

# Revised concept of *Chrysoperla mediterranea* (Hölzel), a green lacewing associated with conifers: courtship songs across 2800 kilometres of Europe (Neuroptera: Chrysopidae)

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**Abstract.** The Holarctic *carnea*-group of *Chrysoperla* green lacewings consists of numerous cryptic, sibling species, distinguished principally by vibrational courtship songs. A European member of this species-group, *C. mediterranea* (Hölzel), was originally diagnosed by its dark green coloration, red band across the front of the head, narrow wings, minute basal dilation of the pretarsus and ecological association with pines. Here we describe its courtship and mating song, which uniquely distinguishes the species from all other members of the *carnea*-group in Europe. Males and females both produce a single type of song, characterized by a long train of short, closely spaced volleys and nearly constant carrier frequency. Measured song features are nearly invariant across a large geographical range that extends well outside that previously known for *C. mediterranea*. Principal components analysis confirms the uniqueness of the *mediterranea* song in comparison with five other sympatric song species of the *carnea*-group. Populations which show the *mediterranea* song phenotype do not necessarily retain all of the traditional morphological features of the species, particularly in eastern parts of the range. Specifically, the green coloration may be less intense, the wings broader and the pretarsal basal dilation larger, resulting in significant morphological overlap with other song species. Larval morphology and ecophysiology also fail to distinguish *C. mediterranea* from other song species. Based on unique song phenotype, however, we conclude that *C. mediterranea* is a valid biological species, which can also be recognized in museum collections when a suite of physical attributes is used.

## Introduction

The Holarctic green lacewing species *Chrysoperla carnea* (Stephens), although tolerably well constrained morphologically (Tjeder, 1960), has been shown to be a complex of many cryptic biological species, reproductively isolated from one another principally by distinctive, substrate-borne courtship songs (Wells & Henry, 1992a,b). In North

America, four species have been formally recognized within the *carnea*-group based on their songs (Henry *et al.*, 1993; Henry, 1993b), while several others remain to be described (Henry, 1994). Eurasia presents a similar but more complicated picture. Within what has become known in Europe as the '*C. carnea* complex' (Thierry & Adams, 1992), numerous song-based species are being discovered (Wells & Henry, 1998), which must be reconciled with existing descriptions of additional species of uncertain validity that have been carved out of '*C. carnea*' over the years. Two such named 'species' within the *C. carnea* complex of Europe are better defined than the others, and possess suites of morphological and ecophysiological traits

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**Table 1.** European collecting sites for living *Chrysoperla mediterranea* used in song analyses 1984–95.

Local site (with nearest city or region)	Country	Altitude (m)	Latitude	Date
Huetor Santillan <sup>1</sup> (Granada)	Southern Spain	1080	37°13'	19.vii.1995
La Mora (mountain pass, Granada)	Southern Spain	1390	37°13'	19.vii.1995
Mojacar <sup>1</sup> (Alicante, on pine)	Southern Spain	0–10	38°25'	9.vii.1989
Cabrera (Madrid)	Central Spain	1050	40°58'	20.vii.1995
St Vincente <sup>1</sup> (Santander)	Northern Spain	sea level	43°27'	22.vii.1995
Col de Peyreson (Pyrenées, on pine)	Southwestern France	1400	42°35'	20.xii.1988
Estagel (Perpignan)	Southern France	110	42°42'	16.vii.1995
Bise-Minervois (Béziers)	Southern France	68	43°21'	16.vii.1995
Carcès (Var)	Southern France	180	43°20'	viii.1994
Monte Caslano (Ticino region, S. slope of Alps)	Southern Switzerland	275	46°30'	vii.1993
Sierre (Pfywald region, N. slope of Alps, pine forest)	Southwestern Switzerland	600	46°30'	1984, 1989, 1993
Caprileone (at coast, on pine)	Northeastern Sicily	0–10	37°48'	vii.1993
Xilokastron (at coast, pine forest)	Greece	0–10	38°	vi.1994
Megara (on pine)	Greece	50	38°	vi.1994
Brückl (leg, Hölzel)	Austria	1000	47°25'	10.x.1994
Duchonka, Topolcany (at light)	Slovakia	350–500	48°40'	8.iv.1994

<sup>1</sup>Confirmed *C. mediterranea* song, but songs not analysed.

that are consistently present over broad geographical regions. One of these, *C. lucasina* (Lacroix), has been validated recently by a comprehensive study of mating signals and morphology across the full extent of its known range (Henry *et al.*, 1996). The other is *C. mediterranea* (Hölzel), erected in 1972 and delimited by its intense green coloration, red markings on the front of the head (Brooks, 1994), relatively narrow wings (but with rounded apices) and small/triangular basal dilation of the claw (Hölzel, 1972; Brooks, 1994; Malet *et al.*, 1994). In addition, *C. mediterranea* is strongly associated with conifers (Monserrat & Marin, 1994), typically in dry localities, and retains its bright green coloration during winter diapause (Canard, 1987; Canard *et al.*, 1994). Despite its distinctiveness, it is nonetheless very closely related to other members of the *C. carnea* complex and the more inclusive *carnea*-group. For example, when not given a choice of mates, *C. mediterranea* will hybridize freely in the laboratory with '*C. carnea*' (undetermined song type), producing fertile hybrids (Duelli, 1987).

Although possessing a recognizable *gestalt*, *C. mediterranea* is imprecisely defined morphologically and geographically, and has been the focus of some controversy among lacing specialists. Originally considered a faunal element principally of the western Mediterranean region (Canard, 1987; Leraut, 1991), the species has also been reported from the relict pine forest of Switzerland (Duelli, 1987) and as far east as Hungary (personal communication to S. Brooks from Szentkirályi, Brooks, 1994). However, Duelli's determination of Swiss 'pine *carnea*' as *C. mediterranea* was challenged by Cianchi & Bullini (1992), based on results from multilocus electrophoresis. While confirming that Duelli's sympatric 'pine *carnea*' and 'oak *carnea*' were distinct and probably reproductively isolated, the authors also showed that 'pine *carnea*' did not

cluster with *C. mediterranea* obtained from southern France, and concluded that Duelli's pine-associated population was a distinct but undetermined species. H. Hölzel (personal communication to P. Duelli, 1995) agreed with this conclusion, expressing the view that *C. mediterranea* does not occur north of the Alps.

The present study describes the vibrational mating signals of *C. mediterranea*, and analyses the variation in song features present among six selected populations found in different parts of Europe. We also survey the morphological variation that exists among those populations, considering both adults and larvae. Using the results of those analyses, we test the hypothesis that *C. mediterranea* is a valid taxon. In order to address that issue directly, we have obtained specimens generally conforming to the traditional view of *C. mediterranea* from areas well outside the previously accepted range of the species, including several sites in Switzerland, Greece, Austria and Slovakia. We are therefore able to define *C. mediterranea* with greater precision, and place its song phenotype and morphology in the broader framework of song species within the *C. carnea* complex and the *carnea*-group as a whole.

## Materials and methods

### Collecting, rearing and identification

Living adults of *C. mediterranea*, and other *Chrysoperla* spp., were collected by the authors and many collaborators at numerous sites and elevations across Europe from 1984 to 1997 (Table 1). All insects were shipped or handcarried to Storrs, Connecticut, for maintenance, rearing and song analysis. Protocols are described fully in other papers (summarized in Henry *et al.*, 1996).

**Table 2.** Values at  $25 \pm 1^\circ\text{C}$  of the song characteristics of *Chrysoperla mediterranea* from six geographical areas in Europe, including comparisons of males to females. Each value is the mean of the means of  $n$  individuals in the population subsample,  $\pm$  SD (one standard deviation). Pairwise comparisons were tested for statistical significance using a  $t$ -test for independent samples. Levels of significance are shown using numbers of asterisks: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ . SRU = shortest repeated unit exchanged between individuals while duetting.

	Time measures of volleys (ms)														
	Frequency measures of volleys (Hz)			Start of SRU			Middle of SRU			End of SRU			SRU features		
	Start of SRU	Middle of SRU	End of SRU	Duration	Period	Duration	Period	Duration	Period	Duration	Period	Duration	Period	Duration (s)	Number of volleys
All songs ( $n=56$ )	79.72 $\pm 3.50$	79.36 $\pm 3.93$	74.58 $\pm 3.95$	98.91 $\pm 11.05$	202.77 $\pm 20.22$	83.57 $\pm 11.01$	131.81 $\pm 11.95$	73.91 $\pm 9.87$	124.33 $\pm 12.44$	7.39 $\pm 3.18$	54.61 $\pm 25.57$				
Males ( $n=28$ )	81.49*** $\pm 3.01$	80.81*** $\pm 3.34$	75.24 $\pm 3.49$	94.97** $\pm 10.59$	196.57* $\pm 17.75$	86.67* $\pm 11/95$	133.83 $\pm 12.81$	75.96 $\pm 11.82$	126.54 $\pm 14.11$	5.60*** $\pm 2.21$	40.45*** $\pm 17.29$				
Females ( $n=28$ )	77.95*** $\pm 3.08$	77.90** $\pm 4.00$	73.92 $\pm 4.33$	102.85** $\pm 10.22$	208.97* $\pm 20.94$	80.47* $\pm 9.16$	129.79 $\pm 10.89$	71.86 $\pm 7.07$	122.12 $\pm 10.31$	9.17*** $\pm 3.02$	68.77*** $\pm 24.86$				

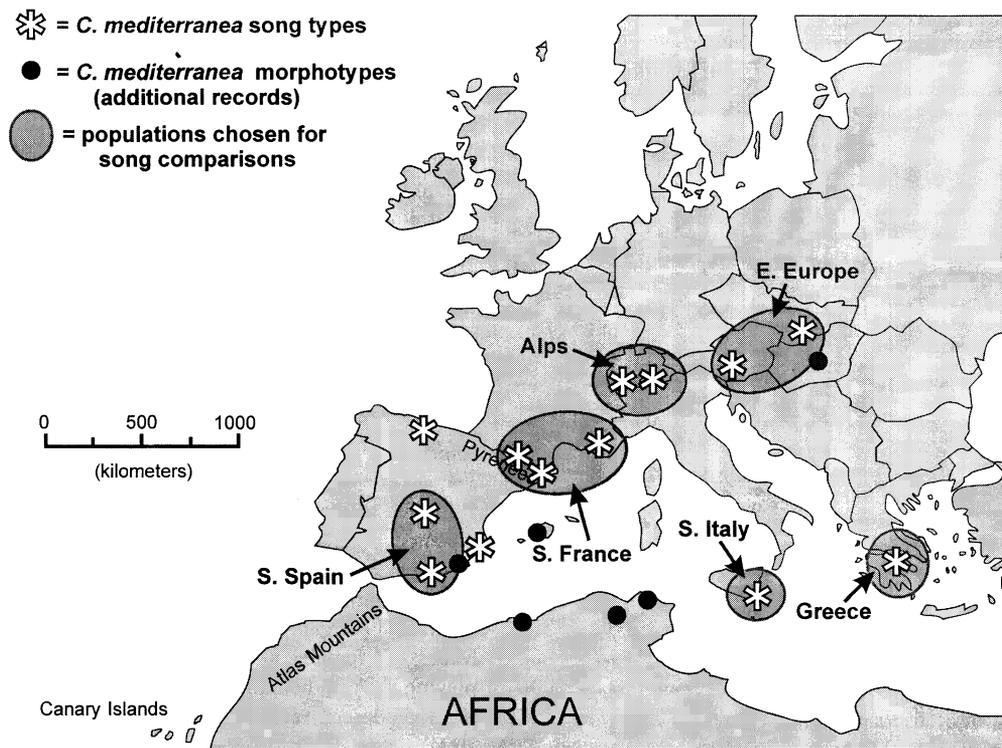
Individuals of *C. mediterranea* were identified initially by their courtship songs, using computer playback of a complete array of previously recorded European song types to each insect (see below). Once identified we looked at each individual for the key morphological features of the species, and checked all other song morphs for possession of those features. All insects with 'mediterranea' songs were also determined to be *C. mediterranea* based on possession of at least a few of the morphological traits currently defining the species. Insects with non-*mediterranea* songs did not display the morphological *gestalt* of *C. mediterranea*. Larval studies were confined to the progeny of individual adults confirmed to be *C. mediterranea* by song analysis.

#### Analysis of songs

Three to seven complete courtship songs (= shortest repeated units or SRUs) of each presumed member of *C. mediterranea* were recorded on cassette tape (Dolby<sup>TM</sup> off) and then digitized and analysed with hardware and software on a personal computer, as described fully in Henry *et al.* (1996). Males and females in the laboratory were induced to sing by playing back to them pre-recorded songs of conspecifics, using methods described in earlier papers (Wells & Henry, 1992b, 1994).

The songs of *Chrysoperla* Steinmann green lacewings consist of volleys of abdominal vibration repeated with a regular period. Each volley can also exhibit internal carrier frequency modulation. Some taxa have relatively simple songs composed of single-volley (monosyllabic) SRUs repeated many times, while others produce complex songs consisting of much longer, multisyllabic SRUs, repeated only in response to other such songs. *Chrysoperla mediterranea* analysed here has a complex song, in which the SRU consists of many similar volleys and can be up to 17 s in duration. Volleys are very short and without internal frequency modulation, but change gradually in duration, period and carrier frequency during the course of the entire SRU (see Fig. 3). Consequently, we measured three song features for each of three different sections of each SRU: start, middle and end (Table 2; Fig. 2). For each feature, measurements on six volleys per section were averaged together. Features were selected for maximum compatibility with the song measurements published for other species, and included carrier frequency of each volley, duration of each volley and volley period (from the start of one volley to the start of the next). Two additional features were measured which apply to the SRU as a whole, including overall duration of SRU and number of volleys per SRU. In all, eleven measures were tabulated (see Table 2).

Analyses were performed on the songs of fifty-six *C. mediterranea* individuals. To avoid temperature effects, all songs were recorded at  $25 \pm 1^\circ\text{C}$ . Although many populations throughout Europe were sampled (Table 1), we grouped those into six regional areas (Fig. 1): southern Spain (four individuals), southern France (eight), the Alps (fifteen), Sicily (eleven), Greece (eight) and eastern Europe (ten). Most statistical analyses were performed on these larger geographi-



**Fig. 1.** Collecting sites of *C. mediterranea* in Europe, with ellipses drawn around the discrete geographical areas which were used for song comparisons.

cal units. For each of three to seven songs (SRUs) per individual, we calculated the mean of each song feature and then took the mean (i.e.  $n$  = number of repeated song measurements per individual) of those means as the representative value of the feature for each individual. Coefficients of variation (CV) were calculated for each song feature, first for each individual (within-individual variation) and then for the entire population (between-individual variation). An analysis of variance (ANOVA) was performed on individual averages using geographical region as the independent variable. Scheffé's contrast test (Scheffé, 1953) was used to compare population means for significant differences.

Another approach to analysing song variation among populations utilizes principal components analysis (PCA). Here, several principal components of variance (factors) are extracted from a larger number of measured variables. In PCA, strong correlations between variables can bias the analysis, so a conservative approach is to eliminate one of each pair of variables exhibiting a high correlation coefficient ( $R \geq 0.80$ ). The feature retained is chosen for its lower correlations, on average, with the other variables. In the analysis of geographical variation in *C. mediterranea*, eleven song variables yielded eight 'uncorrelated' features (specified in Table 3), which were then used to extract the first two principal components. Another two-factor PCA of song differences among *C. mediterranea* and five other song species was performed using six uncorrelated features, chosen as above

from a total of seventeen measurements (additional measurements included six tonal features found only in species with longer, frequency-modulated volleys and were therefore represented in *C. mediterranea* as redundant data). To explore the significance of differences among samples, ANOVAs and Scheffé's contrast tests were applied to the scores of the first two factors extracted in each principal components analysis. Finally, for completeness, each of the two PCA/ANOVA procedures described above was run again on all variables, ignoring correlation structure. All statistical analyses were performed using *Statistica/Win 5.0*.

#### *Analysis of adult morphology*

A total of thirty-one males and thirty-four females identified acoustically as *C. mediterranea* was examined for external morphological features that might vary among populations across the geographical range. An additional four males and three females from The Natural History Museum, London, U.K., were identified morphologically as *C. mediterranea* and studied similarly. Genitalia of preserved males were dissected, stained and mounted using established techniques (Bram & Bickley, 1963; Brooks, 1994). Specimens were examined from the following localities and, unless otherwise stated, they were identified acoustically as *C. mediterranea*: ALGERIA, Constantine, 23.x.1894, Eaton (1♂ identified morphologically);

**Table 3.** Factor loadings of the first two principal components from a principal components analysis of eight uncorrelated measurements of the songs of *C. mediterranea*. Asterisks indicate loadings where the correlation coefficient  $R > 0.700$ .

Variable	Factor 1	Factor 2
Volley duration, SRU start	-0.193	0.774 **
Volley period, SRU start	-0.627	0.479
Volley duration, SRU middle	0.211	0.899 **
Volley duration, SRU end	0.290	0.806 **
Volley number per SRU	-0.667	-0.304
Carrier frequency, SRU start	0.866 **	0.071
Carrier frequency, SRU middle	0.898 **	-0.162
Carrier frequency, SRU end	0.747 **	-0.124
Explained variance	3.117	2.427
Proportion of total variance	0.390	0.303

Majorca, C'An Pastilla, 14–31.viii.1961, E. W. Classey (1♀ identified morphologically); SPAIN, Totana, 29.xii.1979, V. J. Monserrat (1♂ identified morphologically); SPAIN, Mojacar, 0–10 m, 9.vii.1989, on pine, P. Duelli (3♂♂, 1♀); FRANCE, Carcès, 180 m, viii.1994, P. Duelli (2♂♂, 2♀♀); FRANCE, Col de Peyreson, Pyrénées, 1400 m, vii.1988, M. Canard (2♂♂, 1♀ identified morphologically); MALTA, Gozo, 11.ix.1990, on pine, P. Duelli (1♀ identified morphologically); ITALY, Sicily, Caprileone, 0–10 m, vii.1993, on pine, P. Duelli, J. Johnson, L. Wilson (10♀♀); SWITZERLAND, Sierre, Pfywald, 600 m, 3.x.1997, P. Duelli (1♂, 3♀♀); GREECE, Xilokastron, 0–10 m, vi.1994, on pine, P. Duelli, J. Johnson, L. Wilson (6♂♂, 7♀♀); GREECE, Megara, 50 m, † vi.1994, on pine, P. Duelli, J. Johnson, L. Wilson (11♂♂, 3♀♀); AUSTRIA, Brückl, 1000 m, 10.x.1994, H. Hölzel (2♂♂); SLOVAKIA, Duchonka, 350 m, 8.iv.1994, P. Duelli (6♂♂, 7♀♀).

Adults were examined for the states of eighteen characters: ground colour of body, extent of markings on stipes, colour of palps, extent and colour of genal markings, clypeal markings, frontal markings, markings on post ocular region, relative abundance and distribution of black and blonde setae on pronotum, relative size of basal dilation of tarsal claw expressed as the ratio of the length of basal dilation of claw to total width of claw (Fig. 7), extent to which fore wing is rounded or tapered at apex, relative width of fore wing expressed as the ratio of length to breadth at widest point, presence or absence of black markings on wing veins, length of costal setae, presence or absence of black setae on abdominal sternites, length of abdominal setae, shape of lip at apex of sternite 8+9 in males (Fig. 8), relative size of medial lobe (acumen) of tignum in male genitalia expressed as a ratio of length of arm of tignum to length of acumen (Fig. 9) and presence or absence of dark brown stripe on pleural membrane of second abdominal segment.

#### Analysis of larval morphology

We examined a total of eight-seven first-instar, eighty-four second-instar and ninety-eight third-instar larvae from seven

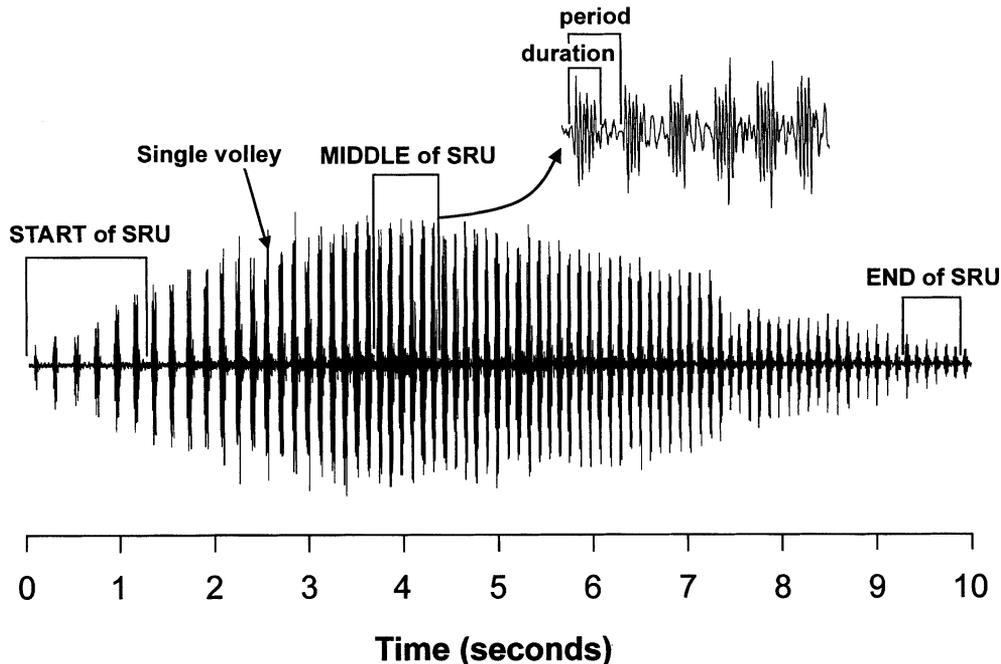
acoustically identified populations distributed from southern France north to Switzerland and east to Slovakia and Greece. Determined larvae were available from all regions used in song analysis, except southern Spain. Larvae were the offspring of adults collected from a subset of the localities listed in the preceding section on adult morphology. Specific sites and sample sizes ( $n$ =first, second, third instars) included Carcès, southern France ( $n$ =4, 6, 16); Col de Peyreson, Pyrénées, France ( $n$ =5, 4, 4); Caprileone, Sicily, Italy ( $n$ =25, 25, 25); Sierre, Pfywald area, Switzerland ( $n$ =9, 10, 18); Brückl, Austria ( $n$ =11, 14, 5); Duchonka, Slovakia ( $n$ =8, 5, 5); and Xilokastron, Greece ( $n$ =25, 20, 25). When possible, larvae were boiled in 100 ml distilled water with a drop of liquid detergent, allowed to cool, and then transferred to 70% ethanol + 5% glycerol + 25% distilled water for storage. Some previously collected larvae had unavoidably been placed in 70% ethanol, resulting in greater deterioration.

Adults and larvae of *C. mediterranea*, pinned or in preserving fluid, have been deposited in the personal collection of Charles S. Henry, Storrs, Connecticut; the Connecticut State Museum of Natural History (CSMNH) at the University of Connecticut, Storrs; The Natural History Museum, London; the personal collection of Peter Duelli, Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland; and the W. F. Barr Museum, University of Idaho, Moscow. Additional verified specimens of the species were deep frozen at minus 70–100°C for molecular systematics studies.

#### Results

Results of ANOVAS (not shown) indicated that equivalent levels of variation existed in *C. mediterranea* among the songs of a single individual, and among the individual averages of any given population of individuals. Within single individuals, the coefficient of variation averaged 28.69% for the eight temporal song features and 7.98% for the three frequency features, while between individuals in a population, mean CV measured 20.34% and 6.78%, respectively. Therefore, we felt that it was valid to compare sex-delimited or geographical populations using individual averages, as has been done in previous studies of song variation in lacewing species (Henry & Wells, 1990).

Adult males and females of *C. mediterranea* produced a single type of song (=SRU), which was used both in solitary calling and dueting. The SRU was multisyllabic, consisting of a series of 20–130 short, similar volleys separated from each other by very short intervals (Fig. 2, Table 2). Volleys were longer and spaced further apart at the beginning of the SRU, averaging 100 msec in length and about 200 msec in period at 25°C. Volleys became progressively shorter and more closely spaced during the course of the SRU, so that by its end they averaged 74 msec in length and 124 msec in period. Volleys were too short to exhibit internal changes in carrier frequency. That frequency remained reasonably constant at around 80 Hz (25°C) throughout most of the SRU, but declined slightly to 75 Hz in the terminal volleys (Table 2, Fig. 3). Each SRU was also characterized by a progressive increase and decrease in



**Fig. 2.** Oscillograph of a typical song of *Chrysoperla mediterranea*, showing the entire SRU and a 700 msec detail of six volleys from the middle of the SRU. Song features discussed in the text are labelled.

intensity, such that maximum amplitude was achieved in the middle portion of the SRU (Figs 2, 3).

Duetting was of the 'polite' type, seen in some other lacewings with multisyllabic SRUs (e.g. *C. downesi* (Smith)/ 'mohave' (Banks); Henry, 1980, 1993b). In this type of duet, each partner answers the other only after an SRU has been completed.

#### *Sex differences in songs*

Males and females were similar but not identical with respect to the eleven features measured (Table 2, asterisks). Females generally displayed longer SRUs and slightly lower carrier frequencies than males, and showed somewhat greater amounts of overall change in volley duration and period during the course of each SRU. However, sex differences were judged small enough to warrant pooling all individuals in other comparisons.

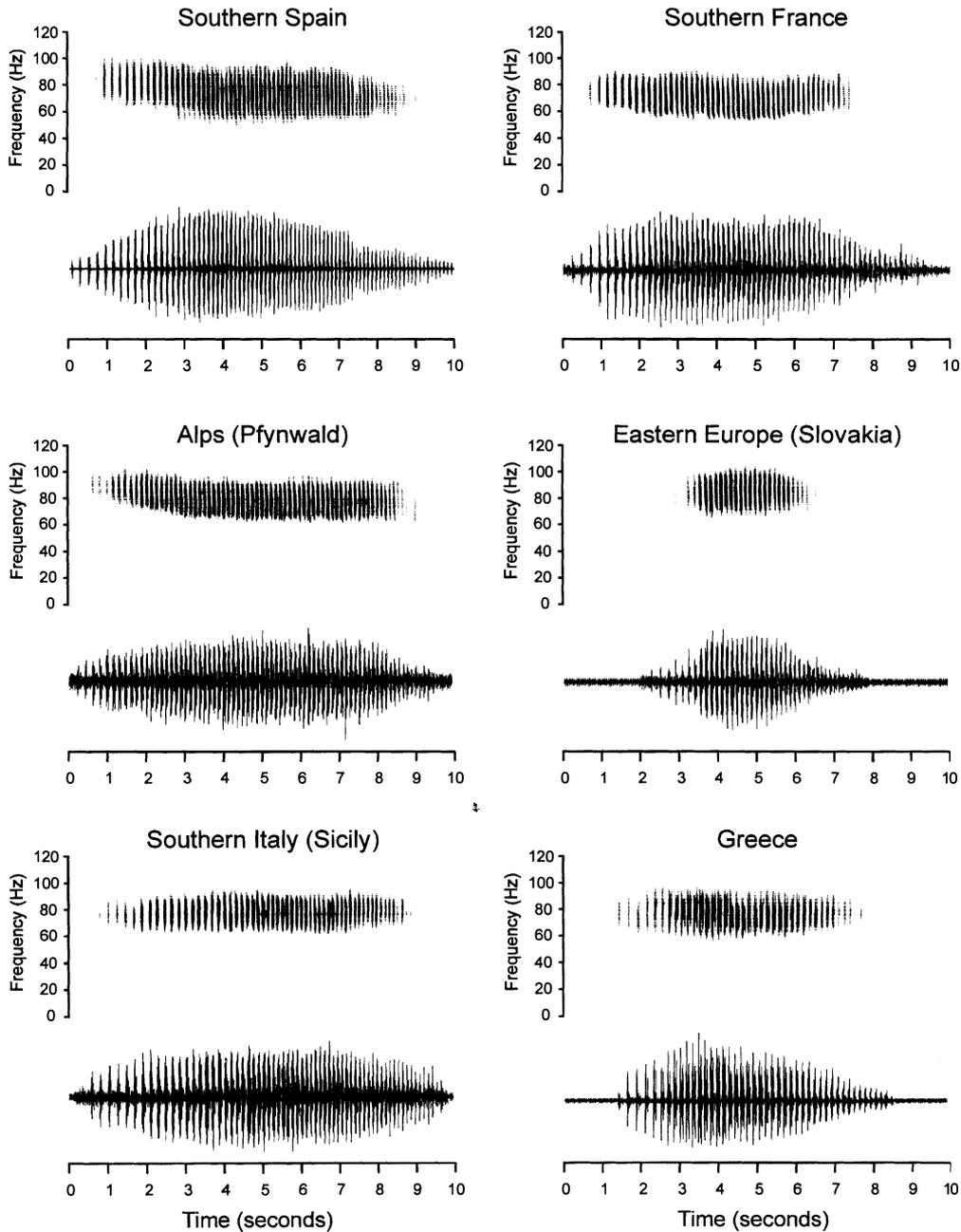
#### *Other variation in songs*

Populations of *C. mediterranea* from six geographical areas of Europe were compared with respect to measurements of eleven song features, nine of which are graphed in Fig. 4. Although differences among populations were not large, they were significant for at least some pairwise comparisons in eight of the nine features shown (different letters above bars in Fig. 4). In general, the population from

Greece was the most distinct of the six with respect to volley duration and periodicity, while eastern Europe showed the largest consistent deviation in carrier frequency. Specifically, volleys of Greek individuals were up to 20% longer and spaced as much as 12% further apart than those of individuals from other parts of Europe (Fig. 4, volley duration and period). Eastern European specimens, on the other hand, vibrated their abdomens at slightly (up to 8%) higher frequencies than did individuals from other geographical areas (Fig. 4, volley frequency).

Principal components analysis of geographical variation is shown in Tables 3 and 4, and Fig. 5. Factor loadings (Table 3) indicated that factor 1 extracted information principally about carrier frequency, while factor 2 represented temporal measurements. The fraction of total variance explained by the first two factors in this analysis was 69.3%. The geographical populations of *C. mediterranea* were poorly separated (Fig. 5), although the results supported the greater distinctiveness of Greek and eastern European individuals. Statistical analysis of the two factors with respect to geography confirmed that populations from Greece and eastern Europe were the most divergent in their song phenotypes (Table 4). Figure 3 illustrates the song of a typical individual from each geographical region.

PCA was also applied to a larger data set which consisted of mean song measurements of individuals of *C. mediterranea* and five other song species of the *carnea*-group (Fig. 6). Factor loadings (not shown) indicated that temporal and frequency measurements contributed significantly to both factors. The fraction of total variance explained by the first two factors in



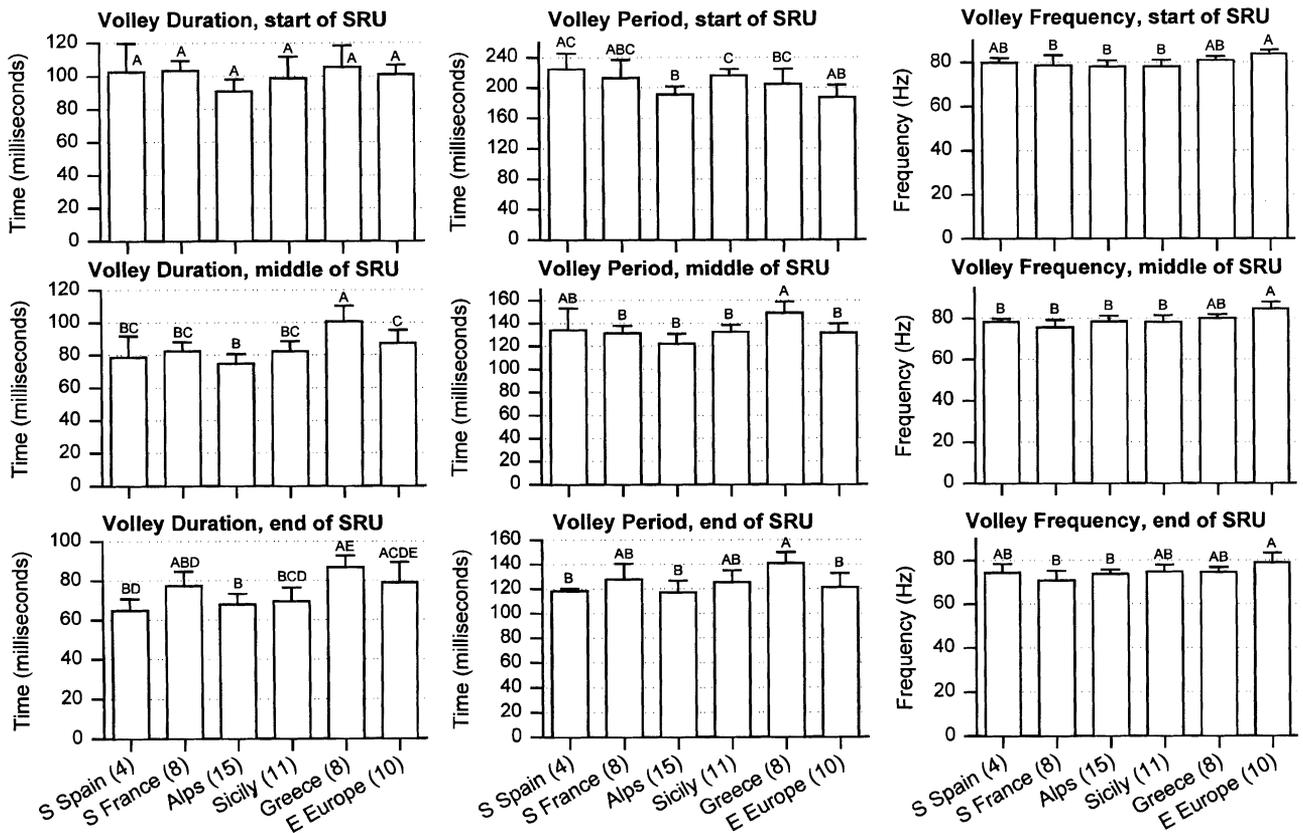
**Fig. 3.** Oscillographs and sonographs (above each oscillograph) of typical songs of *C. mediterranea* from six geographical regions of Europe shown in Fig. 1. The short SRU illustrated from eastern Europe was not typical for that region, but was the best recording in our possession.

this analysis was 75.8%. Each song species formed a very distinct cluster, exhibiting little overlap with other species, and each species was significantly different from every other species, usually with respect to both principal components (results of ANOVA and Scheffé's test not shown). The only exceptions, where significant differences were not found in pairwise comparisons, were *C. adamsi* Henry, Wells & Pupedis and *C. johnsoni* Henry, Wells & Pupedis for factor 1, *C. adamsi* and *C. downesil'mohave*' for factor 2, and *C.*

*plorabunda* (Fitch) and *C. mediterranea* for factor 2 (see also Fig. 6).

When correlation structure was ignored prior to applying each PCA/ANOVA procedure and *post-hoc* comparison, results (not shown) differed little from those described above and illustrated in Figs 5 and 6, although the placement of clusters relative to each other in factor space was altered.

Although formal behaviour experiments were not conducted, it was observed throughout this study that



**Fig. 4.** Bargraphs comparing six distinct geographical populations of *C. mediterranea* with respect to the means (bars) and standard deviations (error bars) of nine features of courtship song. Both sexes were included in the calculations. Populations with significantly different means share no letters (determined by ANOVA and *post-hoc* Scheffé's tests,  $P \leq 0.05$ ).

**Table 4.** Comparison of six geographical populations of *C. mediterranea* using the scores of the first two factors of a principal components analysis of eight uncorrelated song measurements. Results of an ANOVA and *post-hoc* Scheffé's tests are summarized by superscripted letters: populations with significantly different means share no letters ( $P \leq 0.05$ ).

Geographical region ( <i>n</i> )	Factor 1, mean $\pm$ SD	Factor 2, mean $\pm$ SD
Southern Spain (4)	-0.526 $\pm$ 0.175 <sup>BD</sup>	-0.180 $\pm$ 1.155 <sup>AB</sup>
Southern France (8)	-0.820 $\pm$ 1.085 <sup>B</sup>	0.328 $\pm$ 0.417 <sup>BC</sup>
Alps (15)	-0.272 $\pm$ 0.707 <sup>BC</sup>	-0.898 $\pm$ 0.548 <sup>A</sup>
Sicily (11)	-0.459 $\pm$ 0.692 <sup>BD</sup>	-0.098 $\pm$ 0.758 <sup>ACD</sup>
Greece (8)	0.505 $\pm$ 0.507 <sup>ACD</sup>	1.333 $\pm$ 0.921 <sup>B</sup>
Eastern Europe (10)	1.376 $\pm$ 0.494 <sup>A</sup>	0.197 $\pm$ 0.823 <sup>BD</sup>

individuals of *C. mediterranea* responded only to conspecific songs in playback trials. In apparent disregard of the song differences detected among geographical populations, playbacks of songs from any of the six regions

elicited normal dueting responses from individuals obtained anywhere in Europe.

#### Adult morphology

**Ground colour of body.** In most specimens examined the body was dark green. However, in the majority of those examined from Greece, the ground colour was paler green.

**Extent of markings on stipes.** The stipes were marked with a broad black stripe along the entire length in all specimens.

**Colour of palps.** The palps were entirely black in all specimens examined.

**Extent and colour of genal markings.** The gena was marked with a broad black band in all specimens.

**Clypeal markings.** In most specimens the clypeus was marked laterally with a narrow black band. In some specimens from Greece the clypeus was unmarked.

**Frontal markings.** In most specimens the frons and labrum were marked extensively with a red or orange-red band which

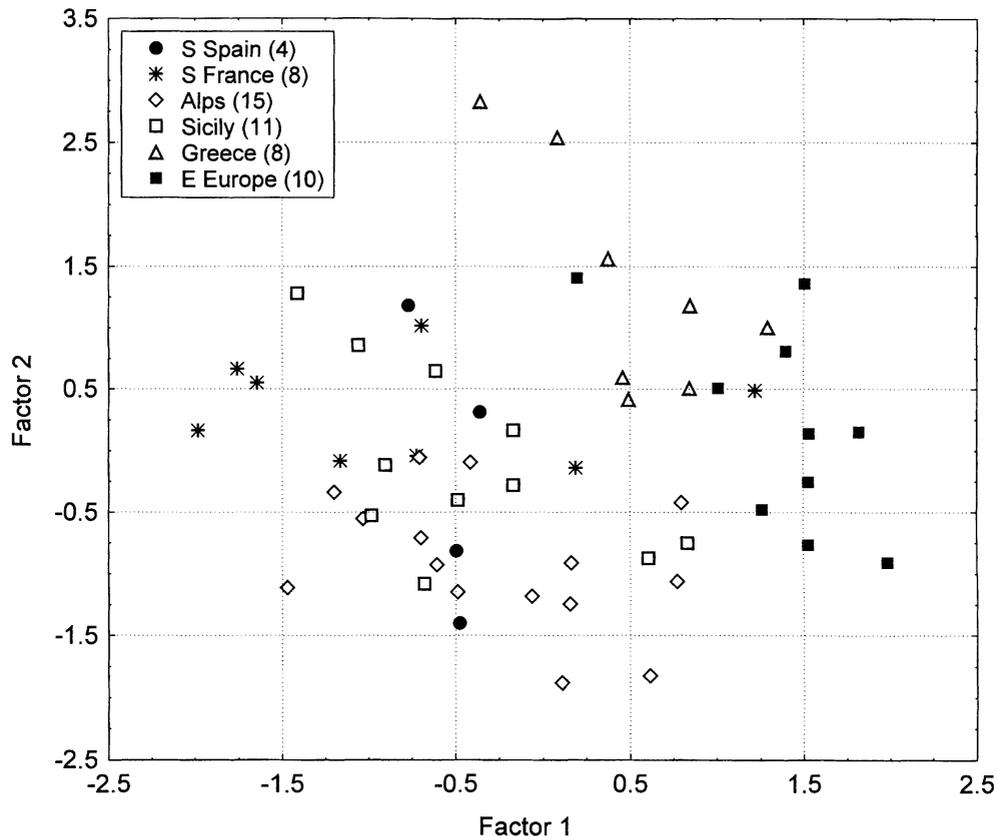


Fig. 5. Scatterplot of the first two factors of a principal components analysis of eight uncorrelated measurements of the songs of *C. mediterranea*. Each data point represents a single individual, coded by geographical regions shown in Fig. 1.

extended across the front of the head. In some specimens this band was interrupted medially.

**Markings on post ocular region.** The post ocular region was either unmarked or marked with a red band. Variation in markings did not appear to be related to regional distribution.

**Relative abundance and distribution of black and blonde setae on pronotum.** In most specimens the pronotal setae were entirely stout and black. In some specimens from Greece, blonde setae were present in the mid-line. In the specimen (male) from Algeria the pronotal setae were entirely blonde.

**Relative size of basal dilation of tarsal claw expressed as a ratio of total width of claw (B) to length of its basal dilation (A)** (Fig. 7, Switzerland). Specimens from the western part of the distributional range (Algeria, Spain, Majorca, southern France and Sicily) had a minute basal dilation to the claw (mean  $\pm$  SD =  $6.28 \pm 1.49$ ,  $n = 19$ , range = 4.27–9.80). Specimens from the eastern part of the range (Greece, Austria and Slovakia) had a larger basal dilation (mean  $\pm$  SD =  $2.65 \pm 0.40$ ,  $n = 36$ , range = 2.04–4.00). Swiss specimens were intermediate in basal dilation (mean  $\pm$  SD =  $3.86 \pm 1.43$ ,  $n = 4$ , range = 2.68–5.75; see Fig. 7).

**Extent to which fore wing is rounded or tapered at apex.** Fore wing rounded at apex.

**Relative width of fore wing expressed as a ratio of length to breadth at widest point.** The fore wing was relatively narrow (mean  $\pm$  SD =  $3.32 \pm 0.19$ ,  $n = 65$ , range = 2.83–3.72).

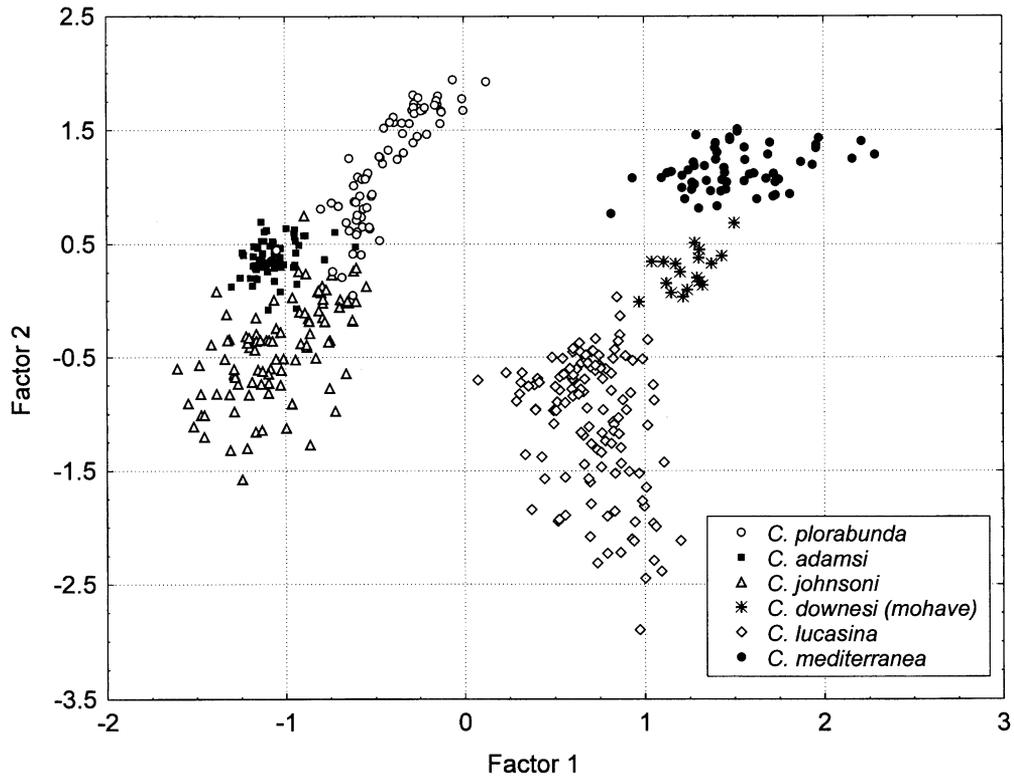
**Presence or absence of black markings on wing veins.** The wing veins were entirely green in all specimens except those from the eastern part of the range (Switzerland, Greece, Austria, Slovakia), in which the major crossveins at the base of the fore wings were marked black.

**Length of costal setae.** Costal setae were short and inclined apically.

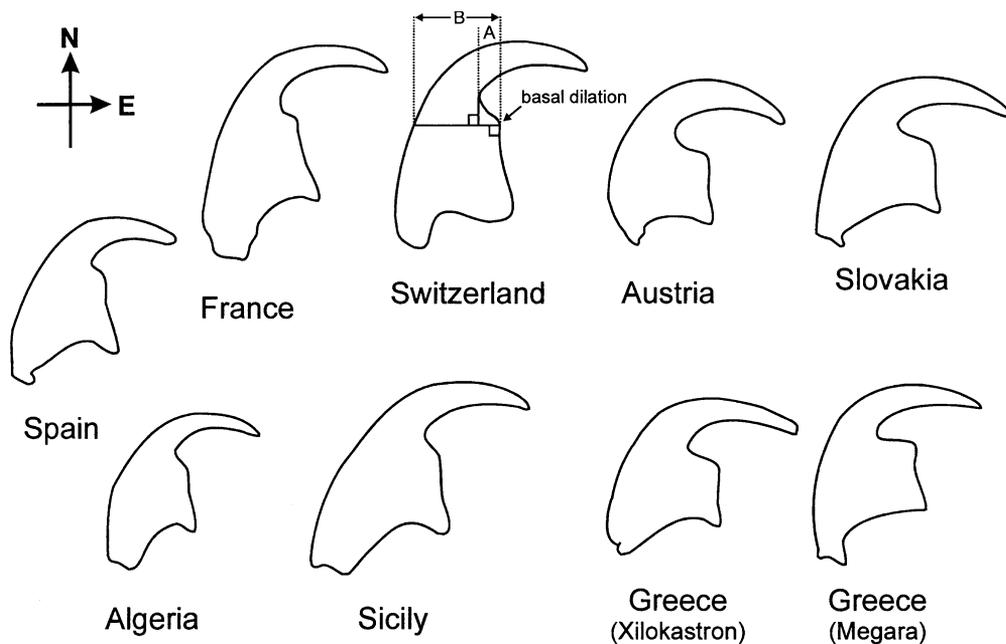
**Presence or absence of black setae on abdominal sternites.** Specimens from Switzerland, Greece, Slovakia and Austria tended to have a greater proportion of black setae on the abdominal sternites than those specimens from western localities.

**Length of abdominal setae.** Abdominal setae were considerably longer in male specimens from Switzerland, Greece, Slovakia and Austria than in males from western localities (females not analysed).

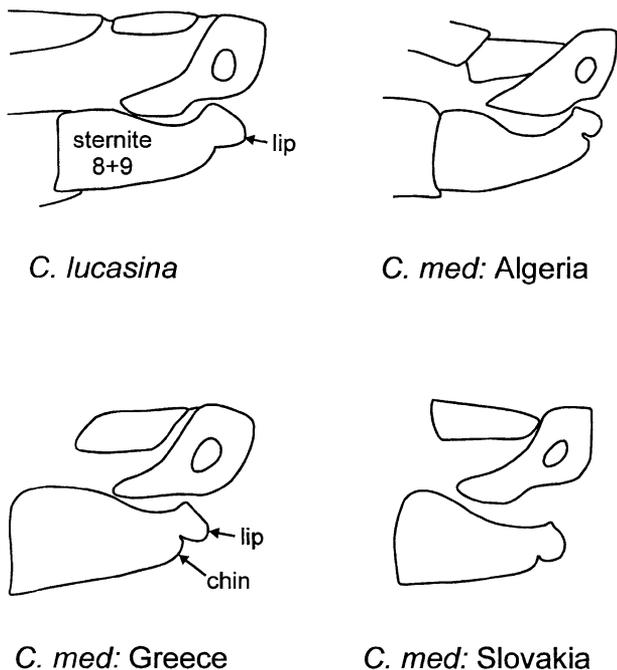
**Shape of lip at apex of sternites 8 + 9 in males.** The lip was relatively short and narrow. In specimens from the western part of the distributional range (Spain, southern France and



**Fig. 6.** Scatterplot of the first two factors of a principal components analysis of six uncorrelated measurements of the songs of six distinct song species of the *carnea*-group. Each data point represents a single individual, coded by species.



**Fig. 7.** Claw shapes of *C. mediterranea* from various sites throughout Europe displayed on a rough compass grid, illustrating the change in proportions of the basal dilation in western vs. eastern populations. Shape is quantified as the ratio  $B/A$  (illustrated on claw from Switzerland), where line 'B' is drawn perpendicular to the outer margin of the basal dilation.



**Fig. 8.** Diagnostic lip and chin at apex of sternites 8+9 in males, showing those features in three geographical populations of *C. mediterranea* vs. the condition typically seen in *C. lucasina*.

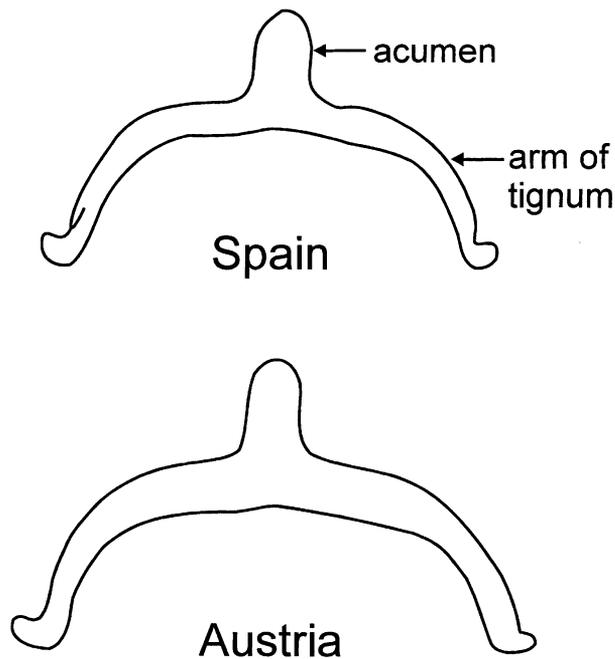
Algeria) the lip was not markedly downturned and hardly protruded beyond the apex of the sternite, which was formed as a distinct 'chin' (Fig. 8, Algeria). Specimens examined from Switzerland, Greece, Austria and Slovakia had a more protruding, downturned lip, and a less pronounced and rather receding chin (Fig. 8, Greece and Slovakia).

*Relative size of medial lobe (acumen) of tignum in male genitalia expressed as a ratio of length of arm of tignum to length of acumen.* The acumen was relatively long and narrow (range = 3.09–3.49; Fig. 9).

*Presence or absence of dark brown stripe on pleural membrane of second abdominal segment.* There was no stripe on the pleural membrane of the second abdominal segment.

#### Larval morphology

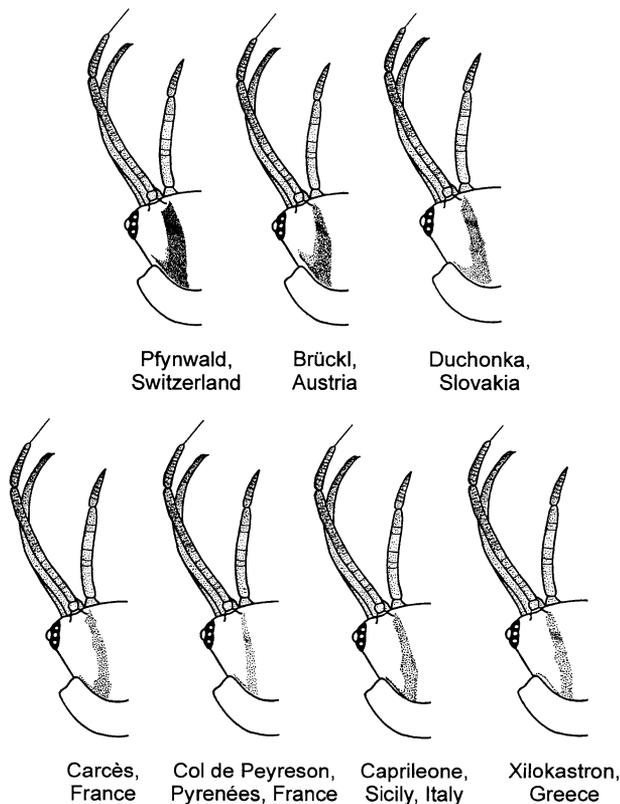
*Chrysoperla mediterranea* larval head capsule markings were dominated by a pair of longitudinal, dorso-lateral brown stripes, each with a baso-lateral expansion extending toward the eyes (Fig. 10). First-instar larvae showed the palest and broadest (relative to head width) longitudinal stripes and the least developed baso-lateral expansions. Within a given population, second instars were intermediate and third instars had the darkest and relatively narrowest longitudinal stripes and the most prominent baso-lateral expansions. Because all three instars showed variation and similar geographical trends, the remainder of the results focuses on third instars.



**Fig. 9.** Tignum of male genitalia of *C. mediterranea* from a western and an eastern population in Europe.

In all populations ( $n=4-25$ ), there was variation in the width, regularity of the margins, and colour intensity of the longitudinal stripes. The latter could have been influenced by the time elapsed since the last moult, or by length and type of preservation. There were also examples of variation within specific populations. Larvae from Carcès (France) and the three northern populations (Switzerland, Austria and Slovakia) varied in the amount of pigmentation between the longitudinal stripes and baso-lateral expansions. Additionally, four (of sixteen) of the third instars from Carcès had a short, narrow antero-medial spot on the frons and three (of eighteen) larvae from Switzerland had a small, pale spot mesad of the apex of each longitudinal stripe. These two latter patterns were unique among the populations studied, but were not illustrated because they represented a minority of individuals within each population.

Variation between populations was also evident. The longitudinal stripes were paler and more slender in the southern populations, becoming slightly darker to the east (Fig. 10). These stripes were distinctly broader and darker in the Swiss and Austrian populations. The Slovakian population was intermediate, with the stripes relatively narrow, but distinctly darker than the coastal populations. The southern populations also had little (Carcès) or no (Col de Peyreson, Sicily and Greece) shading between the longitudinal stripes and baso-lateral expansions, causing the latter to resemble an arm off each longitudinal stripe (Fig. 10). All three northern populations had a substantial, but variable amount of pigmentation between the stripes and baso-lateral expansions, causing the expansions to resemble lobes rather than arms.



**Fig. 10.** *Chrysoperla mediterranea*, third-instar larval head capsules from selected localities across Europe, grouped into northern (upper three) vs. southern (lower four) populations.

Another source of variation between populations was the presence or absence, and in some cases prominence, of an oblique, darker band within the longitudinal stripes mesad of the eyes. This band was evident in the three northern populations and was present, but not conspicuous, in the populations from Col de Peyreson and Greece (Fig. 10). The last significant variation observed was that the area around the eyes was suffused with purple in the Swiss population. This was omitted from the illustration (Fig. 10) to avoid confusion with the standard, brown pigmentation.

### Ecology

Our knowledge of the ecology of *C. mediterranea* is limited. What is known about habitat preference, hibernation and oviposition has been gleaned principally from one population endemic to the Pfywald, site of the largest (800 ha) pine forest in Switzerland (Duelli, 1987). There, in patches dominated by *Pinus sylvestris* Linnaeus, *C. mediterranea* is by far the most abundant lacewing species. It is usually strictly limited to pine trees, but after storms with strong winds some individuals can be collected on deciduous trees within the forest. During the day, adults sit on pine needles, with their heads tucked into the angle between the base of the needle and the twig. Populations of *C. mediterranea* elsewhere in Europe are also associated

with pines. However, where there are no pines, as in Brückl, Austria, the species may live on other conifers, such as the Norway spruce *Picea abies* (Linnaeus) (H. Hölzel, personal communication). As mentioned earlier, *C. mediterranea* adults remain green during winter diapause.

Eggs are deposited singly on the tips of needles, often with several eggs on the same twig. In winter, dense clusters of hibernating *C. mediterranea* can be found under the loose bark of dead pine trees. Life-cycle traits such as diapause induction in mid-August and resumption of sexual activity in early spring coincide temporally with those of other sympatric, cryptic species of the *C. carnea* complex, including *C.c.2* 'slow motorboat' and *C.c.4* 'motorboat', which are found almost exclusively on deciduous trees (note that the nomenclature for undescribed species follows that proposed by Duelli *et al.*, 1996). Also like other *C. carnea* spp., two generations develop annually in the Pfywald.

### Discussion

*Chrysoperla mediterranea* shares some basic attributes of its song with several other song species of the *carnea*-group. For example, among the six fully described species, only *C. johnsoni* and *C. downesi/mohave'* in North America and *C. lucasina* in Europe have multisyllabic SRUs (Henry, 1980, 1993a; Henry *et al.*, 1996). Of those three species, only *C. downesi/mohave'* has short, rapidly repeating volleys of the type found in *C. mediterranea*. In other important aspects of song phenotype, however, *C. downesi/mohave'* is quite unlike *C. mediterranea* (Henry, 1993b).

Principal components analysis places each described song species of the *carnea*-group in a distinct cluster (Fig. 6). *Chrysoperla mediterranea* is positioned closest to *C. downesi/mohave'* in factor space, consistent with the phenetic similarity of their songs noted above. The six taxa also fall into two larger groups, one of which comprises *C. mediterranea*, *C. downesi/mohave'* and *C. lucasina*. Those three species are all characterized by long SRUs composed of many volleys, while the other group of species has SRUs of variable length but consistently made up of just a few long volleys.

Two other, undescribed or undetermined, song species belonging to the *C. carnea* complex of Europe resemble *C. mediterranea* in possessing long, multisyllabic SRUs consisting of short repeating volleys. However, one repeats much more rapidly (*C.c.4* 'motorboat') and the other much more slowly (*C.c.2* 'slow motorboat') than *C. mediterranea* (Duelli *et al.*, 1996). Also, neither of these two taxa has frequency characteristics that match those of the *C. mediterranea* song very closely (C. S. Henry, unpublished data).

To summarize, *C. mediterranea* has a unique song phenotype, some aspects of which superficially resemble features of the songs of four other known species in the *carnea*-group, one from North America (*C. downesi/mohave'*) and three from Eurasia (*C. lucasina*, *C.c.4* 'motorboat' and *C.c.2* 'slow motorboat').

### Song variation

Measures of within- and between-individual variation are low in *C. mediterranea*, suggesting that song features are highly constrained, presumably by stabilizing selection acting on the specific mate recognition system (Paterson, 1986; Butlin & Ritchie, 1994). Males differ from females only slightly, and then principally in the shorter average duration of their SRUs (Table 2). This quality characterizes several other described and undescribed/undetermined song species in Europe, including *C. lucasina* (male 'short songs', Henry *et al.*, 1996), *C.c.4* 'motorboat', and *C.c.2* 'slow motorboat' (C. S. Henry, unpublished data; Duelli *et al.*, 1996). It results from the tendency for males of these species to sing more readily than females when alone, producing a truncated version of the long courtship song that is useful in starting up duets with potential partners in the vicinity. A similar sex effect is found in the multisyllabic North American species *C. johnsoni* (Henry, 1993a), but not in *C. downesi*'*mohave*' where it might also be expected (Henry, 1980).

Among populations of *C. mediterranea* selected from different parts of Europe, only small differences are found in any song feature (Table 2; Fig. 4), and principal components analysis shows weak clustering of individuals by geographical location (Tables 3, 4; Fig. 5). The differences which exist are most apparent in peripherally located populations such as Greece and eastern Europe, as expected in a widely distributed species such as this (Fig. 1). Nothing in our results indicates that *C. mediterranea* is confined to regions south of the Alps, as once believed. The Pfywald population from Switzerland is unambiguously intermediate in most of its song features when compared with the other populations, while Austrian and Slovakian representatives of *C. mediterranea* deviate only slightly from average values for the species (Figs 4, 5). Figure 3 graphically highlights this fundamental geographical homogeneity of *C. mediterranea* courtship songs. More importantly, the insects themselves fail to discriminate between local songs and those recorded from individuals obtained from distant geographical locations.

The status of Duelli's 'pine *carnea*' from the Pfywald of Switzerland remains problematical (Duelli, 1987). Duelli concluded that it represented a relict population of *C. mediterranea*, far outside the range of the species as then delineated. As mentioned above, our results support its identity as *C. mediterranea*, but there is no longer any reason to consider it a relict population. On the other hand, we cannot account for Cianchi & Bullini's (1992) results, showing genetic differentiation and apparent reproductive isolation between Pfywald *C. mediterranea*, French *C. mediterranea* and Pfywald 'oak *carnea*'. Before that problem can be addressed, the source population of *C. mediterranea* from southern France must be examined carefully for its morphological and acoustical characteristics.

Another result of the principal components analysis of geographical song variation in *C. mediterranea* is the partitioning of variance for temporal versus frequency features into different factors (Table 3). For species recognition in insects, the temporal qualities of an acoustical mating signal

are usually more critical than its carrier frequency characteristics (Fulton, 1952; Walker, 1963; Ewing & Bennet-Clark, 1968; Bailey, 1976; Bailey, 1991). Thus, such aspects as pulse or phrase duration, interval and period are under stronger stabilizing selection and will show less variation than carrier frequency (Butlin, 1995). In the present analysis, temporal features contribute principally to factor 2, which in PCA always accounts for less of the total variance than factor 1. Such a result, indicating less variation in temporal song features, suggests that *C. mediterranea* is similar to other acoustical organisms in the pre-eminence of phrasing over frequency in its mating signal system.

### Song types and evolution

Resemblance between the song of *C. mediterranea* and those of other song species could result from ancestry, adaptive convergence or chance.

Resemblance due to ancestry can result either because of common membership in a monophyletic clade united by the derived (apomorphic) song trait, or by retention of the ancestral (plesiomorphic) character state in separate lineages that shared a common ancestor long ago. A phylogenetic hypothesis for most of the cryptic song species of the *carnea*-group, reconstructed by sequencing the COII gene of the mitochondrial genome, does not support a close relationship between *C. mediterranea* and any other singers with similar songs (Wells & Henry, 1998). For example, *C. downesi*'*mohave*' and *C. mediterranea* belong to different major clades, associated with different continents. However, the molecular phylogeny leaves open the possibility that trains of short volleys are the basal or ancestral condition in *Chrysoperla*.

Adaptive convergence of songs could occur if species associated with different habitats evolve substrate-specific signals which propagate optimally in those substrates. Thus, two unrelated (or related but allopatric) species which share habitat preferences would evolve convergent mating signals (Endler, 1993; Butlin, 1995). Most members of the *carnea*-group appear to be habitat generalists and therefore not likely to exhibit adaptive convergence of songs (Henry *et al.*, 1993; Monserrat & Marín, 1994). However, *C. downesi* in North America and *C. mediterranea* in Europe are unusual in showing a strong preference for conifers (Smith, 1932; Bram & Bickley, 1963; Monserrat & Marín, 1994), and those two species in fact share several important song characteristics. Before adaptive significance can be ascribed to this similarity, it must be demonstrated that song features shared by *C. mediterranea* and *C. downesi* actually enhance propagation of signals in conifer needles versus leaves, grass or stems. Documenting a habitat preference for conifers in *C.c.4* 'motorboat' and *C.c.2* 'slow motorboat' would further support an adaptive explanation of song convergence, because those two European song species also bear some acoustical resemblance to *C. mediterranea*. However, existing collecting records do not currently substantiate the existence of conifer

specialization in either *C.c.4* or *C.c.2* (C. S. Henry, S. J. Brooks, P. Duelli & J. B. Johnson, unpublished data).

Chance can play a role in song similarities if the genetic basis of song phenotype is relatively simple and single mutations can alter the signals dramatically. For example, if song features are controlled by few loci, there exists a significant probability that the same allele could become fixed in two unrelated lacewing populations, producing similar phenotypes. Hybridization studies have been performed on two North American species-pairs, *C. plorabunda* × *C. downesi* (Henry, 1985) and *C. plorabunda* × *C. johnsoni* (Wells & Henry, 1994; C. S. Henry, unpublished data). Results suggest that pairs of alleles at relatively few loci can explain the patterns of variation in  $F_1$ ,  $F_2$  and backcross progeny, supporting the possibility of chance convergence of songs in the *carnea*-group.

In summary, further work must be done before it will be possible to choose among hypotheses explaining song similarities in lacewings. On the other hand, all hypotheses are testable, and such tests will provide insight into the patterns and processes of song evolution in the *carnea*-group and other *Chrysoperla* species.

#### Morphology of adults

As mentioned above, other authors (Hölzel, 1972; Monserrat, 1977; Aspöck *et al.*, 1980; Duelli, 1987; Brooks, 1994; Thierry *et al.*, 1998) have distinguished *C. mediterranea* from superficially similar species in the *C. carnea* complex using morphology, in particular by the shape of the tarsal claw and wings. In the present study those two characters, together with a suite of sixteen additional traits, were examined in *C. mediterranea* specimens that had been segregated from other *C. carnea* complex individuals on the basis of their courtship songs.

In earlier studies (Hölzel, 1972; Brooks, 1994), *C. mediterranea* was distinguished by its narrow fore wings. We found that, on average, the fore wing of *C. mediterranea* is indeed narrower (mean length/width=3.32) than in other species of the *C. carnea* complex (mean=3.02, Brooks, 1994). However, there is also considerable overlap between the two taxa in this measurement (2.83–3.72 in *C. mediterranea*; 2.7–3.1 in the *C. carnea* complex), so the character is not diagnostic for *C. mediterranea*.

The minute basal dilation of the claw tooth has also been regarded as diagnostic for *C. mediterranea* (Fig. 7). This character holds for specimens from the western part of the range. However, in specimens examined from Greece, Slovakia and Austria, the basal dilation is relatively large and falls within the range of other species in the *C. carnea* complex (Brooks, 1994; Henry *et al.*, 1996). Measurements of basal dilation in Swiss specimens are intermediate between values characteristic of eastern and western populations. The character is therefore not diagnostic for specimens of *C. mediterranea* from the eastern part of the range. The recent, multivariate statistical analysis of claw morphometrics by Thierry *et al.* (1998), which shows a clear separation of *C.*

*mediterranea* from four other 'species' of the *C. carnea* complex, nonetheless suffers from this same problem of narrow geographical perspective. All specimens of *C. mediterranea* used in that study were collected at one site in south-west Europe, near Valencia, Spain, and cannot be expected to show the full range of sizes for the basal claw dilation typical of the species.

Other morphological characters also distinguish specimens from the eastern part of the range from western specimens. In specimens from Switzerland, Greece, Austria and Slovakia the crossveins between veins *Psc* and *Psm* at the base of the fore wing are black, whereas those veins are green in specimens from western Europe. Eastern specimens also possess longer abdominal setae than those from western sites.

The presence of a lip at the apex of sternite 8+9 in males is autapomorphic for *Chrysoperla* (Brooks & Barnard, 1990). A survey of this character throughout the song taxa so far segregated within European *C. carnea s.l.* has shown the shape of the lip to be diagnostic in most species. In *C. mediterranea*, the lip is shorter and narrower than in *C. lucasina* and, instead of being directed posteriorly, it is downturned (Fig. 8). However, again, eastern and western populations of *C. mediterranea* differ in the relative length of the lip and extent of protrusion of the 'chin'. In western specimens, the lip is shorter and the chin more protruding than in eastern individuals (Fig. 8).

A consistent morphological trait characteristic of *C. mediterranea* specimens from all parts of its distribution appears to be the extensive red markings across the front of the head. Other species in the *C. carnea* complex have black markings restricted to the gena and lateral clypeus and lack markings across the middle of the frons and labrum (Brooks, 1994).

#### Morphology of larvae

The basic pattern of larval head capsule markings for *C. mediterranea* is a pair of longitudinal, dorso-lateral brown stripes, each with a baso-lateral expansion. This pattern appears to occur throughout the *carnea*-group (Tauber, 1974; Henry *et al.*, 1996; C. S. Henry, S. J. Brooks, P. Duelli & J. B. Johnson, unpublished data). The variation observed in first-instar larvae over the wide geographical range of *C. mediterranea* does not support Thierry *et al.*'s (1992) suggestion, based on a study of three other *Chrysoperla* spp. in France, that first instars are significantly more conservative than later stadia.

*Chrysoperla mediterranea* larvae from the four southern populations studied are distinct from those of other species of the *carnea*-group: their longitudinal stripes are the narrowest and palest of any populations yet seen, and, more definitively, the baso-lateral expansions are present as slender arms. Outside of the *carnea*-group, one finds similar slender baso-lateral arms in larvae of *C. mutata* (McLachlan), but *C. mutata* specimens seen by the authors have an antero-medial spot on the frons, which is generally lacking in *C. mediterranea*. In addition, *C. mutata* larvae

are easy to distinguish from *C. mediterranea* by their robust body form, typical of members of the *pudica*-group to which *C. mutata* belongs (Brooks, 1994). The pale or absent oblique, darker band within each longitudinal stripe also seems to be unique to *C. mediterranea* among members of the *carnea*-group from southern Europe. However, absence of the antero-medial spot is not helpful for identification in these populations, because it is not universal and some samples of *C. lucasina* and *C.c.3* 'maltese' from the Mediterranean coast also lack this spot.

The three northern populations of *C. mediterranea* are similarly problematic. Their wider, darker stripes, each with a broad, basal lobe, closely resemble several other species, particularly *C. lucasina* and *C.c.3* 'maltese' (Henry *et al.*, 1996; C. S. Henry, S. J. Brooks, P. Duelli & J. B. Johnson, unpublished data). In addition, the oblique, darker band within each stripe is common among northern *Chrysoperla* spp. populations (Henry *et al.*, 1996; C. S. Henry, S. J. Brooks, P. Duelli & J. B. Johnson, unpublished data). However, *C. mediterranea* is unique among the northern populations examined, to date, in lacking the antero-medial spot on the frons.

Thus, as has been found true for *C. lucasina* (Henry *et al.*, 1996), there is no character or suite of characters that will allow recognition of all *C. mediterranea* larvae, but within a region larval head capsule markings may prove useful. The remaining challenge with regard to *C. mediterranea* larvae is to link more closely the northern and southern populations. This effort is most likely to be successful with more collecting in the eastern portion of the species' range, between Slovakia and Greece.

## Conclusion

Morphological analyses of adults suggest that *C. mediterranea* falls into two distinct population groups, one in south-western European (Algeria, Spain, Majorca, southern France and Sicily) and the other in central and south-eastern Europe (Switzerland, Greece, Austria, Slovakia). However, larval morphological variation seems to follow a north-south gradient. Song features deviate the most from average in specimens from eastern Europe and Greece, but the precise geographical pattern of variation in songs is not congruent with that seen either in adult or larval morphology (see Results and Figs 4, 5). In addition, song differences are much less pronounced than latitudinal and longitudinal variation in adult or larval morphology, and are insufficient to prevent normal behavioural responses even between individuals from distant geographical populations. On the basis of song homogeneity alone, *C. mediterranea* should be considered a single, valid species, using the same reasoning that has been applied to other song species within the *carnea*-group (Henry *et al.*, 1993). Plasticity in morphological characters over a wide geographical range is perhaps to be expected, rather than variation in the courtship songs, which are part of the specific mate recognition system and thus under strong stabilizing selection pressure (Paterson, 1986; Butlin & Ritchie, 1994).

*Chrysoperla mediterranea* should be redefined as a species within the *C. carnea* complex closely associated with coniferous vegetation and distributed across nearly all of southern and central Europe. It possesses a unique, multi-syllabic vibrational mating signal (song), which shows very little variation among individuals or between the sexes across the distributional range. Morphologically, *C. mediterranea* is best characterized by its brilliant to dark green coloration and the prominent red markings on the front of the head. Larval features overlap those found in several other closely related species. Once promising adult characters, including the size of the basal dilation of the claw and relative narrowness of the wings, cannot be used reliably to discriminate *C. mediterranea* from other members of the *carnea*-group.

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