

Calling Song Structure and Geographic Variation in *Cicada orni* Linnaeus (Hemiptera: Cicadidae)

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Gabriela Pinto-Juma, Paula C. Simões, Sofia G. Seabra, and José A. Quartau (2005) Calling song structure and geographic variation in *Cicada orni* Linnaeus (Hemiptera: Cicadidae). *Zoological Studies* 44(1): 81-94. An analysis of the structure of the calling song of *Cicada orni* Linnaeus, 1758 over a selected part of its distribution range in the Mediterranean area was carried out in order to better understand the pattern of its geographic variation and population history. Calling songs of males from several localities in western and southeastern Europe were recorded from 1995 to 2003, and were analysed in time and frequency domains. The calling songs analyzed constituted a relatively homogeneous group. However, there was some tendency of songs from southeastern Europe (Greece) to group apart from those of western Europe (Iberian Peninsula and France). The inter-echeme interval duration was the variable that contributed most to this separation, but males from Greece showed significant differences in almost every acoustic variable in relation to the remaining studied regions. This acoustic differentiation is in agreement with introductory genetic results still under investigation. Echeme duration proved to be quite constant across the geographic range of this cicada and, as such, is probably one of the most important parameters encoding species-specific information for species recognition. Conversely, the inter-echeme interval was quite variable, and so it is expected that this variable is not an important parameter for species recognition and isolation in *C. orni*. The observed distinctiveness of the populations of the Aegean area may be the result of repeated cycles of isolation in southern refugia through the mountain ranges of the Balkans during the ice ages. Furthermore, it is hypothesized that the Aegean area and West Asia Minor might constitute the main area of origin of *C. orni*. <http://www.sinica.edu.tw/zool/zoolstud/44.1/81.pdf>

Key words: *Cicada orni*, Calling song, Acoustic signals, Acoustic divergence, Geographic variation.

Cicada orni Linnaeus is one of the most abundant and common cicadas throughout the Mediterranean area, being very familiar for the striking calling songs produced by males during summertime. It is distributed from the Iberian Peninsula in western Europe to Greece and Turkey and some countries in the near East (Nast 1972), as well as around the Black Sea (Popov 1975). This cicada is known to occur in closed high shrubland and woodland (Patterson et al. 1997, Puissant and Sueur 2001, Sueur et al. 2004), more commonly occurring in olive trees, pine trees, oak trees, and also eucalyptus and vineyards.

As in other cicadas, males of *C. orni* produce loud airborne acoustic signals by means of a tymbal mechanism. The tymbals are ribbed cuticle membranes located dorsolaterally in the 1st segment of the abdomen that are distorted by the action of powerful muscles (e.g., Pringle 1954, Popov 1975, Bennet-Clark 1998). Male cicadas can produce different kinds of acoustic signals (e.g., Alexander 1967, Boulard 1995 2000a, Fonseca 1991), with the calling song being the most common one. This is typically regularly patterned and species-specific, and is involved in mate attraction (as shown in *Magiccicada* spp. (Cooley and Marshall 2001)) as well as in male

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aggregation and chorusing (as in *C. mordoganensis* (Simões et al. 2000) or in other cicadas as referred to by, for instance, Villet (1992) and Fonseca and Revez (2002). Therefore, according to Paterson's recognition concept of species, the calling song constitutes a distinct specific-mate recognition system (SMRS) that should remain relatively constant by stabilizing selection across the distribution range of the species (Paterson 1985).

Cicada orni belongs to a complex of species which are morphologically very similar but which differ mostly in the calling songs that males produce. Thus, the study of these acoustic signals is very important in the delimitation of close species, as well as to better understand the speciation process.

Several authors have described the calling song of *C. orni* (e.g., Popov 1975, Joermann and Schneider 1987, Fonseca 1991, Boulard 1995) for a few local populations of this species. However, studies on the acoustic variation across the distribution area of cicadas are rare, with the exception of some work on American cicadas (e.g., Moore 1993). For *C. orni*, introductory comparisons of the calling songs in populations from southern France with those from the former USSR showed no obvious geographic variation (Claridge et al. 1979, Claridge 1985). Later, Quartau et al. (1999), when comparing Portuguese with Greek populations, revealed some geographic variation in *C. orni* songs.

The present paper is a more-thorough analysis of the structure of the calling song of *C. orni* over a wider distribution range of the species in the Mediterranean region in order to complement previously known data and to better understand the

pattern of its geographic variations.

MATERIAL AND METHODS

Males of *C. orni* from several populations of several different regions along the geographic distribution of the species were recorded during the hottest season (June to Aug.) from 1995 to 2003. Eight populations from the Iberian Peninsula region, three from the south of France, seven from continental Greece, and one specimen each from Corsica and Turkey, totaling 176 specimens, were sampled (Fig. 1, Table 1). Some of the French recordings, as well as the recording from Corsica were provided by Jérôme Sueur (Muséum national d'Histoire Naturelle, Paris, France).

Males were first located by their calling songs, and their songs recorded, followed by collection of specimens by hand or by means of a sweep net. Most of the recordings were carried out using a Sony Dat recorder (TCD-D10 ProII and TCD-D8; at frequency ranges of 20~22,000 and 20~20,000 Hz, respectively, and at a sampling frequency of 44.1 kHz) connected to a dynamic Sony F-780 microphone or a Telinga Pro4PiP microphone (with frequency responses of 50~18,000 and 40~18,000 Hz, respectively). Other recordings were made using a UHER 4200 Report Monitor (with a sampling frequency of 44.1 kHz and a frequency range of 20~25,000 Hz) with an AKG D202 dynamic microphone. The microphone was placed at a distance of at least 30 cm from the calling insect. Acoustic recordings and specimens were kept in the Department of Animal Biology with one of the authors (J.A.Q.).

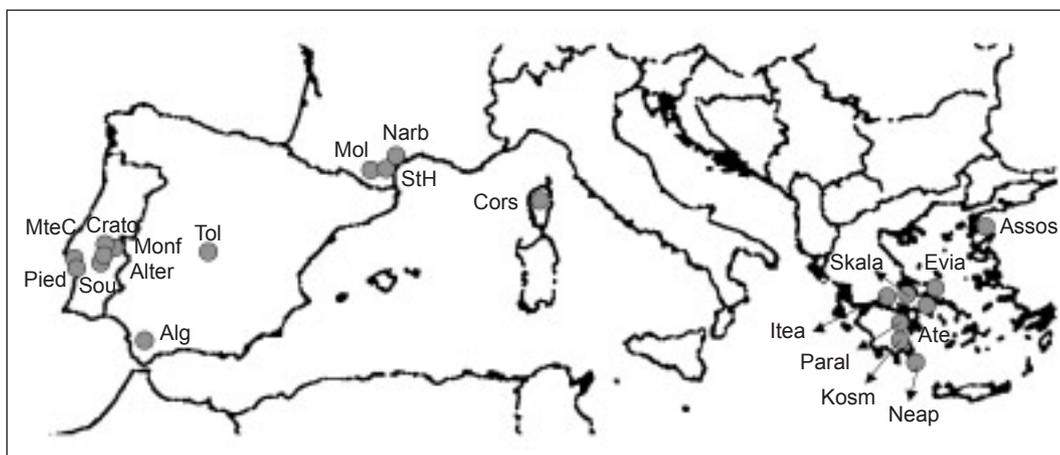


Fig. 1. Map showing sampling localities for *Cicada orni* acoustic signals (for abbreviations see table 1).

Recordings were taken between 09:00 and 18:00 with temperatures ranging from 23 to 38°C.

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, and the time and frequency domains were analyzed. For each specimen, whenever possible, a 1-min recording was used to produce oscillograms, sonagrams (or spectrograms), and mean amplitude spectra (Fig. 2). In the frequency domain, spectra were computed using Fast Fourier transformation with a resolution of 512 points and a Hamming Window.

Avisoft software allows the automatic measurement of time variables derived from the spectrogram, namely the number and duration of acoustic elements (in this case the echemes, which are composed of groups of pulses), and the duration of the interval between them (inter-echeme interval). Spectrum-based variables (peak frequency, minimum frequency, maximum frequency, bandwidths, and quartiles) were also

automatically obtained for each echeme (Fig. 2, Table 2). 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 specimen.

Due to a strong background noise of chorusing cicadas in some samples, which prevented the distinction of pulses, variables like the number of pulses per unit of time were discarded. Moreover, missing data relative to temperature in some populations did not allow us to take this environmental factor into account.

The selected variables were measured for each specimen analyzed. Statistical tests were made using STATISTICA 6.0 software (StatSoft 2001). Variables were not normally distributed, and so nonparametric tests were applied.

The correlation coefficients (Spearman rank order correlations) between all pairs of variables were calculated to determine the relationship

Table 1. Sampled populations of *Cicada orni* with abbreviation names (Abbrev.), number of specimens recorded for sound analyses (*n*), dates of recording, and ambient temperatures

Locality	Abbrev.	<i>n</i>	Dates of recording	Temperature range (°C)
IBERIAN PENINSULA				
Algeciras (Andalucía, Spain)	Alg	10	5 Aug. 2001	31~34
Sousel (Alto Alentejo, Portugal)	Sou	11	27 June 2003	27~30
Alter-do-Chão (Alto Alentejo, Portugal)	Alter	7	6~9 Aug. 1997	25~30
Monforte (Alto Alentejo, Portugal)	Monf	16	25 July~7 Aug. 1997	23~38
Crato (Alto Alentejo, Portugal)	Crato	8	27 June 2001	24~26
Piedade (Arrabida, Estremadura, Portugal)	Pied	10	19 July~12 Aug. 1995	-
Monte-da-Caparica (Estremadura, Portugal)	MteC	7	16~22 Sept. 1997	25~30
Toledo (Castilla-La Mancha, Spain)	Tol	2	5 July 2000	-
FRANCE				
Molitg-les-Bains (Languedoc-Roussillon)	Mol	10	17 July 2001	27
St Hippolyte (Languedoc-Roussillon)	StH	11	14 and 17 July 2001	26~33
Narbonne (Languedoc-Roussillon)	Narb	8	16 July 2001	26
CORSICA				
	Cors	1	-	-
GREECE				
Itea (Athika)	Itea	24	26 and 29 June 2002	-
Skala (Athika)	Skala	4	29 June 2002	-
Evia (Athika)	Evia	12	29 June 2002	-
Athens (Athika)	Ate	18	9~10 July 1997; 15 July 1998	-
Paralio (Peloponnese)	Paral	7	24 June 2002	-
Kosmas (Peloponnese)	Kosm	2	24 June 2002	-
Neapolis (Peloponnese)	Neap	7	25 June 2002	-
TURKEY				
Assos (Aegean coast)	Assos	1	27 June 2003	33
TOTAL		176		

between each pair of variables.

To compare the amount of acoustic variation in specimens, populations, or regions, the coefficient of variation (CV) corrected for small samples (Sokal and Rohlf 1981) was applied: $CV = 100 \times (1 + 1/4N) \times SD/Average$; where N is the sample size and SD is the standard deviation. Since the CV is simply the standard deviation expressed as a per-

centage of the mean, it allows comparisons of variation between sets of data even when the averages greatly differ. The coefficients of variation for each variable were compared at the intra-individual (CVind), intra-population (CVpop), and intra-regional (CVreg) levels.

Altogether, 176 cicadas were analyzed for 13 acoustic variables. In order to reduce the dimen-

Table 2. Description of the acoustic variables analyzed for *Cicada orni* calls

Variable	Description
No. of echemes/s	Number of elements per second
Echeme duration	Duration of each element from start to end (ms)
Inter-echeme interval	Duration between the end of one element and the beginning of the following one (ms)
Echeme period	Duration between the start of one element and the beginning of the following one (ms)
Echeme/inter-echeme interval ratio	Ratio between the echeme duration and the inter-echeme interval
Peak frequency	Frequency of the maximum amplitude on the spectrum
Minimum frequency	Lowest frequency having an amplitude exceeding the threshold (-20 dB)
Maximum frequency	Highest frequency having an amplitude exceeding the threshold (-20 dB)
Bandwidth	Difference between the maximum and minimum frequencies (Hz)
25% Quartile	Frequency below which 25% of the total energy of the spectrum resides
50% Quartile	Frequency below which 50% of the total energy of the spectrum resides (mean frequency of the spectrum)
75% Quartile	Frequency below which 75% of the total energy of the spectrum resides
75% Quartile - 25% Quartile	Difference between the upper (75% quartile) and lower (25% quartile) quartiles of the spectrum (a measure of the pureness of the sound)

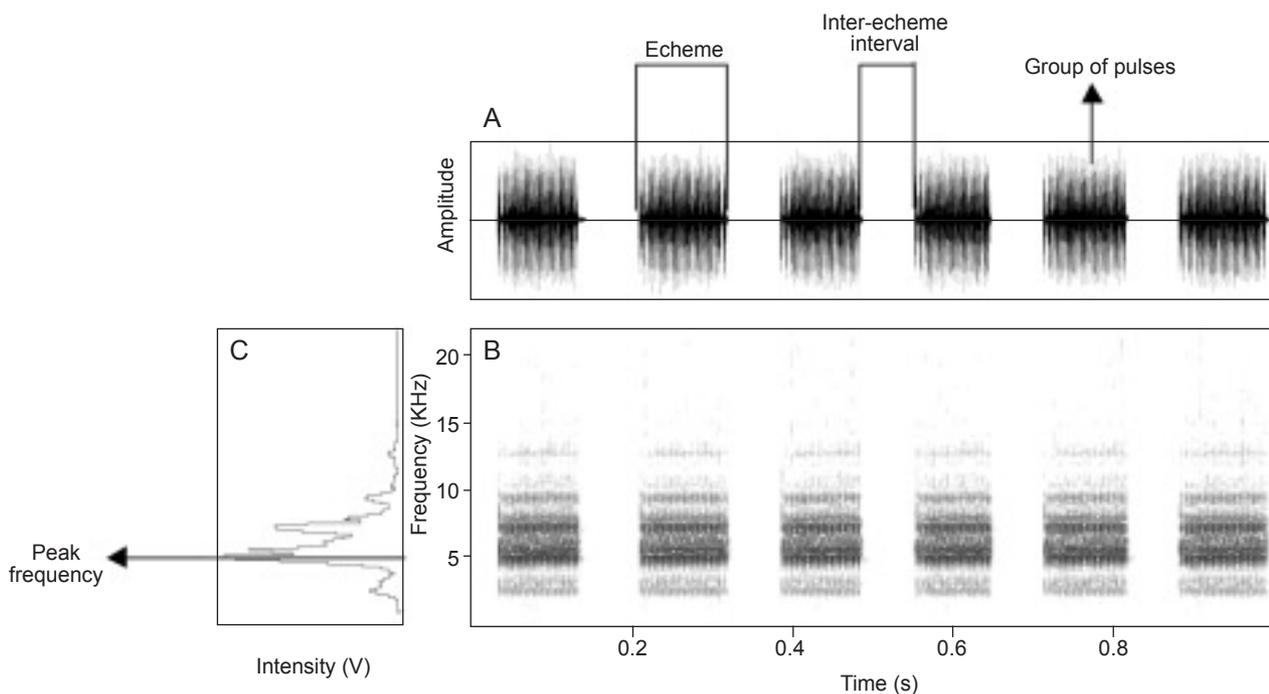


Fig. 2. Calling song of *Cicada orni*. (A) Oscillogram (amplitude vs. time); (B) sonagram or spectrogram (frequency vs. time); and (C) mean amplitude spectrum (frequency vs. amplitude).

sionality of the data matrix and to test for intraspecific acoustic differentiation among these specimens, an ordination through principal component analysis (PCA) was carried out. Data were standardized. The Kaiser criterion was used to retain only components with eigenvalues greater than 1. After this analysis, Kruskal-Wallis (KW) nonparametric tests were used to compare component scores between different regions. When results were significant ($p < 0.05$), Mann-Whitney U tests (MW) were performed to compare pairs of regions (Dytham 2003).

Kruskal-Wallis nonparametric tests were also used to compare several independent samples (regions or populations) for each acoustic variable, followed by Mann-Whitney U tests for 2-sample comparisons when the KW tests were significant (Dytham 2003).

RESULTS

The calling song of *C. orni* is produced by males which can sing continuously from a single site for hours, sometimes chorusing with other males. It is made up of a regular repetition of echemes, which are composed of a variable number of groups of pulses (Fig. 2).

Descriptive statistics of all the acoustic variables analyzed for all specimens studied are shown in table 3. This signal can be described in the time domain as having echemes of 0.08 ± 0.03 (average \pm standard deviation) s in duration separated by intervals of 0.15 ± 0.07 s. The spectral

characteristics of the signal showed a peak frequency of 4825 ± 486 Hz, and a bandwidth (at -20 dB) of 7233 ± 1437 Hz. Moreover, the mean frequency was 5544 ± 441 Hz, and the frequency difference between the upper and lower quartiles of these species was relatively small (2357 ± 585 Hz) compared with those of other cicada species. The calling songs were more variable in the time (with CVs which ranged from 27.80% to 75.25%) than in frequency domain (with CVs which ranged from 7.60% to 24.84%).

Strong nonparametric correlations between frequency variables were detected (e.g., R_S (peak frequency vs. 25% quartile) = 0.84; R_S (bandwidth vs. maximum frequency) = 0.97; $p < 0.001$). On the other hand, no significant correlations were found between the time and frequency variables. Also, time variables such as echeme duration and the interval between echemes were weakly but significantly correlated ($R_S = -0.35$; $p < 0.001$).

Recordings taken using either the Sony or UHER recorders were compared for the three French populations for which there were specimens recorded with Sony and others with UHER. It was found that there were some high-frequency components in the recordings taken with UHER which did not appear in the recordings taken with Sony. As such, the acoustic variables of maximum frequency, bandwidth, quartile 75%, and the difference between the upper and lower quartiles were significantly higher for the UHER recordings (MW tests, $p < 0.05$). The opposite was found with the minimum frequency (Fig. 3). On account of this, the UHER recordings for these variables (two from

Table 3. Descriptive statistics of the acoustic variables of *Cicada orni* specimens. n , number of specimens recorded for sound analyses; SD, standard deviation; CV, coefficient of variation

Variable	n	Average \pm SD	Minimum	Maximum	CV (%)
No. of echemes/s	176	4.69 \pm 1.30	2.23	7.49	27.80
Echeme duration (s)	176	0.08 \pm 0.03	0.03	0.21	36.42
Inter-echeme interval (s)	176	0.15 \pm 0.07	0.06	0.36	48.28
Echeme period (s)	176	0.23 \pm 0.07	0.13	0.45	30.82
Echeme/inter-echeme interval ratio	176	0.71 \pm 0.53	0.17	3.31	75.23
Peak frequency (Hz)	176	4824.84 \pm 485.61	3851.22	6584.29	10.08
Minimum frequency (Hz)	158	2191.43 \pm 390.84	1244.27	4074.77	17.86
Maximum frequency (Hz)	158	9430.15 \pm 1397.45	6966.84	13347.91	14.84
Bandwidth (-20 dB) (Hz)	158	7232.92 \pm 1437.02	3626.52	11280.30	19.19
25% Quartile (Hz)	176	4658.30 \pm 353.53	3603.51	5784.14	7.60
50% Quartile (Hz) (mean freq.)	176	5544.42 \pm 440.93	4611.31	6640.51	7.96
75% Quartile (Hz)	158	7038.39 \pm 751.82	5405.18	9948.06	10.70
75% Quartile - 25% Quartile (Hz)	158	2356.54 \pm 584.83	1242.40	5205.60	24.84

Toledo, five from Molitg-les-Bains, six from St. Hippolyte, and three from Narbonne) were removed from the analysis.

Results of the principal component analysis are shown in figure 4. The 1st four components accounted for 89.06% of the total variation. More than half (64.5%) of the variation in the study was explained by the 1st two components, and more than 3/4 (78.08%) by the 1st three components (C1 = 38.83%, C2 = 25.67%, and C3 = 13.58%). There was no complete separation between the three different regions (Iberian Peninsula, France, and Greece) and/or populations for any combination of the 1st three axes, since specimens mostly appeared as a homogeneous group (Fig. 4).

However, when plotting components 1 and 2,

specimens from southeastern Europe (Greece) tended to group on the 3rd and 4th quadrants while those of western Europe (Iberian Peninsula and France) appeared mainly in the 1st and 2nd ones (Fig. 4). Thus, there was a slight separation along the 2nd axis (component 2). In fact, KW nonparametric analysis revealed significant differences for this axis between different regions. MW tests revealed significant differences between Greece and each of the other two regions for component 2, and also for component 1 when comparing Greece with the Iberian Peninsula.

Factor loadings were considerably high (Table 4) for some of the variables and considering that component 2 had the highest loading for inter-echeme interval duration (-0.92), this was the vari-

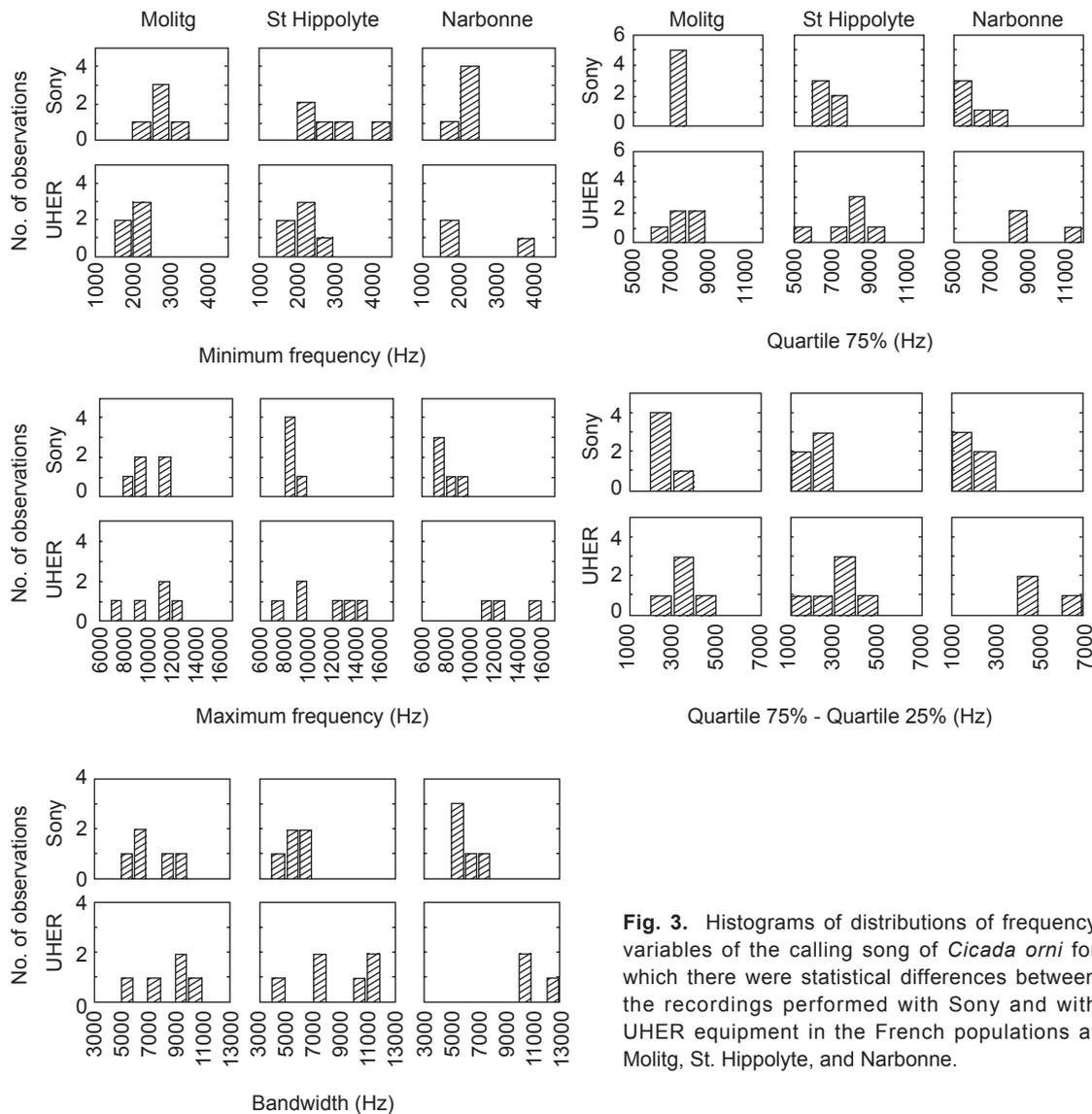


Fig. 3. Histograms of distributions of frequency variables of the calling song of *Cicada orni* for which there were statistical differences between the recordings performed with Sony and with UHER equipment in the French populations at Molitg, St. Hippolyte, and Narbonne.

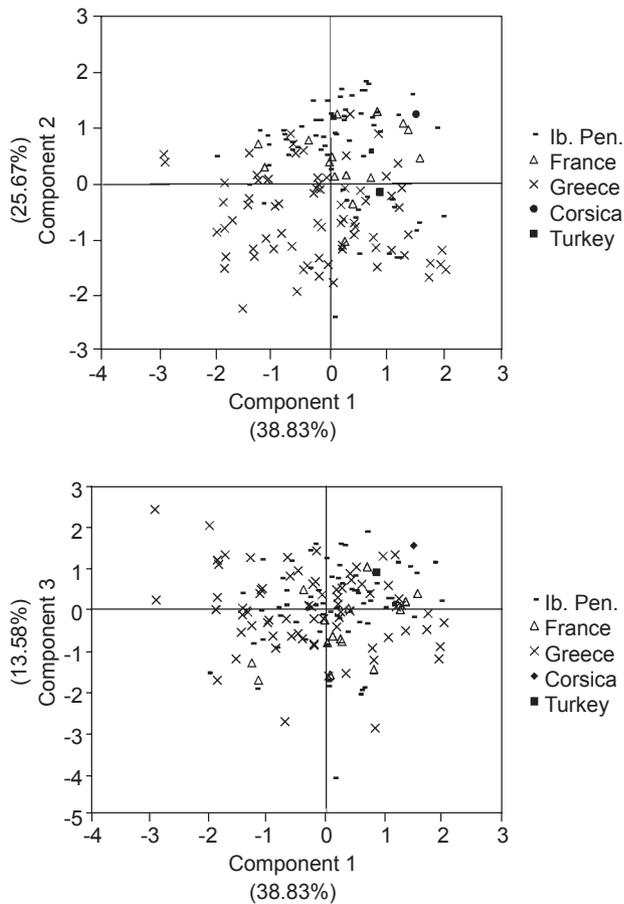


Fig. 4. Bidimensional diagrams of relationships between specimens of *Cicada orni* (176 OTUs) of the principal component analysis based on a correlation matrix between 13 acoustic characters.

able which contributed most to the separation between the populations of Greece and the other two regions. In fact, as shown below, Greek specimens had inter-echeme intervals of longer duration.

There were also significant differences between the populations of France and the Iberian Peninsula, but just for component 3, and which however explained only 13.58% of the total variation.

With respect to the Corsican and Turkish specimens, the Corsican one was grouped with the western European samples, while the Turkish one was grouped with the southeastern ones.

When only uncorrelated variables were used (echeme duration, inter-echeme interval duration, and difference between the upper and lower quartiles), the PCA results were similar (results not shown).

In a comparison of the three regions, the Iberian Peninsula, France, and Greece, the number of echemes per second, inter-echeme interval duration, and echeme period differed for each pair of regions (MW tests, $p < 0.05$). These differences were due to generally longer inter-echeme intervals in Greek cicadas, being intermediate in French cicadas and shorter in the Iberian ones (Fig. 5). The only acoustic variables for which there were no differences between regions (KW tests, $p > 0.05$) were echeme duration, the 75% quartile, and the difference between the upper and lower quartiles. For the remaining frequency vari-

Table 4. Factor loadings of the principal component analysis (PCA) based on a correlation matrix between 13 acoustic variables for *Cicada orni* (using 176 OTUs)

Variable	Component 1	Component 2	Component 3	Component 4
No. of echemes/s	-0.222552	0.851582	0.304946	0.283294
Echeme duration	-0.177807	0.269043	-0.792527	-0.497948
Inter-echeme interval	0.338561	-0.920525	0.026469	-0.086444
Echeme period	0.285371	-0.858498	-0.277505	-0.282083
Echeme/inter-echeme interval ratio	-0.233615	0.672798	-0.584044	-0.323306
Peak frequency	-0.534498	-0.474767	-0.309437	0.388026
Minimum frequency	-0.818619	-0.204684	0.304262	-0.368513
Maximum frequency	-0.747884	-0.314878	-0.298219	0.419953
Bandwidth (-20 dB)	-0.902071	-0.261265	-0.050210	0.145775
25% Quartile	-0.947363	-0.044019	0.075287	-0.044032
50% Quartile	-0.766465	0.136966	0.281331	-0.315707
75% Quartile	-0.258655	0.133888	-0.478907	0.518750
75% Quartile - 25% Quartile	-0.914037	-0.171322	0.175947	-0.230332

ables, Greek samples showed significantly higher values than the others, while samples from the Iberian Peninsula and France were similar to each other. The minimum frequency was an exception; French populations differed from the remaining ones in having higher minimum frequencies. With respect to the ratio of echeme/interval, Greek samples showed lower values.

In Greece all of the variables showed some differences among populations (except for the ratio of the echeme/inter-echeme interval) (KW tests, $p < 0.05$). In the Iberian Peninsula and France, every time variable but not every frequency variable showed some differences among populations (KW tests, $p < 0.05$). The frequency variables revealing significant differences among popula-

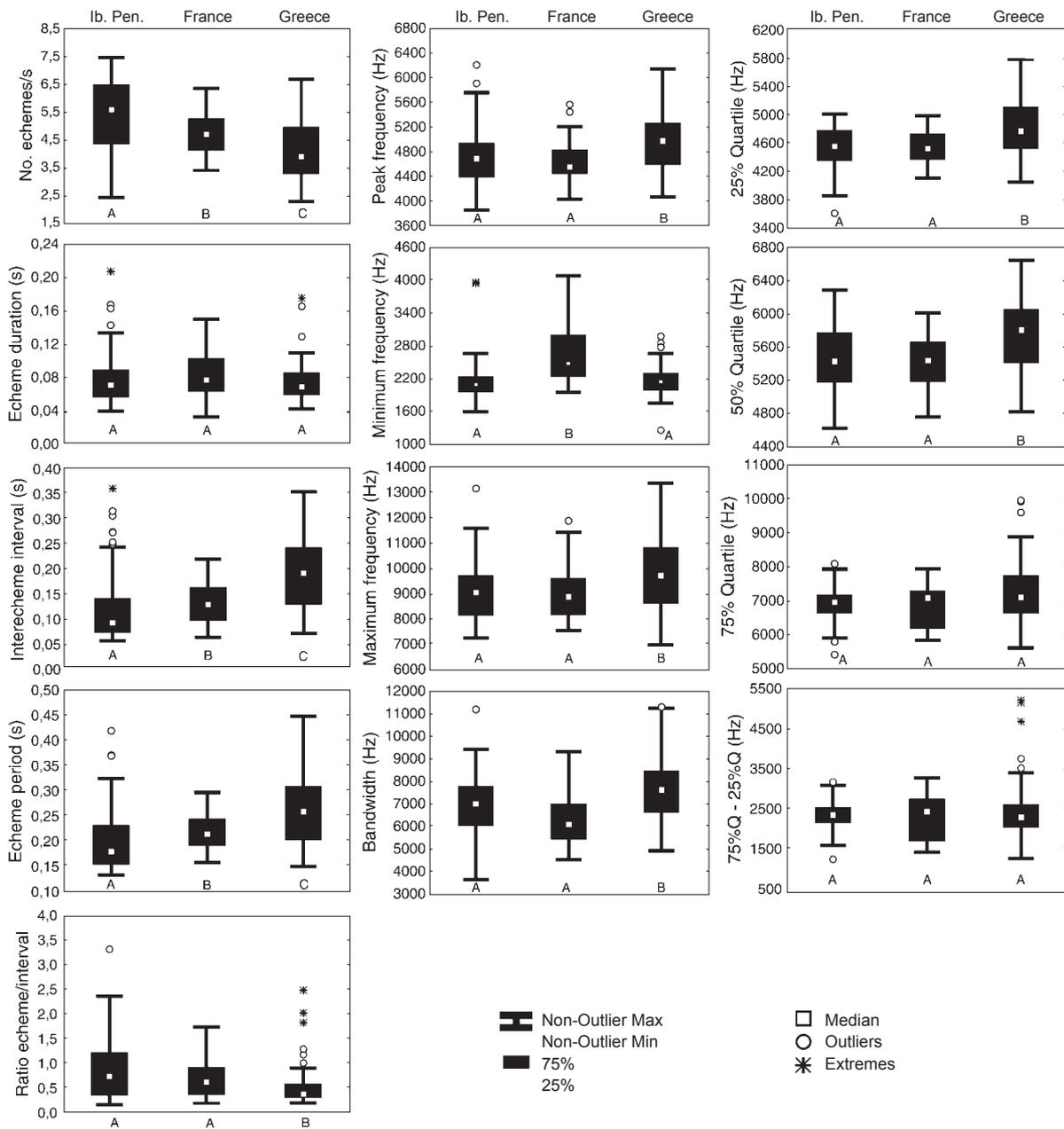


Fig. 5. Boxplots of the 13 acoustic variables of the calling song of *Cicada orni* investigated for the three regions (71 specimens from the Iberian Peninsula, 29 specimens from southern France, and 74 specimens from continental Greece). Different letters indicate that significant differences (Mann-Whitney, $p < 0.05$) exist between those regions.

tions on the Iberian Peninsula were the 25% quartile, the difference between the upper and lower quartiles, and the minimum and maximum frequencies. For the French samples, these variables were the 75% quartile, the difference between the upper and lower quartiles, and the minimum frequency.

Data for the calling song of *C. orni* by Joermann and Schneider (1987) and Popov (1975) for Yugoslavia and southern USSR, respectively, were closer to the values here obtained for the Iberian Peninsula and French cicadas than to the Greek ones, especially for the inter-echeme interval duration. In fact, the range of values of this variable obtained for Yugoslavian samples was 39–127 ms (Joermann and Schneider 1987), while that for southern USSR was 45–87 ms (calculated from Popov (1975) by subtracting the mean echeme duration from the mean period duration of each specimen). These values were closer to the median values obtained for the Iberian Peninsula and France (lower than 150 ms) than to the Greece median value (higher than 150 ms) (Fig. 5).

When comparing populations, some deviated quite obviously from the others for some variables (Fig. 6). The Sousel (Portugal) population specimens had, on average, longer inter-echeme intervals than the remaining Portuguese populations, which was also reflected in longer echeme periods and shorter ratios of echeme/interval (similar to the values found in Greek populations). The Toledo (Spain) population had lower 25% quartile and 50% quartile values, which might have been due to some interference of the recorded material (UHER). In Greece, the Kosmas population showed longer echemes and shorter intervals than the other Greek populations investigated.

In general, the frequency characters were found to be less variable than the time ones, as referred to above. This tendency was true at all three levels of the analysis. The variable presenting the highest coefficient of variation for all levels of comparison was the ratio of echeme/interval ($CV_{ind} > 28\%$, $CV_{pop} > 48\%$, and $CV_{reg} > 43\%$). On the other hand, the variables with the lowest coefficients of variation at all levels were the 25%, 50%, and 75% quartiles ($CV_{ind} < 3\%$ and CV_{pop} and $CV_{reg} < 10\%$) (Table 5). For most variables, the Greek cicadas showed a higher intra-individual coefficient of variation (CV_{ind}), a lower intra-population coefficient of variation (CV_{pop}), and a higher intra-region coefficient of variation (CV_{reg}), than did cicadas from the other regions (Table 5).

The mean intra-individual coefficients of variation were always lower than intra-population ones for all variables in all regions (Table 5), which allowed the use of a mean value of each variable measured for each specimen. In fact, the percentage of specimens presenting a CV_{ind} higher than the CV_{pop} was always lower than 50% (with the exception of echeme duration for Greece) (Table 6).

In contrast, CV_{pop} values were more similar to CV_{reg} ones for most of the variables than were CV_{ind} relative to CV_{pop} values (Table 5). Low percentages of populations with $CV_{pop} > CV_{reg}$ were only found for the inter-echeme interval and echeme period, as well as the minimum frequency in the Iberian Peninsula, while Greece showed low values for echeme duration and the echeme/interval ratio (Table 6). These values were probably due to the atypical populations of Sousel (Portugal) and Kosmas (Greece) which, as referred to before, have very distinct values for these variables (Fig. 6).

DISCUSSION AND CONCLUSIONS

Detailed acoustic analyses of the time and frequency domains revealed some variations in the calling song among specimens and populations of *C. orni* and conform to previously known data. Values obtained in the present study for time and frequency variables of this song are within the range of variation observed in the former Yugoslavia (Joermann and Schneider 1987) and southern USSR (Popov 1975). The results obtained by Boulard (1995 2000b) in Provence (France) also fall within the observed range of the French populations of this study as well as of those reported by Fonseca (1991) for Portugal.

Principal component analysis indicated that in spite of some variations, the calling songs of *C. orni* investigated here constitute a relatively homogeneous group, without clear-cut acoustic differences as expected for conspecifics. However, some significant differences were found among regions, a fact not concordant with the prediction by Paterson (e.g., 1985) that specific mate recognition systems should be invariant within species. Songs from southeastern Europe (Greece) tended to group separately from those of western Europe (Iberian Peninsula and France), and the inter-echeme interval was the variable that contributed most to this separation. In fact, Greek male songs showed longer inter-echeme intervals on average

than the songs of western Europe as found previously by Quartau et al. (1999). An exception is the Portuguese population at Sousel, which generally showed longer inter-echeme intervals. In Greece and Portugal, the influence of temperature is an unlikely explanation for such differences since the recordings were performed within a similar range of temperatures at all sites. Conversely, the population at Kosmas (Greece) showed shorter inter-echeme intervals and longer echemes than the typical Greek populations. As this population was the only one recorded at a high elevation (> 1000 m), more-thorough studies should be carried out at this locality as well as at Sousel to better understand these unusual findings.

In addition to the inter-echeme interval, specimens from Greece showed significant differences in relation to the remaining studied regions for almost every acoustic variable in both the time and frequency domains, as shown in the pairwise comparisons. This acoustic differentiation is in agreement with genetic data, namely, isozymes (Quartau et al. 2001), microsatellites (Seabra in prep.), and mitochondrial DNA (Pinto-Juma in prep.) and is probably due to the considerable isolation of such populations throughout the mountain ranges of the Balkans during the ice ages.

It is interesting to note that the songs of the Corsican specimens as well as the Yugoslavian (Joermann and Schneider 1987) and southern USSR (Popov 1975) ones (for time variables,

especially inter-echeme intervals) were closer to those of the Iberian Peninsula and France. On the other hand, and as expected due to reasons of geographic proximity, the single Turkish male investigated here had greater similarities with the Greek populations than to other populations.

The only time variable that showed no significant differences between each pair of regions, i.e. that proved to be quite constant across the geographic range of this cicada, was echeme duration. In a comparison of *C. orni* with its sibling *C. mordoganensis*, echeme duration is the variable which more-obviously readily distinguishes these species, since there is minimal overlap (Simões et al. 2000). Thus, echeme duration is probably one of the most important parameters encoding species-specific information for species recognition. On the other hand and as referred to previously, the inter-echeme interval is quite variable within *C. orni*, and its range of variation considerably overlaps that of *C. mordoganensis*. Therefore, it is expected that the inter-echeme interval alone is not an important parameter for species recognition and isolation in these species.

Characters for the time domain were found to be more variable (i.e., with higher coefficients of variation) than those for the frequency domain as reported for other cicadas (e.g., Sueur and Aubin 2002). This was expected since the spectrum is especially constrained by the physical properties of the sound-producing organ. In fact, as stated for

Table 5. Coefficients of variation for each acoustic variable analyzed within specimens (CVind) within populations (CVpop) (average \pm standard deviation) and within regions (CVreg) for the three areas investigated

	Iberian Peninsula			France			Greece		
	CVind	CVpop	CVreg	CVind	CVpop	CVreg	CVind	CVpop	CVreg
No. of echemes/s	-	18.36 \pm 8.21	17.48	-	16.08 \pm 0.54	10.26	-	20.11 \pm 5.00	20.58
Echeme duration	15.36 \pm 7.02	30.33 \pm 11.90	30.34	16.10 \pm 7.29	28.10 \pm 3.21	28.93	19.41 \pm 6.76	22.4 \pm 14.47	41.67
Inter-echeme interval	17.88 \pm 8.04	33.39 \pm 14.03	48.37	16.42 \pm 8.36	27.73 \pm 2.64	21.49	27.17 \pm 10.21	29.59 \pm 4.66	34.00
Echeme period	12.18 \pm 7.53	18.98 \pm 8.07	25.62	11.70 \pm 6.08	16.03 \pm 0.97	10.14	18.8 \pm 7.44	20.07 \pm 4.61	22.43
Echeme/inter-echeme interval ratio	28.56 \pm 13.90	54.50 \pm 15.81	50.72	30.59 \pm 19.18	54.59 \pm 17.20	43.97	40.27 \pm 19.13	48.72 \pm 38.42	79.10
Peak frequency	4.97 \pm 4.20	8.34 \pm 3.43	3.4	3.27 \pm 3.14	6.77 \pm 3.40	2.48	5.98 \pm 3.14	6.72 \pm 3.71	6.17
Minimum frequency	4.85 \pm 4.30	12.61 \pm 9.87	10.66	11.85 \pm 9.71	15.90 \pm 9.65	15.12	9.74 \pm 8.58	9.85 \pm 4.57	6.19
Maximum frequency	6.83 \pm 5.99	13.05 \pm 4.96	4.54	4.20 \pm 4.12	10.26 \pm 5.11	12.40	6.02 \pm 3.91	11.68 \pm 5.74	11.48
Bandwidth	9.62 \pm 8.01	18.47 \pm 9.93	6.62	9.73 \pm 6.92	18.4 \pm 4.88	14.69	8.94 \pm 5.52	14.82 \pm 6.05	14.31
25% Quartile	1.06 \pm 0.53	5.54 \pm 1.40	2.77	0.77 \pm 0.45	4.14 \pm 1.32	2.23	1.74 \pm 1.36	5.83 \pm 2.17	5.17
50% Quartile	1.48 \pm 1.08	7.35 \pm 2.32	1.78	1.38 \pm 0.70	6.2 \pm 2.25	5.11	2.06 \pm 1.55	6.42 \pm 3.54	6.50
75% Quartile	1.79 \pm 1.96	7.18 \pm 1.88	2.97	2.16 \pm 1.95	7.75 \pm 2.84	8.71	2.93 \pm 2.49	9.97 \pm 4.87	9.58
75% Quartile - 25% Quartile	4.97 \pm 4.65	14.71 \pm 5.30	8.35	6.57 \pm 6.35	20.14 \pm 7.95	22.07	8.21 \pm 6.44	23.58 \pm 7.95	23.41

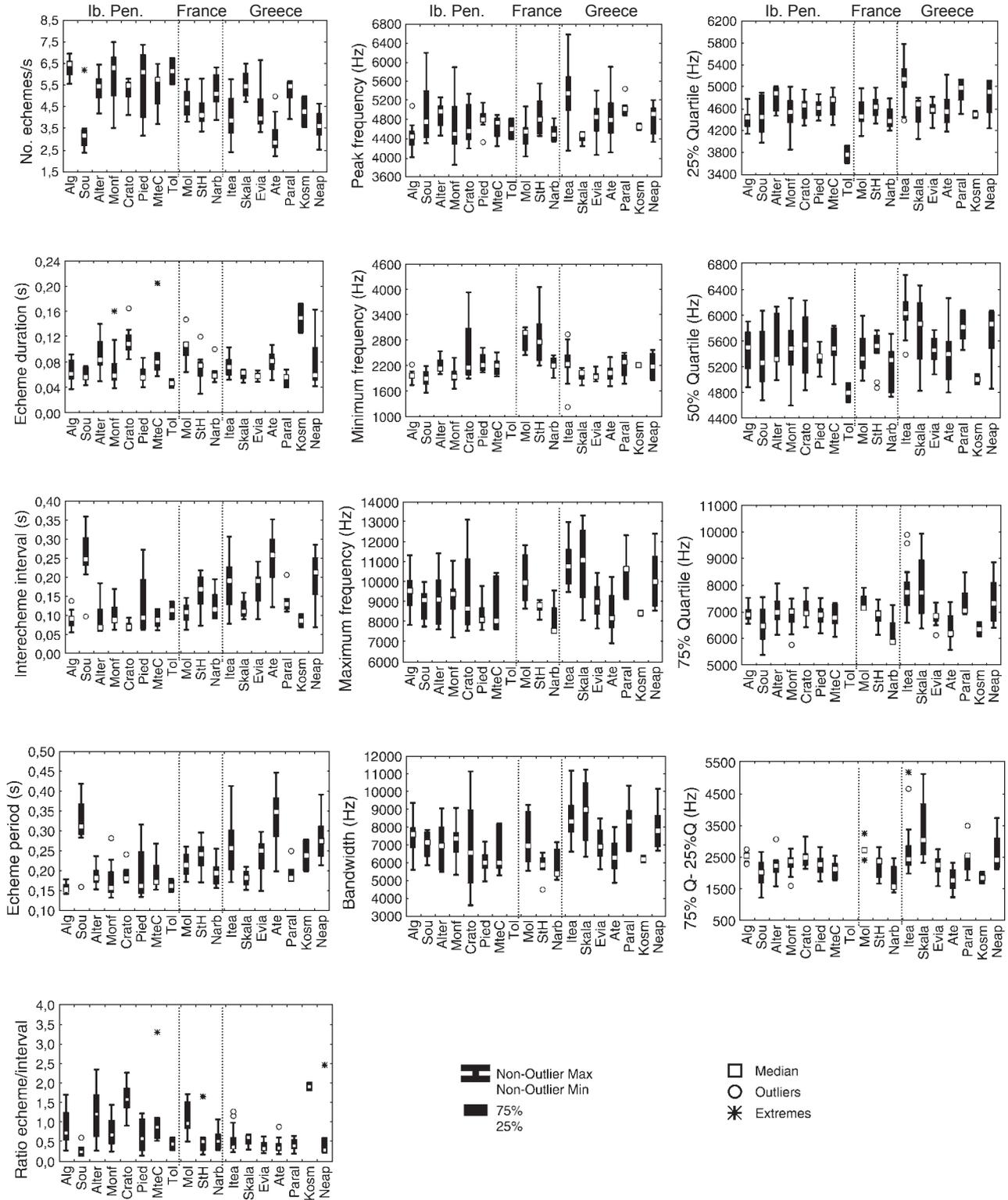


Fig. 6. Boxplots of the 13 acoustic (including five time and eight frequency) variables of the calling song of *Cicada orni* investigated for each population. (for an explanation of the abbreviations see table 1).

insects in general by Stumpner and Helversen (2001), the time pattern of the song is usually more important in the recognition of a conspecific signal than its spectrum, since this latter differs much less between related species.

Intra-individual variability was found to be higher in the Greek populations than in those from the other regions investigated. This might be related to a stronger interaction among specimens due to the higher densities of cicadas found in Greece. Consequently, at the intra-population level, the acoustic variability was low. On the other hand, Greek songs also proved to have a higher level of intra-regional variation than songs from the remaining areas. An explanation for this might be the very high density of cicadas in Greece and consequently a more-pronounced chorus effect which is important for attracting females and pair formation (as has also been shown for *Magiccada* spp. by Karban (1981) and Williams and Kimberly (1991)). Therefore, this chorus effect might have promoted a scattered distribution and population isolation and, as such, enhanced divergence between different cicada populations.

It is likely that significant regional differentiation might have occurred during the glacial periods, when *C. orni* was split into isolated populations in at least two main Mediterranean areas – the Iberian Peninsula in the west and the south-eastern Aegean area (including the mainland and

islands) (Taberlet et al. 1998). Therefore, the extant eastern populations of *C. orni* would have been derived from such isolated Aegean refuge stock, an isolation which would have promoted substantial microevolutionary genetic diversification as in other groups (Avisé and Walker 1998). In such a view, the Aegean area and West Asia Minor might correspond to the original area of dispersal and expansion of *C. orni* during Pleistocene climatic cycles, as probably occurred with many widespread species over the entire Mediterranean basin (Oosterbroek and Arntzen 1992, Oosterbroek 1994). In fact and in corroboration with Hewitt (e.g., 1996 1999) and others, the southern European population genomes of many animal species show greater haplotype diversity. As suggested before, this was probably due to the existence of southern ice age refugia, such as the Aegean coastal zones, valleys, and islands, which were subjected to repeated cycles of isolation through the mountain ranges of the Balkans, and from where expansion cycles took place for colonizing more-northerly and western areas during interglacial warmer periods.

In summary, further investigations on *C. orni* are needed not only at the acoustic but also at the genetic and phylogeographic levels to foster a better understanding of the history of the intriguing distinctiveness of its southeastern populations.

Table 6. Percentage of specimens presenting $CV_{ind} > CV_{pop}$ and percentage of populations presenting $CV_{pop} > CV_{reg}$. CV_{ind} coefficient of variation within individuals; CV_{pop} coefficient of variation within populations; and CV_{reg} coefficient of variation within regions

	Iberian Peninsula		France		Greece	
	$CV_{ind} > CV_{pop}$	$CV_{pop} > CV_{reg}$	$CV_{pop} > CV_{ind}$	$CV_{reg} > CV_{pop}$	$CV_{ind} > CV_{pop}$	$CV_{pop} > CV_{reg}$
No. of echemes/s	-	50%	-	100%	-	57.10%
Echeme duration	9.86%	37.50%	13.79%	33.30%	52.17%	14.30%
Inter-echeme interval	9.86%	25%	10.35%	100%	34.78%	28.60%
Echeme period	15.49%	12.50%	20.69%	100%	34.78%	28.60%
Echeme/inter-echeme interval ratio	9.86%	62.50%	10.35%	66.70%	27.54%	14.30%
Peak frequency	21.74%	100%	26.67%	100%	26.09%	57.10%
Minimum frequency	11.59%	14.30%	33.33%	33.30%	34.78%	71.40%
Maximum frequency	14.49%	100%	13.33%	33.30%	10.15%	42.90%
Bandwidth	15.94%	100%	13.33%	66.70%	14.49%	57.10%
25% Quartile	0%	100%	0%	100%	2.90%	57.10%
50% Quartile	0%	100%	0%	66.70%	2.90%	42.90%
75% Quartile	2.90%	100%	0%	33.30%	4.35%	42.90%
75% Quartile - 25% Quartile	8.70%	85.70%	0%	33.30%	4.35%	28.60%

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