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Calling songs of sympatric and allopatric populations of *Cicada barbara* and *C. orni* (Hemiptera: Cicadidae) on the Iberian Peninsula

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Abstract. Calling songs of the sibling species *Cicada barbara* and *C. orni* were studied in sympatric and allopatric populations on the Iberian Peninsula, where the distribution ranges of both species overlap. No difference was found in any acoustic property for the sympatric and allopatric populations of *C. barbara* studied and only one variable (minimum frequency) was significantly different between sympatric and allopatric populations of *C. orni*. No hybrids with intermediate songs were found and no character displacement in the calling song was detected. It is very likely that these species were already considerably differentiated when they met on the Iberian Peninsula. Particularly, premating (or even postmating) isolating mechanisms (according to Mayr's Biological Species Concept) or different specific-mate recognition systems (in the view of the Paterson's Recognition Concept of Species) were most likely already present, which prevented hybridization between this pair of species. It is assumed that the calling songs are the most important premating isolating mechanism corresponding to the specific-mate recognition systems of these species of cicadas.

INTRODUCTION

Cicada barbara Stål and *C. orni* Linnaeus (Hemiptera, Cicadidae) are a pair of sibling cicada species, very similar in morphology, with only slight differences in external characteristics. Examples are differences in the wing spots (Quartau, 1988; Ribeiro, 1998) and in the male genitalia, namely in the lengths of the pygofer (and its dorsal spine), the tenth abdominal segment and its appendages (which are shorter in *C. barbara*) and in the width of the shaft of the aedeagus (thinner in *C. orni*) (Quartau, 1988). In contrast, the acoustic signals produced by the males of these two species are distinct, since *C. barbara* produces a continuous series of pulses without pauses (Boulard, 1982, 1995; Fonseca, 1991; Quartau & Rebelo, 1994) and *C. orni* a series of pulses forming echemes alternating with intervals of silence (Popov, 1975; Boulard, 1982; Fonseca, 1991; Quartau et al., 1999, 2000; Pinto-Juma et al., 2005). In addition, sequencing of the 12S rRNA mitochondrial gene indicates that these two species constitute distinct evolutionary units (Pinto-Juma et al., unpubl.).

Both *C. barbara* and *C. orni* occur in Mediterranean woodland or shrubland, mainly in olive, oak or pine tree groves, but also on other trees and shrubs and even on scattered vegetation in cities (Quartau, 1995). In Corsica *C. orni* was found by Puissant & Sueur (2001) on two types of vegetation: either it is 0.5 to 2 m high with ligneous plants covering more than 60% of the area or more than 2 m high with ligneous plants covering more than 40% of the area. Only *C. barbara* is present both in North Africa and the Iberian Peninsula. *C. orni* is distributed in south west, central and eastern Europe, western Asia and the Middle East (Popov, 1975; Quartau & Fonseca, 1988; Schedl, 1973, 1999). Moreover the Iberian populations of *C. barbara* are considered by Boulard (1982) to be a subspecies, *C. barbara lusitanica* Boulard, different from the type subspecies, which only occurs in north Africa (*C. barbara barbara* Stål).

The distribution ranges of *C. barbara* and *C. orni* overlap on the Iberian Peninsula, and males of both species in a few localities can be seen singing on the same trees at the same time. However, there is some seasonal displacement as *C. orni* emerges earlier in summer (June) than *C. barbara* (July/August). In allopatric populations, the adults of *C. orni* disappear in September/October, whereas in sympatric populations they usually disappear much earlier (August). *C. barbara* shows less variation: it appears somewhat earlier where it occurs allopatrically than sympatrically but disappears at the same time, in September/October (Ribeiro, 1998; and pers. obs.).

Despite the clear differences in the calling songs of males of *C. barbara* and *C. orni*, the structure of the tymbals and mechanism of sound production in these species is similar, as expected in closely related species of the same genus (Fonseca, 1991, 1994). In both species the inward distortion of one of the tymbals (membranes located in the first segment of the abdomen, which are distorted by the tymbal muscles) produces usually three pulses of sound and the outward distortion another pulse, which is usually superimposed on the inward pulses of the other tymbal (Fonseca, 1991). The tymbals alternate in the production of sound.

Males of *C. barbara* and *C. orni* sing continuously for hours, usually without changing their location. This pattern of behaviour is described in Cooley & Marshall (2001), as one extreme in the range of systems found in acoustically signalling insects, and is observed in other species, such as the Neotropical cicada *Fidicina mannifera* (Cocroft & Pogue, 1996), the Palaearctic *Tibicina* species (Quartau & Simões, 2003; Sueur & Aubin, 2004) and the Australian *Cystosoma saundersii* (Doolan, 1981). Males of both species of *Cicada* studied sing during the hottest part of the day, sometimes even at night if temperatures are well above 30°C.

The calling songs of cicada males are known to attract conspecific females in other species, for example in *Pycna semiclara* and *Azanicada zuluensis* (Villet, 1992), in *Cystosoma saundersii* and *Cyclochila australasiae* (Daws et al., 1997) and in *Magicicada* spp. (Cooley & Marshall, 2001), and are also known to result in the aggregation of males, for example in *Azanicada zuluensis* (Villet, 1992) and *Magicicada* spp. (Cooley & Marshall, 2001).

Experiments on the behaviour of cicadas in general, and *C. barbara* and *C. orni* in particular, are difficult in captivity (Fonseca & Revez, 2002a; Simões & Quartau, unpubl.). In the field, the males of both species often aggregate on the same trees, with a single male song often eliciting other males to sing. Moreover, females are often seen flying to the trunks or branches of trees where males are singing (pers. obs.). Playback experiments carried out with males of *C. barbara* (Fonseca & Revez, 2002a) has shown that females of this species can discriminate conspecific song from that of *C. orni*, as they respond (by singing) more quickly to the conspecific than heterospecific song. Also, altering the temporal pattern of the calling song of *C. barbara* reduce the response of the males, which do not respond to songs with pauses longer than 30 ms, which is more characteristic *C. orni* song, but respond to a modified *C. orni* song without pauses. No experiments were done yet about species discrimination by females.

Where sibling species occur sympatrically it is possible to address questions about isolation mechanisms and recognition processes. According to the Biological Species Concept, species are isolated from each other by "isolating mechanisms" (Dobzhansky, 1951), which include premating or postmating mechanisms (Mayr, 1948, 1963). According to Dobzhansky (1951), if two incompletely reproductively isolated species meet, after a period of allopatry, the reinforcement of isolating mechanisms might evolve by natural selection to prevent heterospecific matings. Under this scenario, sympatric populations would be expected to show more marked reproductive character displacement in mating mechanisms in comparison to allopatric populations. However, Paterson (1985) criticizes this concept since he considers the premating and postmating isolating mechanisms as effects, not evolving to serve the function of preventing hybridization between species. In his Recognition Concept of Species, species are seen as "that most inclusive population of individual biparental organisms which share a common fertilization system" (Paterson, 1985 p. 25). Speciation is seen by Paterson as an incidental effect resulting from the adaptation of the characters of the fertilization system (or Specific-Mate Recognition System – SMRS), among others, to a new habitat. According to the same author, if two populations have interpopulation "sterility" (heterozygote disadvantage) but share a common SMRS, they cannot coexist, since natural selection will act to eliminate the cause of hybrid disadvantage, in which case, reproductive character displacement is not expected (Paterson, 1985).

Evidence of reproductive character displacement is difficult to find. Marshall & Cooley (2000) report a case in one pair of north-American cicada species, *Magicicada tredecim* and *M. neotredecim*. These authors found that *M. neotredecim* produces higher dominant frequency calls when it occurs sympatrically than allopatrically with *M. tredecim*, but the latter species maintains the same frequency throughout their distribution range. This is the expected pattern if there is a process of reinforcement driven by selection against wasteful heterospecific matings (Marshall & Cooley, 2000). Alternatively, this character displacement could be viewed as an adaptation of *M. neotredecim* to the acoustic environment created by *M. tredecim*. In fact, the background noise created by the calling activity of another species may affect the receiver's ability to perceive signals and promote directional selection of the signal produced (Villet, 1995).

In this paper the evidence for hybrids with intermediate characteristics in calling songs between *C. barbara* and *C. orni* was assessed. Differences between the calling songs of sympatric and allopatric populations of each species on the Iberian Peninsula, particularly, whether the songs were more similar or different when species occurred sympatrically, were also investigated.

MATERIAL AND METHODS

Field procedures

Eleven populations of *C. barbara* and eight of *C. orni* were sampled on the Iberian Peninsula where these species occurred allopatrically (six for *C. barbara* and four for *C. orni*) and sympatrically (five for *C. barbara* and four for *C. orni*) (Fig. 1 and Table 1).

Male cicadas were located by their calling songs. Recordings of the acoustic signals were made using a Sony Dat recorder (TCD-D10 ProII; frequency range 20–22000 Hz; sampling frequency 44.1 kHz) connected to a dynamic Sony F-780 microphone (frequency responses 50–18000 Hz). Ambient temperature was taken in the shade at the time of each recording at all localities except Alcalar, Alvor, Monforte, Casalinho and Piedade.

Sound analysis

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. Time and frequency analysis were performed on one-minute recordings of each individual (whenever possible). Fast Fourier transformation with a resolution of 512 points and a Hamming Window was applied to compute the frequency spectra (Fig. 2). The gross-temporal variables analysed in *C. orni* were: number and duration of echemes and duration of the interval between them (interecheme interval) (Fig. 2). Echeme rates, periods and ratios echeme/interval were then calculated. Spectrum-based variables analysed for both species were: peak frequency, minimum frequency, maximum frequency, bandwidths, and quartiles (described in Pinto-Juma et al., 2005). In *C. orni*, all frequency measurements were calculated from the mean spectrum of each echeme. Time and frequency measurements of the echemes were then averaged, and the mean was taken as the value of the variable for each speci-

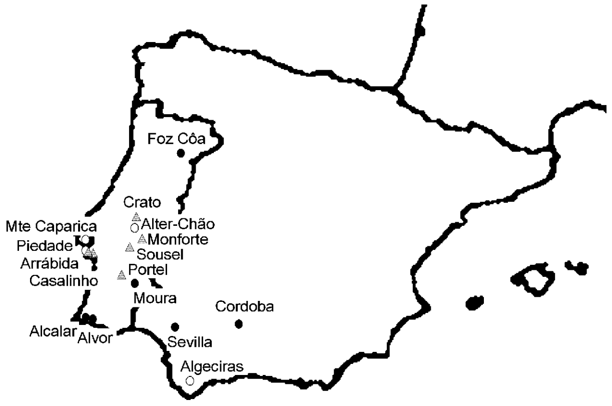

Fig. 1. Allopatric populations of *Cicada barbara* (dark circles) and *C. orni* (empty circles), and sympatric populations of both species (triangles) sampled on the Iberian Peninsula

men. A fine-temporal property of the signals, the syllable rate, was also calculated for both species. The number of groups of pulses (syllables) was counted in 30 echemes for *C. orni* (first and last syllables in each echeme were discarded due to their different characteristics – see Fig. 2) and 30 fragments of c. 0.1 s for *C. barbara*. The average number of syllables per unit of time was calculated for each specimen. These syllable rates correspond to the production of pulses by the two tymbals.

Statistical analysis

Nonparametric Mann-Whitney tests were used to compare each acoustic variable between species and between sympatric and allopatric populations of each species. Coefficients of variation for each acoustic variable were compared between species using the nonparametric Wilcoxon signed rank test. Spearman nonparametric correlations between ambient temperature and each acoustic variable were also computed for each species. The significance of multiple tests was assessed by reducing the critical P value according to the Dunn-Sidák method (Dytham, 2003), from 0.05 to 1–(0.951/k), where k is the number of tests performed.

Multivariate analysis was applied to the data matrix of 9 acoustic variables (the variables common to both species) measured for 158 individuals, namely a Principal Components Analysis (PCA) and a Discriminant Function Analysis (DFA). PCA is used to reduce a large number of variables to a smaller number of factors (or components), with no need to specify a dependent variable. The percentage of variance explained by each of the components is given, as well as the correlation coefficients between the variables and the components (component loadings). The component scores obtained for the individuals can be used to compare groups (in this case there are four groups: allopatric *C. barbara*, sympatric *C. barbara*, sympatric *C. orni* and allopatric *C. orni*) with Kruskall-Wallis and Mann-Whitney tests. In DFA, on the other hand, groups are determined beforehand. It derives discriminant functions that best separate the groups. The statistical significances of the functions in discriminating the groups are given, as well as the correla-

TABLE 1. Populations of *Cicada barbara* and *C. orni* sampled on the Iberian Peninsula in areas where they occurred allopatrically and sympatrically.

Localitites	N	Dates of recording	Temperatures (°C)
C. barbara occurs allopatrically			
Sevilla (Andalucía, Spain)	7	6/8/2001	38–41
Córdoba (Andalucía Spain)	5	6/9/2000	34
Alcalar (Algarve, Portugal)	10	23/8/1995	–
Alvor (Algarve, Portugal)	3	28/8/1995	–
Moura (Baixo Alentejo, Portugal)	11	28/8/2001	31–34
Foz Côa (Beira Alta, Portugal)	3	11/7/1999	34
C. barbara occurs sympatrically			
Portel (Alto Alentejo, Portugal)	10	24/7 and 10/8/2001	31–35
Sousel (Alto Alentejo, Portugal)	11	8/9/2001	33–35
Monforte (Alto Alentejo, Portugal)	6	22/7 to 24/7/1995	–
Crato (Alto Alentejo, Portugal)	14	6/7 to 8/7/1999; 15/7/1999; 1/8 to 3/8/1999	26–41
Casalinho (Estremadura, Portugal)	5	27/7/1995	–
C. orni occurs allopatrically			
Algeciras (Andalucía, Spain)	10	5/8/2001	31–34
Alter-do-Chão (Alto Alentejo, Portugal)	7	6 to 9/8/1997	25–30
Piedade (Arrábida, Estremadura, Portugal)	10	19/7 to 12/8/1995	–
Monte-da-Caparica (Estremadura, Portugal)	7	16 to 22/9/1997	25–30
C. orni occurs sympatrically			
Sousel (Alto Alentejo, Portugal)	11	27/6/2003	27–30
Monforte (Alto Alentejo, Portugal)	16	25/7 to 7/8/1997	23–38
Crato (Alto Alentejo, Portugal)	8	27/6/2001	24–26
Arrábida (Estremadura, Portugal)	4	18/8 and 10/9/1997	30
TOTAL	158		

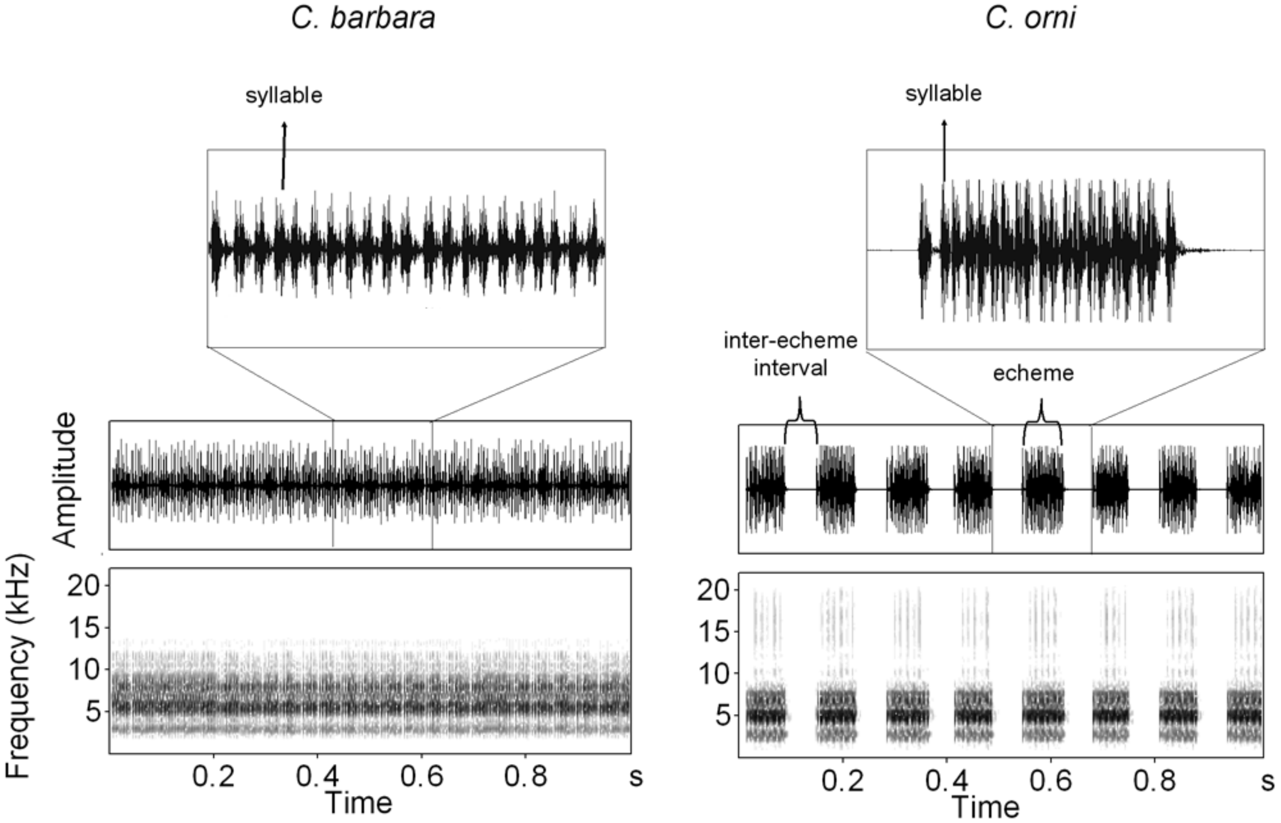

Fig. 2. Oscillograms (amplitude vs. time) and sonagrams (frequency vs. time) of the calling songs of *Cicada barbara* and *C. orni*.

tions of each variable to each discrimination function. All statistical analyses were carried out in SPSS Version 10.0.

RESULTS

Cicada barbara and *C. orni* differed significantly in every acoustic variable compared (Mann-Whitney, $p < 0.001$). The average values of frequency variables of *C. orni* were always lower than those of *C. barbara*. The peak frequency was 4709.8 Hz ± 452.44 (average ± standard deviation) in *C. orni* and 6283.5 ± 476.40 in *C. barbara*. On the other hand, the syllable rate was on average higher in *C. orni* (224.3 ± 25.00) than, in *C. barbara* (201.4 ± 18.78) (Table 2, Fig. 2). The syllable periods, derived from the syllable rates, ranged from 3.96 to 7.27 ms in *C. barbara* and from 3.84 to 6.72 ms in *C. orni*. The syllable rates for only one of the tymbals are half and the syllable periods double of these values.

The inter-individual coefficients of variation of the frequency variables varied from 6.24% to 20.85% in *C. barbara* and from 6.15% to 18.79% in *C. orni*. The syllable rate has a coefficient of variation of 9.35% in *C. barbara* and of 11.20% in *C. orni*. No significant difference between species was found in the coefficients of variation for the acoustic variables (Wilcoxon test, $p = 0.859$). The inter-individual coefficients of variation for the temporal variables in *C. orni* were higher than the frequency variables and ranged from 24.90% to 68.38%.

No significant differences in any of the acoustic variables were found between sympatric and allopatric populations of *C. barbara* (Mann-Whitney tests). In *C. orni*, only the minimum frequency differed significantly between sympatric and allopatric populations (Mann-Whitney test, $p = 0.002$), with lower values when it occurred sympatrically than allopatrically (values for sympatric populations deviating more from those of *C. barbara*) (Fig. 3). This difference was not due to differences in temperature as no frequency variable significantly correlated with temperature. On the other hand, ambient temperature was found to be positively correlated with the syllable rate in both *C. barbara* and *C. orni* (Fig. 4) ($r_S = 0.360$, $p = 0.006$ for *C. barbara* and $r_S = 0.714$, $p < 0.001$ for *C. orni*). The correlation for *C. barbara* was only marginally significant after applying the Dunn-Sidák correction (critical *P* value = 0.0057), while that for *C. orni* was still strongly significant (critical *P* value = 0.0037). In *C. orni*, the echeme duration was negatively correlated ($r_S = -0.631$, $p < 0.001$), the echeme rate was positively correlated ($r_S = 0.574$, $p < 0.001$) and the echeme period negatively correlated ($r_S = -0.574$, $p < 0.001$) with temperature. The correlations between inter-echeme interval and temperature, and between the ratio echeme/interval and temperature were not significant ($r_S = 0.030$, $p = 0.827$ and $r_S = -0.329$, $p = 0.012$, respectively) after applying the Dunn-Sidák correction.

In the PCA, the percentage of variation explained by the first four components was 95.3% (64.1% by the first component alone, 80.0% by the first two and 89.2% by the first three). The component loadings were high for all variables in the first component (from 0.642 for band-

Table 2. Average ± standard deviation (SD), minimum (Min.), maximum (Max.) and coefficient of variation (CV, corrected for small size samples as in Sokal & Rohlf (1981)) of the acoustic variables of the calling songs of *Cicada barbara* and *C. orni* recorded on the Iberian Peninsula.

Acoustic variables	*C. barbara*					*C. orni*				
	N	Average ± SD	Min.	Max.	CV(%)	N	Average ± SD	Min.	Max.	CV(%)
Peak frequency (Hz)	85	6283.5 ± 476.40	5080	7660	7.60	73	4709.8 ± 452.44	3851	6199	9.64
Minimum frequency (Hz)	85	1830.7 ± 337.53	2150	4730	11.96	73	2125.5 ± 372.79	1583	3952	17.60
Maximum frequency (Hz)	85	10840.5 ± 1699.39	7920	17910	15.72	73	9100.2 ± 1185.43	7239	13144	13.07
Bandwidth (Hz)	85	8006.2 ± 1664.69	5160	15330	20.85	73	6970.2 ± 1305.07	3626	11174	18.79
Quartile 25% (Hz)	85	5839.1 ± 421.82	4300	6460	7.24	73	4572.4 ± 280.27	3854	5011	6.15
Quartile 50% (Hz)	85	6665.2 ± 414.62	5680	7490	6.24	73	5472.0. ± 420.26	4611	6280	7.71
Quartile 75% (Hz)	85	8543.2 ± 715.16	6460	9730	8.40	73	6894.6 ± 517.50	5405	8085	7.53
Quart75%–Quart25% (Hz)	85	2704.1 ± 452.18	1300	3620	16.77	73	2321.9 ± 363.30	1242	3170	15.70
No. syllables/s	82	201.4 ± 18.78	137.6	252.3	9.35	53	224.3 ± 25.00	148.7	260.3	11.20
No. Echemes/s						73	5.37 ± 1.333	2.38	7.49	24.90
Echeme duration (s)						73	0.078 ± 0.0308	0.039	0.207	39.58
Inter-echeme interval (s)						73	0.123 ± 0.0720	0.058	0.362	58.53
Echeme period (s)						73	0.201 ± 0.0649	0.134	0.420	32.34
Ratio echeme/interval						73	0.894 ± 0.6093	0.166	3.314	68.38

width to 0.969 for quartile 75% in frequency variables and –0.484 for syllable rate). All the variables were significantly correlated, except for minimum frequency *vs*. bandwidth. There were no significant differences in the component scores of Component 1 and 2 between sympatrically and allopatrically occurring specimens of the same species (Mann-Whitney test, $p > 0.05$), but the sympatrically occurring specimens had a greater dispersion in the scatterplot (Fig. 5) than the allopatrically occurring ones in both species on both axis. This greater dispersion is probably the reason for the slight proximity observed between some sympatric specimens of one species and those of the other species.

DFA gave an overall correct classification rate of 62.96%. Three functions were computed but only one was significant (Wilk's $\lambda = 0.178$, $p < 0.0001$) and accounted for 97.6% of the variation. The structure matrix indicated that the frequency variables quartile 25%, peak frequency, quartile 50%, quartile 75% and minimum frequency were the most important in determining Function 1. The classification table (Table 3) showed that almost every *C. barbara* and *C. orni* individual where correctly classified to species, with the exception of three individuals (one sympatric *C. barbara* was classified as allopatric *C. orni*; one sympatric *C. orni* was classified as sympatric *C. barbara*; and one allopatric *C. orni* was classified as sympatric *C. barbara*). However, a substantial number of individuals of both allopatric and sympatric groups were misclassified. Individuals of *C. orni* that occurred sympatrically were mostly classified in the allopatric group. When cross-validating, only 48.9% of the grouped samples were correctly classified, which indicates that this analysis did not discriminate between these groups.

When performing the Discriminant Analysis separately for each species, the discriminant function separating sympatric and allopatric *C. barbara* was significant (Wilk's $\lambda = 0.828$, $p = 0.044$) and correctly classified 65.9% of the original samples and 53.7% of cross-validated samples. The variables that were more highly correlated with the discriminant function were quartile 50% (0.621), peak frequency (0.531), syllable rate (–0.504) and minimum frequency (0.493). When the Discriminant Analysis was applied to allopatric and sympatric *C. orni*, the discriminant function was not significant (Wilks' $\lambda = 0.872$, $p = 0.479$, when the nine variables common to both species were used; and Wilks' $\lambda = 0.743$, $p = 0.260$, when all 14 variables were used).

DISCUSSION

The calling songs of *Cicada barbara* and *C. orni* are easily distinguished by the human ear since the first spe-

Table 3. Classification of groups using the Discriminant Function Analysis. Values are percentages of samples (individuals) in the actual group predicted to belong to each group. Number of samples (individuals) in parentheses.

Actual Group	Predicted group membership			
	allopatric Cb	sympatric Cb	sympatric Co	allopatric Co
allopatric *C. barbara*	56.8% (21)	43.2% (16)	0.0% (0)	0.0% (0)
sympatric *C. barbara*	26.7% (12)	71.1% (32)	0.0% (0)	2.2% (1)
sympatric *C. orni*	0.0% (0)	3.3% (1)	76.7% (23)	20.0% (6)
allopatric *C. orni*	0.0% (0)	4.3% (1)	56.5% (13)	39.1% (9)

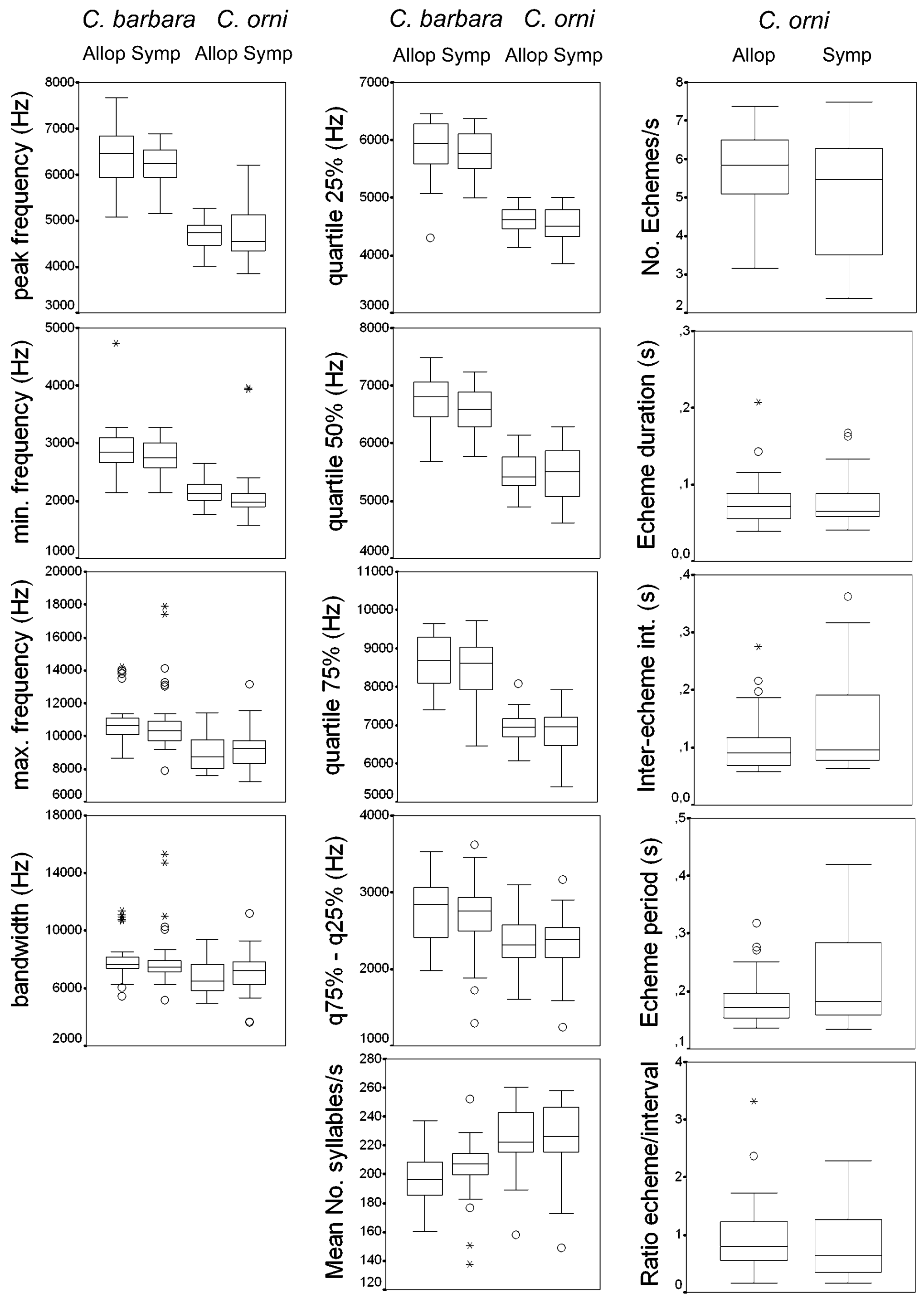

Fig. 3. Boxplots of the acoustic variables of the songs of *Cicada barbara* and *C. orni* occurring allopatrically (Allop) and sympatrically (Symp).

cies produces a continuous shrill and the latter a successive series of short shrills alternating with short pauses. At the acoustic frequency level these species are also generally distinct, *C. barbara* produces a higher peak frequency (average of 6.3 kHz) than *C. orni* (average of 4.7 kHz), with a difference of more than 1 kHz between the averages for each species. However, this is not a totally diagnostic characteristic because there is an overlap between species (see Fig. 3). In fact, some *C. orni* males produce a sound with a peak frequency above 6 kHz and some *C. barbara* males a peak frequency as low as 5 kHz. Also the syllable rates of the two species overlap substantially, despite the average being significantly higher in *C. orni* than in *C. barbara*. In fact, the presence of a few specimens that, in some song variables, were similar to those of the other species caused the Principal Components Analysis and the Discriminant Function Analysis to indicate an incomplete separation between the species.

Differences in the frequency of the sounds are generally related to the size of the sound producing or resonator

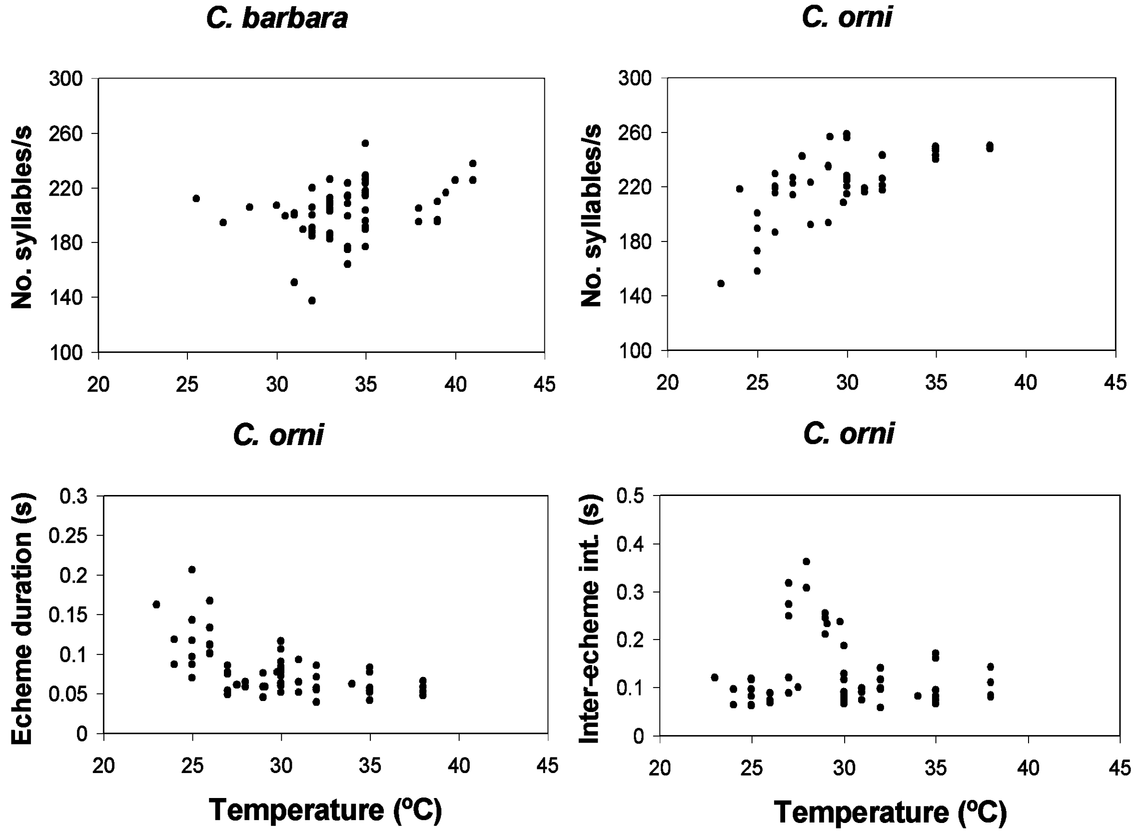

Fig. 4. Scatterplots of the number of syllables per second relative to temperature in *Cicada barbara* and *C. orni* and of both echeme duration and inter-echeme interval relative to temperature in *C. orni*.

organ (Young & Josephson, 1983). A significant negative correlation is usually found between body length and the dominant song frequency in cicada species, with larger species producing lower dominant frequency songs (Bennet-Clark & Young, 1994). This also appears to be the case in the pair of species analysed since *C. barbara* is usually smaller than *C. orni* (Ribeiro, 1998).

Variability in the frequency characteristics of calling songs of cicadas of these species was relatively low (coefficient of variation usually below 20%), as expected since these characteristics are constrained by physical properties of the sound-producing organ. On the other hand, the variability of the gross-temporal characteristics in *C. orni* was high, as also found by Pinto-Juma et al. (2005). However, the fine-temporal characteristic of the songs of both species, the syllable rate, had similar coefficients of variation to the ones of the frequency variables. According to Gerhardt (1994), many female insects choose males whose signals have species-typical values of fine-temporal properties, such as pulse rate or duration. This would lead to stabilizing selection on these characteristics of the song.

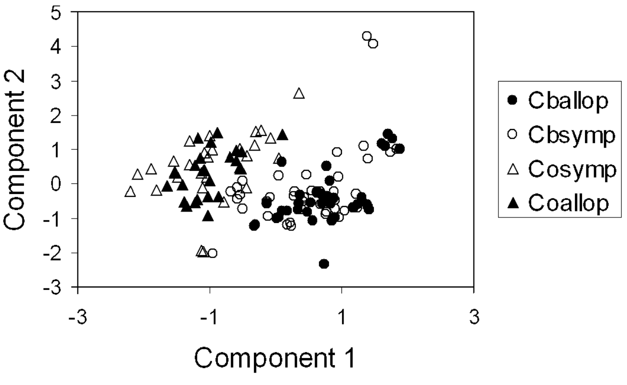

Fig. 5. Scores of the first two PCA components extracted from a data matrix, which was composed of 9 acoustic variables measured for 158 individuals of *Cicada barbara* (Cb) and *C. orni* (Co) occurring allopatrically (allop) and sympatrically (symp).

However, the variability of the characteristics of each song should be analysed with caution, since the estimate of the variability is dependent on our ability to measure it, which might be different in scaling from the perceptual systems of the intended receivers (McGregor, 1991).

No cicadas with songs intermediate between *C. barbara* and *C. orni* were found in this study. All male cicadas were clearly identified by the gross-temporal differences in their songs (*C. barbara* song is continuous and that of *C. orni* discontinuous). The overlap between species in some song variables is believed to be due to natural variation and not hybridization. Moreover, there was no evidence of character displacement in the acoustic variables studied. Only one acoustic variable (minimum frequency) was significantly different in allopatric and sympatric populations of *C. orni* showing a deviation from the *C. barbara* values. The multivariate analyses did not indicate any consistent differentiation between sympatric and allopatric populations at the acoustic level.

Also, previous morphometric work, based on the head, wings and male genitalia of *C. barbara* and *C. orni*, did not reveal differences between sympatric and allopatric populations (Ribeiro, 1998).

It is likely that the calling songs, which are most certainly part of the Specific-Mate Recognition Systems in these species, were already sufficiently differentiated before these species came into contact, and so, no hybridization occurred. In fact, at the mtDNA level, *C. barbara* and *C. orni* are highly divergent distinct lineages (Pinto-Juma et al., unpubl.). In terms of isolating mechanisms, the calling song is a prezygotic isolating mechanism that prevents hybridization between species. Since this mechanism was already differentiated before the species came into contact, acoustic character displacement was not necessary to ensure the correct selection by conspecific females. According to Gerhardt (1994), if the signals are already differentiated before the species come into contact, selection would only need to sharpen the selectivity of females, an aspect that should also be studied in these cicadas. *C. barbara* males can discriminate the frequency and temporal characteristics of their song and react preferentially to conspecific songs than to *C. orni* song (Fonseca & Revez, 2002a), but the preferences of females are unknown.

The overlap in song frequencies between the two species could interfere with the communication in these species, particularly, *C. barbara* song could "mask" *C. orni* song since it is continuous and make the temporal characteristics of *C. orni* song more difficult to perceive by receivers. The fact that late in the summer season, *C. orni* is not or rarely found where they occur sympatrically while it remains abundant where it occurs on its own, might be a result of "acoustic competition". Different ecological adaptations could eventually reduce this competition. For instance, different singing positions in the vegetation of cicada species that occur sympatrically is described for *C. orni/Lyristes* (Claridge et al., 1979) and for *C. orni/Tibicina haematodes* (Sueur & Aubin, 2003). Such segregation may also occur between *C. orni* and *C. barbara*, as observed qualitatively by Ribeiro (1998). Boulard (1982) records in Arrábida *C. barbara* singing on the seaward side and *C. orni* on the inland side of the mountain. However, in olive orchards at some sampling sites (e.g. Crato, Portel and Sousel) it was common to see males of both species singing on the same trunks or branches. A study of the micro-habitats occupied by these species (including their singing position on the trees) throughout the summer season (when only *C. orni* is present, when both species are present and when only *C. barbara* is present) is needed to test for ecological adaptations.

In order to exclude any potential effect geographic isolation, only Iberian Peninsula populations were analysed in this study. In fact, as detected previously, populations of these species from other areas differ from the Iberian Peninsula populations (Quartau et al., 1999, Pinto-Juma et al., 2005; Pinto-Juma et al., unpub.). The inter-echeme interval in *C. orni* song is longer in Greek than in Iberian Peninsula populations and the peak frequency is on average higher in Greece (Pinto-Juma et al., 2005). All frequency variables were higher in *C. barbara* from Morocco than from the Iberian Peninsula. It is most likely that these differences in *C. orni* populations are due to geographic distance, since the calling songs in France are similar to those in the Iberian Peninsula. In *C. barbara,* the differences could also be due to geographic isolation between North African and Iberian cicadas. Ecological factors, such as habitat and climatic conditions could be responsible for the direct or indirect selection of certain sound frequencies, specifically the lower frequencies found in both species on the Iberian Peninsula.

In the genus *Cicada* this is the only pair of species known to occur sympatrically. Other species like *C. mordoganensis* Boulard and *C. cretensis* Quartau & Simões, which are present in the Aegean area, have a calling song very similar in temporal and frequency pattern to that of *C. orni*, which is also present in the same region (Simões et al., 2000; Quartau & Simões, 2005), but they are not known to occur sympatrically with *C. orni*. It would be interesting to know if *C. lodosi* Boulard, which has a continuous song like *C. barbara*, but occurs in Turkey (Boulard, 1979), occurs sympatrically with any of the species with discontinuous songs.

The syllable rate increased significantly with temperature in *C. orni*, but only marginally significantly in *C. barbara*. In another study on more populations of *C. barbara* this correlation is significant (Pinto-Juma et al., in prep.). Also, Fonseca (1991) records a temperature dependent syllable period in *C. barbara* and an increase in syllable rate with temperature is described for other cicada species, such as *Tettigeta argentata*, *Tettigeta josei* and *Tympanistalna gastrica* (Fonseca & Revez, 2002b).

In *C. orni* the echeme duration decreased significantly with increasing temperature and there was a very low non-significant correlation between the inter-echeme interval and temperature. In a previous study of one population of *C. orni* at Crato, several recordings per individual at different temperatures showed the same variation with temperature in echeme duration and inter-echeme interval as recorded in this study, though the significance values are marginal for echeme duration and below the critical 0.05 value for inter-echeme interval (echeme duration: $r_S = -0.248$, $p = 0.079$; inter-echeme interval: $r_S = 0.339$, $p = 0.015$) (Quartau et al., 2000). These differences could be due to a sampling effect or other factors. In fact, as reported before by Pinto-Juma et al. (2005), the population at Sousel had longer inter-echeme intervals than other *C. orni* populations, which is not a temperature dependence outcome, since longer intervals would be expected at higher temperatures (Quartau et al., 2000) and Sousel individuals were sampled at temperatures ranging from only 27°C to 30°C. Excluding this population, the results in terms of correlations remained the same (inter-echeme interval positively but not significantly correlated with temperature). The conditions under which *C. orni* population at Sousel were recorded might have been unusual and not detected by the researchers. Nevertheless, Sousel is an area where *C. orni* and *C. barbara* occur sympatrically, and thus, a character displacement might be occurring in which longer silent pauses occur between echemes in the song of *C. orni*, making it more different from the song of *C. barbara*, which has no silent pauses. However, this hypothesis is not consistent with the findings for other areas where these species occur sympatrically. Furthermore, some cicadas from Piedade, where *C. orni* occurs allopatrically have also long inter-echeme intervals compared to other populations (Pinto-Juma et al., 2005). However, this is also inconclusive since the environment temperature was not taken at the time of the recording in this population.

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