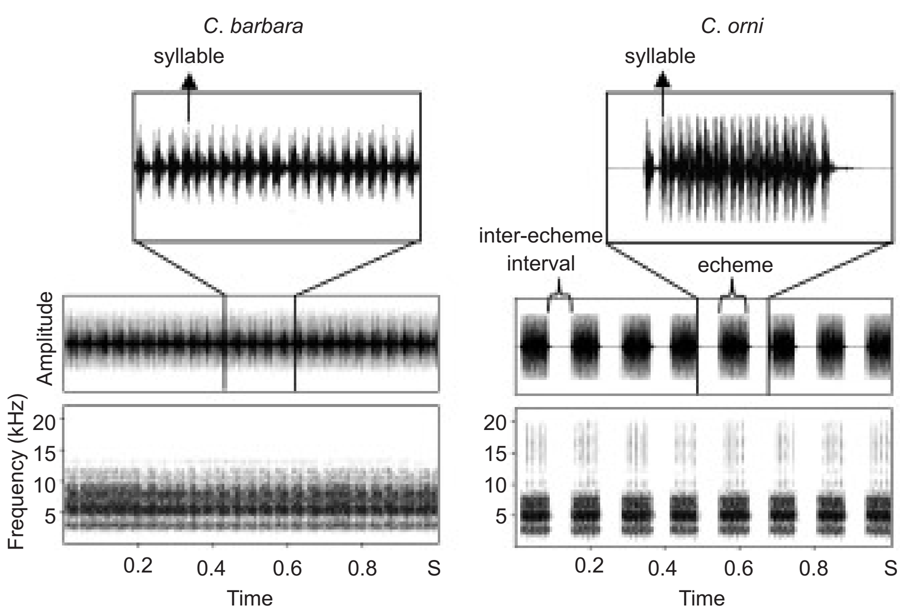

Fig. 1. Oscillograms (amplitude-time waveform) and frequency-time spectrograms of the calling songs of *Cicada barbara* and *C. orni*.

quency of the maximum amplitude on the spectrogram, also called the carrier frequency or dominant frequency) was also automatically obtained for both species. A fine-temporal property of the signal, the syllable rate, was obtained for both species by manual screening measurements (using the computer cursor) on the oscillograms. A syllable is a group of sound pulses recognizable on the oscillogram that corresponds to the movement of one of the 2 tymbals. The number of syllables was counted in 30 echemes in *C. orni* (the 1st and last syllables in each echeme were discarded due to their different characteristics) and in 30 fragments of about 0.1 s each in *C. barbara*.

Within-individual variations

Males of both species from Crato, Portugal in 1999 were recorded at least twice during the day (Table 1) from 09:00 to 19:00, with recordings separated by an hour or more. The ambient temperature was measured at each recording time. The recordings of some *C. orni* males of the population in Crato in 1999 were previously analyzed (Quartau et al. 2000), but only temporal variables of the song were considered in that analysis, and it was based on a much more restricted number of echemes.

The average and standard deviation of each acoustic variable were calculated for each individual cicada. In this study, we were mostly interested in comparing the variability of each acoustic variable within individuals with the variability among individuals (within populations). The coefficient of variation (CV) corrected for small samples (Sokal and Rohlf 1981) was calculated as: CV = 100 x (1 + 1/4N) x SD/Average; where N is the sample size and SD is the standard deviation.

Nonparametric Spearman correlations were calculated between each acoustic variable and the

Table 1. Number of recordings (*n*) of the calling song of males of *Cicada barbara* (Cb) and *C. orni* (Co) obtained at different times of day and temperatures at Crato, Portugal in 1999

Species	Male ID	Date (day/month)	*n*	Local time of day (clock time)	T (°C)
C. barbara	Cb720	06/07	7	11:00-18:00	27.5-33.0
	Cb725	07/07	2	10:00-11:00	32.0-33.0
	Cb730	08/07	5	10:00-14:00	33.0-40.0
	Cb731	08/07	2	17:00-18:00	40.0-41.0
	Cb766	14/07	6	11:00-18:00	33.0-38.0
	Cb771	15/07	3	16:00-18:00	38.5-39.5
	Cb851	01/08	5	11:00-17:00	28.0-35.0
	Cb852	01/08	3	12:00-17:00	30.5-35.0
	Cb854	01/08	2	16:00-17:00	34.0-35.0
	Cb855	02/08	5	10:00-17:00	25.5-34.0
	Cb856	02/08	6	11:00-17:00	27.0-34.0
	Cb858	03/08	5	11:00-16:00	25.0-32.5
C. orni	Co699	01/07	6	10:00-17:00	35.0-39.0
	Co700	01/07	4	12:00-16:00	36.5-39.0
	Co707	30/06	2	10:00-11:00	34.0
	Co709	30/06	3	13:00-14:00	36.0-39.0
	Co712	30/06	2	15:00-16:00	38.0-40.0
	Co713	30/06	2	17:00-18:00	38.0-39.0
	Co715	02/07	8	11:00-19:00	28.0-35.0
	Co716	02/07	6	12:00-18:00	30.0-34.5
	Co719	06/07	7	11:00-18:00	26.5-32.5
	Co724	07/07	3	09:00-11:00	30.0-33.5
	Co726	07/07	4	15:00-18:00	38.0-39.0
	Co729	08/07	7	09:00-17:00	30.0-41.0
	Co765	14/07	7	11:00-18:00	33.0-38.0
	Co767	15/07	4	10:00-13:00	30.0-40.5
	Co768	15/07	3	10:00-12:00	30.5-40.0

ambient temperature for all observations of all individuals as well as for each individual separately (those with more than 3 observations).

Among-year variations in each population

Calling songs of males were recorded from 3 populations of *C. barbara* and 5 populations of *C. orni*, in at least 2 different years for each population (Table 2). Nonparametric Kruskal-Wallis or Mann-Whitney tests were used to compare the acoustic variables from different years at the same locality and from different populations. A parametric analysis of covariance (ANCOVA) with the covariates of temperature and time of day was also applied. However, controlling for temperature was not complete because the ambient temperature was not measured at some localities in some years.

All statistical analyses were performed using SPSS vers. 10.0 (SPSS 1999). The significance of multiple tests was assessed to reduce the critical p value according to the Dunn-Sidák method (Dytham 2003) from 0.05 to 1 - (0.95^1/k), where k is the number of tests performed.

RESULTS

Within-individual variation

There were significant differences among individuals of both species from Crato (in 1999) for every acoustic variable (Kruskal-Wallis tests, $p < 0.003$). In *C. barbara*, the average coefficients of variation were generally higher among individuals than within individuals (Fig. 2). In *C. orni*, the average coefficients of variation were also generally higher among individuals than within individuals, except for echeme duration and interval, which

Table 2. Number of males (*n*) of *Cicada barbara* (Cb) and *C. orni* (Co) recorded in each locality each year with the range of temperature (T) at the time of the recording

Species	Locality	Year	Population	*n*	T (°C)
C. barbara	Crato (Alto Alentejo)	1995	CbCra95	4	-
		1996	CbCra96	5	-
		1997	CbCra97	7	25.0-35.0
		1999	CbCra99	12[a]	25.0-41.0
		2001	CbCra01	3	35.0
	Portel (Baixo Alentejo)	1999	CbPor99	8	35.0-37.0
		2001	CbPor01	10	31.0-35.0
	Sousel (Alto Alentejo)	2001	CbSou01	11	33.0-35.0
		2003	CbSou03	8	29.0-33.0
C. orni	Alter-do-Chão (Alto Alentejo)	1997	CoAlt97	7	25.0-30.0
		1998	CoAlt98	8	30.0-34.0
	Crato (Alto Alentejo)	1996	CoCra96	4	-
		1997	CoCra97	3	25.0-30.0
		1998	CoCra98	3	26.0
		1999	CoCra99	15[a]	26.5-41.0
		2001	CoCra01	8	24.0-26.0
	Monforte (Alto Alentejo)	1995	CoMon95	4	-
		1996	CoMon96	2	-
		1997	CoMon97	16	23.0-35.0
	Monte-da-Caparica (Área Grande Lisboa)	1996	CoMte96	4	-
		1997	CoMte97	7	25.0-30.0
		1998	CoMte98	3	25.0-29.0
	Piedade (Arrábida, Estremadura)	1995	CoPie95	10	-
		1996	CoPie96	3	-

[a]Each of these males was recorded more than once during the course of the day (at different times of day and temperatures, see Table 1): CbCra99 had a total of 51 recordings, while CoCra99 had a total of 68 recordings.

were very similar (Fig. 3).

The relationships of each acoustic variable with temperature and time of day were analyzed from recordings made at Crato from 1999, when several individuals were recorded at least twice during the course of a day. Among all recordings of *C. barbara*, temperature was positively and significantly correlated with the syllable rate (Table 3A). When the time of day (partial correlations) was controlled for, the correlation was still positive but non-significant, and the non-significant correlation with the peak frequency was maintained. However, when considering correlations for each male individually (only for males with more than 3 recordings), the results were inconsistent among individuals, except that the syllable rates of all individuals were positively correlated with temperature (Table 3A, Fig. 4).

In *C. orni*, the results were very discrepant among individuals (Table 3B). Using all observations, significant correlations with temperature were found for the echeme duration (negative), inter-echeme interval (positive), and echeme/interval ratio (negative) (Fig. 5). The correlation of the syllable rate with temperature was very low and non-significant (Table 3B, Fig. 5), but when male Co729 was excluded (see Fig. 5), the correlation increased and became significant (r_S = 0.357, p = 0.005). When controlling for the time of day (partial correlation), the results were similar (Table 3B). Again, when male Co729 was excluded, the partial correlation of the syllable rate with temperature (controlling for time of day) was significant and positive (r_S = 0.503, p < 0.001).

The time of day (when temperature was controlled for) was significantly correlated with the echeme rate (r_S = 0.415, p < 0.001) and echeme period (r_S = -0.404, p < 0.001) in *C. orni*.

Among-year variation in each population

No differences were found in any acoustic variable among years within a given locality for *C. barbara* (Kruskal-Wallis test for Crato and Mann-Whitney test for Portel and Sousel, Table 4A). When comparing localities (including all individuals from different years at the same locality in the same group), there were significant differences among localities in the peak frequency, with peak frequency significantly higher in Sousel than in the other populations (Mann-Whitney tests), but there were no significant differences in the syllable rate. The results were similar when controlling for temperature and time of day (ANCOVA) (Table 4A).

In *C. orni*, there were no significant differences in the acoustic variables among years at Mte Caparica and Monforte (Table 4B). In Alter-do-Chão, the syllable rate showed significant differences among years (Mann-Whitney test; Table 4B), which could partly be explained by temperature: some cicadas were recorded in 1997 at lower

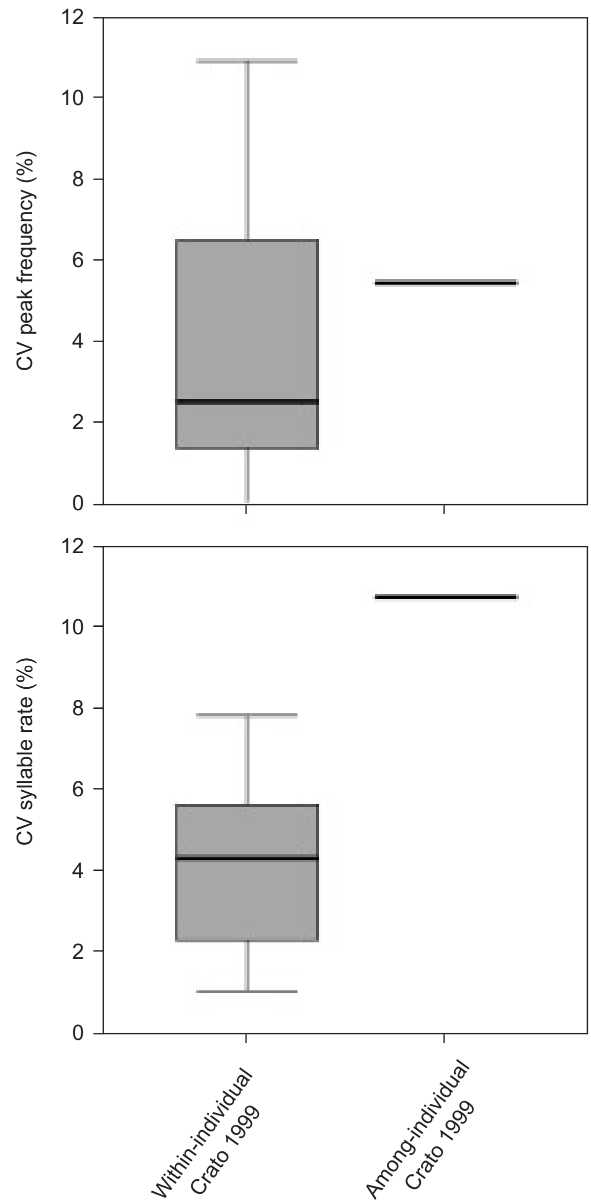

Fig. 2. Boxplots of the coefficients of variation (CVs), in percentages, of each acoustic variable within individuals and the value of among-individual CVs in the population of *Cicada barbara* at Crato, Portugal (in 1999). The rectangular box delimits the 25% and 75% quartiles, with median value shown as a horizontal line; whiskers indicate the non-outlier maximum and minimum.

temperatures than those recorded in 1998. In Piedade, the peak frequency showed significant differences among years. In Crato, the peak frequency showed no significant difference among years, but all of the time variables did (Kruskal-Wallis test; Table 4B), and results were similar when controlling for temperature and time of day (ANCOVA). There were significant differences in all temporal variables among localities (with different years taken together at each locality). Temperature data were missing from the Piedade and Mte Caparica populations, and thus ANCOVA was not used. When Crato was excluded from comparisons among localities, only the echeme duration significantly differed among the 4 localities, with Monte-da-Caparica and Alter-do-Chão having longer echemes than Monforte and Piedade. It was not possible to test for the effect of temperature or time of day due to missing data.

DISCUSSION

Variation in both frequency and time acoustic variables measured in the calling song was usually higher among individuals than within individuals. A similar pattern was reported by Pinto-Juma et al. (2005) for *C. orni*, but the intra-individual variation in that study was calculated from the same song bout, while we calculated it from different song bouts separated by at least 1 h. Differences in premating mechanisms among males could affect a male's relative mating success. Gerhardt (1991) categorized the properties of the calling sounds of tree frogs as static (with a within-male coefficient of variation of < 5%) or dynamic (with a within-male coefficient of variation of > 12%). According to this classification, several studies on insect and anuran species showed that fine-temporal properties of songs are usually static while gross-temporal properties are usually dynamic (Gerhardt and Huber 2002). Moreover, female-choice studies have generally shown that preferences based on static properties are stabilizing or weakly directional, while those based on dynamic properties are strongly directional (Gerhardt and Huber 2002). In both *C. barbara* and *C. orni*, the syllable rate (a fine-temporal variable) showed median within-individual CVs of < 5% (thus classified as being static) and median among-individual CVs of < 12%. The peak frequency also showed low variation in both species, which was most likely due to functional

Table 3. Spearman correlation coefficients of each acoustic variable with the ambient temperature for each individual cicada and for all observations of *Cicada barbara* (A) and *C. orni* (B) from Crato, Portugal in 1999. Partial correlations (parametric) of the acoustic variables and temperature controlled for time of day are also given. *Significant after applying the Dunn-Sidák correction

(A)

	Individuals							All individuals	
	Cb720	Cb730	Cb766	Cb851	Cb855	Cb856	Cb858	Simple correlation	Partial correlation
Peak frequency	-0.612	-0.154	0.247	0.224	0	-0.926*	0.949*	-0.311	-0.336
Syllable rate	0.286	0.800	0.714	0.300	0.400	0.829	0.500	0.616*	0.263

(B)

	Individuals									All individuals	
	Co699	Co700	Co715	Co716	Co719	Co726	Co729	Co765	Co767	Simple correlation	Partial correlation
Peak frequency	0.714	0.800	0.59	0.771	0.883	0.316	0.847	-0.306	0.600	0.244	0.185
Syllable rate	0.429	-0.800	0.843	0.886	0.523	-0.632	-0.721	0.144	0.800	0.192	0.141
Echeme rate	0.714	-0.800	0.735	0.771	0.955*	0.632	0.342	0.811	0.200	-0.056	-0.174
Echeme duration	0.771	-0.800	-0.916*	0.086	-0.577	-0.632	-0.559	-0.559	0.400	-0.405*	-0.420*
Inter-echeme interval	-0.829	0.600	-0.048	-0.6	0.18	-0.316	0.577	0.324	-0.200	0.439*	0.522*
Echeme period	-0.714	0.800	-0.735	-0.771	-0.955*	-0.632	-0.342	-0.811	-0.200	0.055	0.142
Ratio echeme/interval	0.829	-0.800	-0.723	0.257	-0.541	0.316	-0.505	-0.342	0.200	-0.481*	-0.576*

constraints of the sound-producing mechanism. In *C. orni*, the gross-temporal characteristics of the song, echeme duration, and inter-echeme interval showed high median values of both within- and among-individual coefficient of variation (> 12%), and were thus classified as being dynamic.

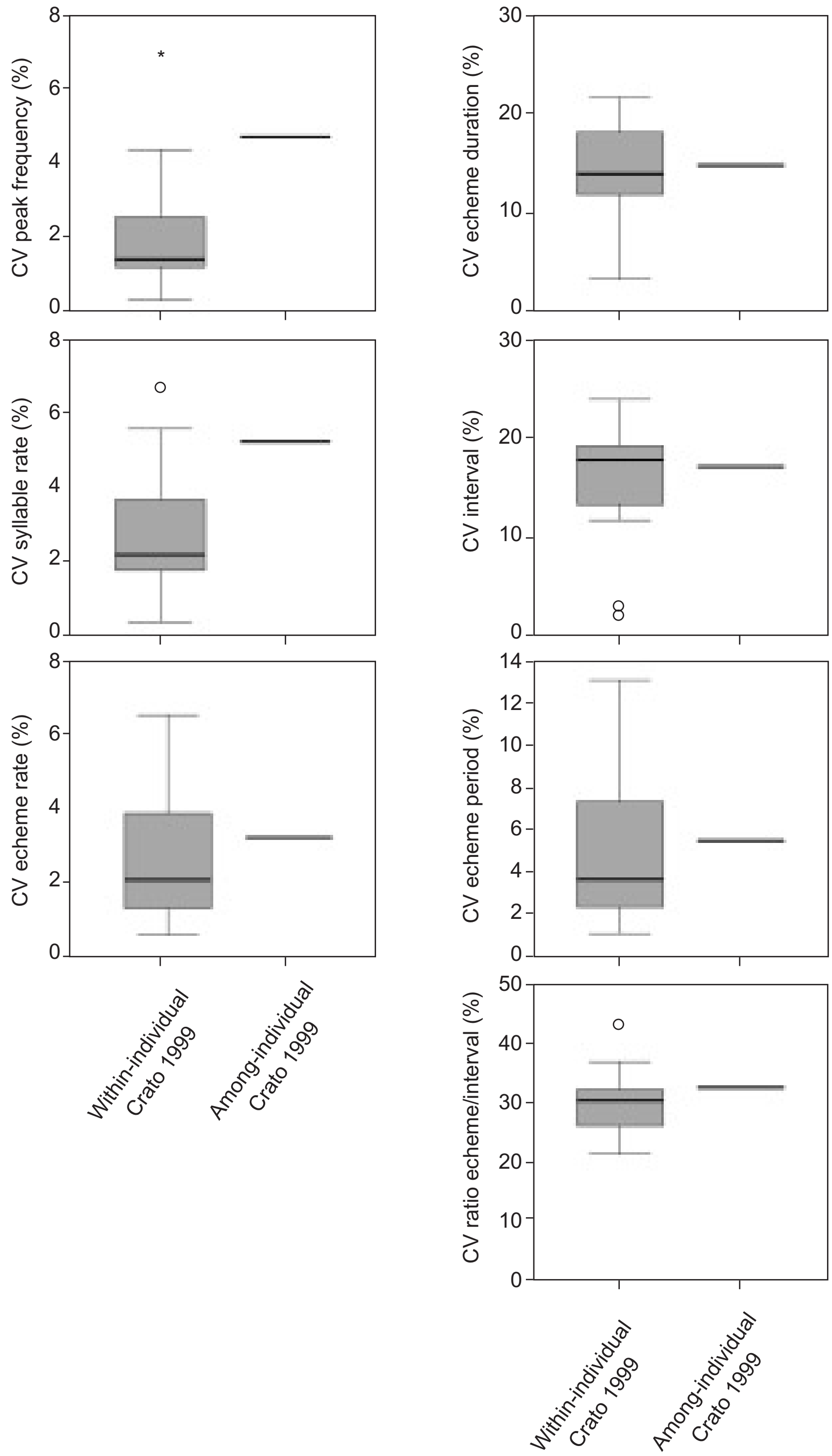

Fig. 3. Boxplots of the coefficients of variation (CVs), in percentages, of each acoustic variable within individuals and the value of among-individual CVs in the population of *Cicada orni* at Crato, Portugal (in 1999). The rectangular box delimits the 25% and 75% quartiles, with median value shown as a horizontal line; whiskers indicate the non-outlier maximum and minimum, the points are outliers, and the stars are extremes.

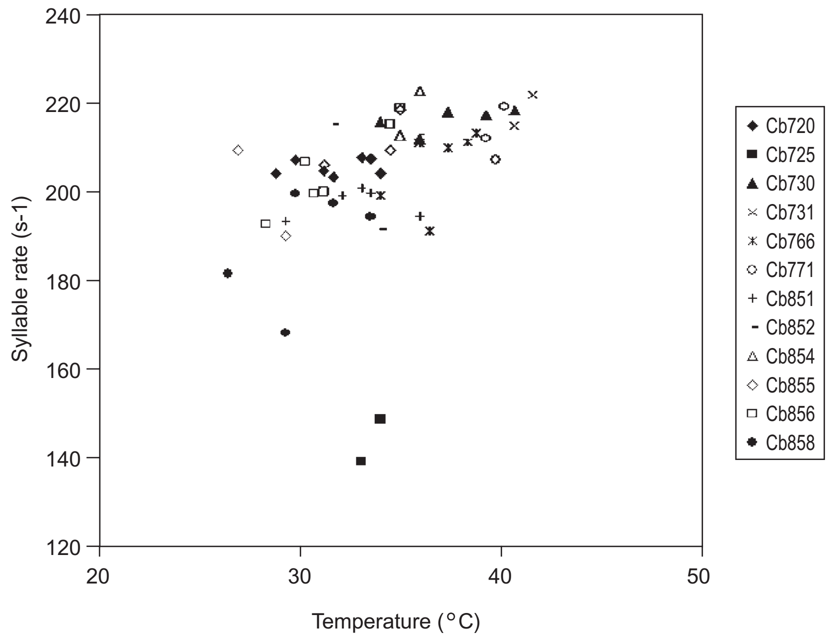

Fig. 4. Scatterplot of the syllable rate and ambient temperature for each individual of *Cicada barbara* at Crato, Portugal (in 1999) with 2 or more recordings during the course of a day.

Table 4. *p* values obtained from nonparametric tests (using the Mann-Whitney test for 2 samples and the Kruskal-Wallis test for more than 2 samples) comparing sampling years within each locality and localities for each species, *Cicada barbara* (A) and *C. orni* (B). ANCOVA results are also given, with the covariates temperature and time of day. *Significant after applying the Dunn-Sidák correction

(A)

	Among years at a locality			Among localities	After controlling for temperature and time of day
	Crato (5 yr)	Portel (2 yr)	Sousel (2 yr)	(3 localities)	
Peak frequency	0.447	0.965	0.545	0.004*	0.006*
Syllable rate	0.087	0.573	0.536	0.951	0.865

(B)

	Among years at a locality					Among localities	
	Alter-do-Chão (2 yr)	Piedade (2 yr)	Mte. Caparica (3 yr)	Crato (5 yr)	Monforte (3 yr)	(5 localities)	(4 localities, excluding Crato)
Peak frequency	0.397	0.007*	0.165	0.624	0.394	0.749	0.700
Syllable rate	0.001*	0.018	0.951	0.000*	0.516	0.001*	0.024
Echeme rate	0.694	0.692	0.597	0.000*	0.159	0.000*	0.050
Echeme duration	0.336	0.692	0.223	0.000*	0.329	0.000*	0.001*
Inter-echeme interval	0.955	0.937	0.093	0.003*	0.112	0.000*	0.312
Echeme period	0.694	0.692	0.597	0.000*	0.159	0.000*	0.052
Ratio echeme/interval	0.694	0.937	0.057	0.004*	0.236	0.000*	0.058

In contrast, the echeme rate and echeme period showed low levels of within-individual variations (with median values of < 5%) and among-individual variations (< 6%). Studies of mate preference in these species are necessary to assess the biological significance of the variation found in these calling-song properties.

Differences in values of calling-song properties among populations were generally higher than among years within the same population. Accordingly, in a genetic study based on microsatellites, no genetic structure was found among years within populations (Seabra et al. unpublished results). For *C. orni*, differences in song properties between populations were mainly due to the population at Crato. Also, every time variable from Crato showed significant differences between years, which could not be explained by temperature differences. However, the small sample sizes may have been responsible for these differences.

Effects of temperature

When analyzing variability levels of the acoustic properties of songs, it is important to be aware that they may be constrained by environmental characteristics, such as temperature (Gerhardt and Huber 2002). Time variables were more consistently related to temperature than to the frequency variable, as previously reported for both species (Seabra et al. 2006). The sound frequency of the songs is independent of temperature in cicadas, as expected from its sound-producing system (Sanborn 2006). In contrast, time variables, particularly the pulse rate, are influenced by body temperature, since tymbal muscles contract more rapidly and with greater force as the temperature of the muscle increases (Sanborn 1997 2006). In this study, we measured ambient temperatures but not body temperatures, which are not always the same in cicadas. In fact, several cicada species are known to thermoregulate (Sanborn 1997). Temporal properties are known to be dependent on ambient temperatures in some species but not in others (reviewed in Sanborn 2006).

Some of the recordings of *C. orni* made at Crato 1999 were previously analyzed for time variables using only a 10 s sample to calculate the

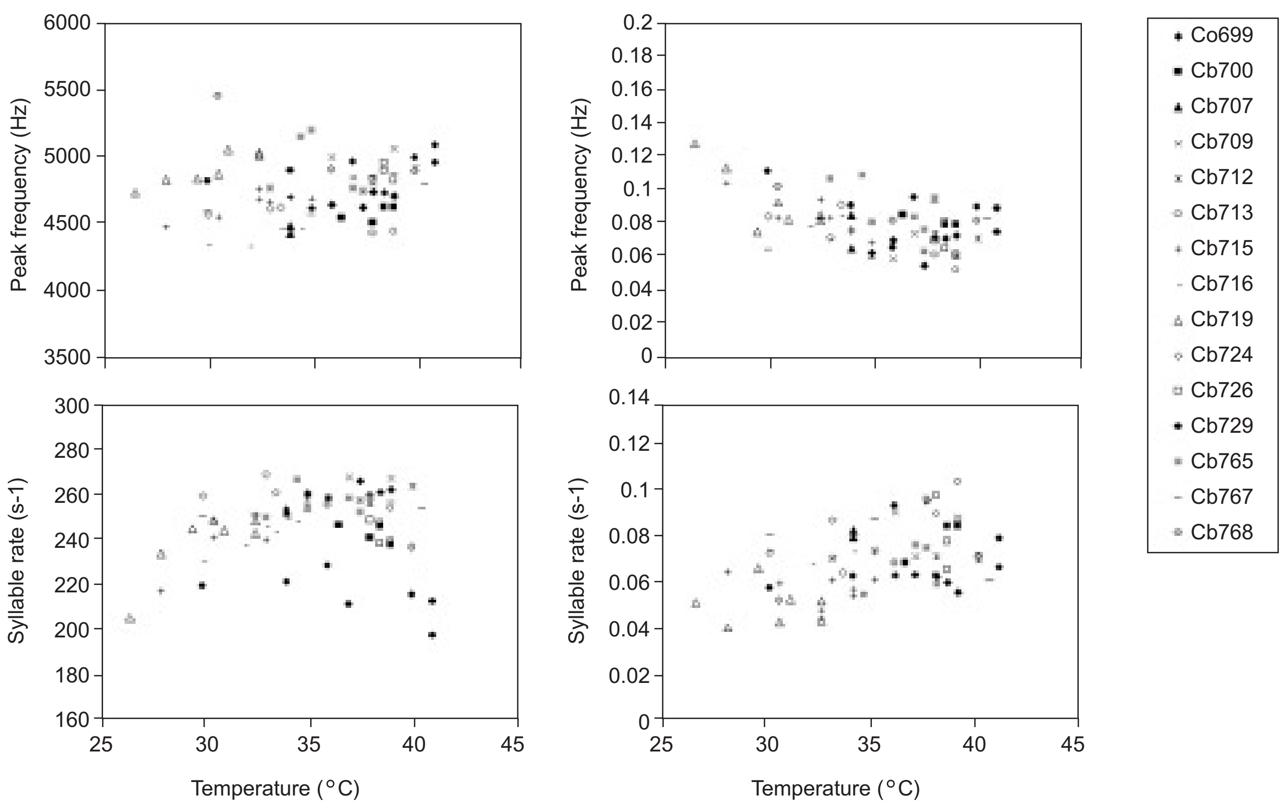

Fig. 5. Scatterplot of each acoustic variable and ambient temperature for each individual of *Cicada orni* at Crato, Portugal (in 1999) with 2 or more recordings during the course of a day.

echeme rate and 15 echemes to calculate the echeme and inter-echeme interval durations (Quartau et al. 2000). Even with such a small sample, the results were very similar to the ones obtained here (no correlation of ambient temperature with the echeme rate, a significant positive correlation with the inter-echeme interval, and a negative correlation with echeme duration, even though not significant in the last case at p = 0.079).

Generally, temperature was positively correlated with the syllable rate in both species. The echeme rate and echeme period in *C. orni* were not correlated with temperature but were correlated with the time of day (when controlling for temperature), which may indicate some "motivational" difference at different times of the day.

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