

# ***Chrysoperla lucasina* (Lacroix): a distinct species of green lacewing, confirmed by acoustical analysis (Neuroptera: Chrysopidae)**

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**Abstract.** The existence of cryptic, sibling species, distinguished principally by vibrational courtship songs, has been confirmed for the *carnea*-group of *Chrysoperla* green lacewings in Europe and western Asia. One member of this species-group, *C. lucasina* (Lacroix), is characterized by several morphological traits as well, but its taxonomic validity has been considered uncertain due to the subtle and variable nature of those traits. Here, we describe the calling and courtship songs of *C. lucasina*, which uniquely distinguish that taxon from all other members of the *carnea*-group in Europe. The male of *C. lucasina* produces two slightly but consistently different types of songs, one while calling and the other while dueting with a female. The female produces just one type of song, identical to the male's calling song, which is used for both calling and dueting. Measured features of the songs of *C. lucasina* are nearly invariant over a broad geographical area, from the Canary Islands to Cyprus and from northern Africa to the U.K. Similarly, at least one morphological feature, the presence of a dark stripe on the pleuron of the second abdominal segment, characterizes all adult individuals identified acoustically as *C. lucasina*. Furthermore, larval head markings vary little among different populations of the song species. We conclude that *C. lucasina* is a valid biological species, which can also be recognized in museum collections by a suite of morphological attributes. We also briefly compare the songs and adult morphology of *C. lucasina* with five other currently unnamed song species of the *carnea*-group whose geographic ranges overlap extensively with it.

## **Introduction**

Although the green lacewing genus *Chrysoperla* Steinmann is well defined (Brooks & Barnard, 1990), its constituent species have proven to be particularly difficult to distinguish from one another. Consequently, the systematics of the genus as a whole has been neglected. Brooks' (1994) recent taxonomic review of *Chrysoperla* addressed and largely corrected this problem, but the study was not able to resolve many cases of cryptic, sibling species that occur in North America and Eurasia.

One of the most useful new tools for identifying biological species within *Chrysoperla* is song analysis. The genus is

characterized by substrate-borne vibrational courtship signals, which are produced by vertical oscillations of the abdomen (Henry, 1979). These tremulation songs are produced by both males and females of a species in essentially identical form, and are highly species-specific (Henry, 1985). A sexually receptive male and female must establish a vibrational duet as a precursor to and prerequisite of copulation. Experiments testing female choice of recorded and computer-synthesized courtship songs confirm that the songs have a powerful reproductive isolating effect and probably serve as the principal barriers to hybridization between different song species (Wells & Henry, 1992a,b, 1994).

Brooks (1994) recognizes four distinct species groups within *Chrysoperla*, based mainly on morphology of the male genitalia and wings: the *carnea*-group, the *comans*-group, the *nyerina*-group and the *pudica*-group. The *pudica*-group corresponds to what is known in North America as the *rufilabris*-group (Bram & Bickley, 1963). The *carnea*-group is the least derived of the

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species groups (Brooks, 1994) and exhibits the best developed singing behaviour (Henry, 1984). Because songs are so important to the reproductive isolation of closely related species in that group, the problem of ambiguously defined cryptic, sibling species is greatest there.

For the *carnea*-group in North America, progress has begun toward identifying sibling 'song' species within single morphological species. From the species *C. plorabunda* (Fitch), for example, three additional species [*C. adamsi* Henry *et al.*, *C. johnsoni* Henry *et al.* and *C. downesi* (Smith)] have been formally recognized, based on their highly distinctive vibrational courtship songs (Henry, 1993b; Henry *et al.*, 1993). However, before the current study, song analysis had not been systematically applied to any Palaearctic members of the *carnea*-group. Earlier, preliminary acoustical work showed that *C. carnea* (Stephens) was certainly not the same species as Nearctic *C. plorabunda*, although that view had been prevalent at the time (Henry, 1983). We are now in a position to refine our concept of Palaearctic *C. carnea*, based on detailed studies of acoustically defined members of that morphological species which we have collected in Europe and parts of Asia. Specifically, we begin this effort with a detailed description of the tremulation songs of *C. lucasina* (Lacroix), which is now the best defined of the many morphologically or ecologically based 'species,' subspecies, varieties, ecotypes and colour morphs of Europe that are currently embedded within the larger taxonomic entity known as *C. carnea*.

### Current systematic status of *C. carnea* in Europe

At least eighty-one different species names have been applied to all or parts of the morphological species, *C. carnea*. Most are not valid, because the named taxa are poorly defined, highly variable, synonymous with other taxa, or overlapping in their defining characteristics with other forms. However, several recent studies have supported the existence of sibling species in Europe, and some attempt has been made to assign consistent, legitimate names to those species. Such studies include morphological surveys of localized fauna (Leraut, 1991), electrophoretic and genetic analyses (Bullini & Cianchi, 1983; Bullini *et al.*, 1984; Cianchi & Bullini, 1992), detailed morphometric examination of male genitalia (Séméria, 1992), and multivariate analysis of morphological and ecophysiological character states in adults and larvae (Thierry & Adams, 1992; Thierry *et al.*, 1992, 1995).

In the Loire Valley of France at least, a modest consensus has emerged which recognizes three sympatric species, *C. carnea s. str.*, *C. kolthoffi* (Navás) and *C. lucasina*, putatively separable using morphological and life history traits (Thierry *et al.*, 1995). Problems arise, however, when one extends these systematic determinations to other geographic areas. Although Thierry *et al.* (1996) argued that the three species are easily recognized throughout Europe, Brooks (unpublished data, March, 1995) was unable to assign many individuals from Western Europe and the U.K. definitively to *C. carnea* or *C. kolthoffi*, because of conflicting character attributes. Only *C. lucasina* could be identified reliably over broad areas of

Europe, using a combination of morphological criteria (Brooks, unpublished data, and see below).

### Current systematic status of *Chrysoperla lucasina*

*Chrysoperla lucasina* was first described from Algeria by Lacroix (1912), synonymized with *C. carnea* by Aspöck *et al.* (1980), reinstated as a valid species by Leraut (1991), and then synonymized again with *C. carnea* by Brooks (1994). It is considered a southern element in Europe, and is often a dominant member of the *Chrysoperla* fauna of the Mediterranean region (Brooks, 1994; Thierry *et al.*, 1996). Although presently lacking formal recognition, the species is potentially well defined by its morphology (at least in living or fresh specimens), possessing a thin dark brown stripe on the pleural sutures of the second abdominal segment, a dark brown band over most of the maxillary stipes, short costal hairs, pointed fore-wing apex, and long dark hairs on the abdominal venter. In addition, and in contrast to *C. carnea* and *C. kolthoffi* as defined by Thierry *et al.* (1995), *C. lucasina* does not change colour in the autumn and winter, remaining green throughout the adult overwintering period (Thierry *et al.*, 1995).

When we began our study of the songs of the *Chrysoperla* species of Europe in 1993, we intentionally made no attempt to segregate individuals by their morphological traits. Subsequent song analyses revealed six discrete song types across the region (Henry, 1994). All of these song 'species' are physically very similar and difficult to distinguish from one another without careful study of subtle morphological features (C. S. Henry, S. J. Brooks, P. Duelli and J. B. Johnson, unpublished data). One of the easiest among the six to identify was *C. lucasina*, which showed a consistent suite of morphological traits (see above) that correlated well with the acoustical signal.

The present study describes the sexual songs of *C. lucasina*, and analyses the variation in song features present among eight selected populations found in different parts of Europe. In addition, we survey the morphological variation that exists among those populations, in both adults and larvae. Using the results of these analyses, we test the hypothesis that *C. lucasina* represents a valid taxon, deserving of formal recognition. We also discuss some preliminary observations of songs and morphology in several additional, currently unnamed song species of the Eurasian *carnea*-group, to help place *C. lucasina* in a broader systematic context.

### Materials and Methods

#### *Collecting, rearing, and identification*

Living individuals of *C. lucasina*, and other *Chrysoperla*, were collected by the authors and many collaborators at numerous sites and elevations across Eurasia, the British Isles and Finno-Scania from 1981 to 1995 (Table 1). All insects were shipped or hand-carried to Storrs, Connecticut, U.S.A for maintenance, rearing and song analysis. They were segregated by sex and locality, placed in groups of 10–14 individuals in

**Table 1.** European collecting sites for living *Chrysoperla lucasina* used in song analyses, 1981–95

Local Site (with nearest city or region)	Country	Altitude	Latitude	Year
Tenerife	Canary Islands	sea level	28°41'	9 Aug. 1988
Agadir, Morocco	NW Africa	100 m	30°30'	1985
Sierra Nevada (Granada)	S Spain	2000 m	37°	14 Aug. 1989
Huetor Santillan (Granada)	S Spain	1080 m	37°13'	19 Jul. 1995
La Mora (mountain pass, Granada)	S Spain	1390 m	37°13'	19 Jul. 1995
Alicante	S Spain	sea level	38°20'	17 Jul. 1995
Cabrera (Madrid)	C Spain	1050 m	40°58'	20 Jul. 1995
N Burgos	N Spain	925 m	42°20'	21 Jul. 1995
St Vincente (Santander)	N Spain	sea level	43°27'	22 Jul. 1995
Salvatierre (Vitoria)	N Spain	NA	42°43'	23 Jul. 1995
Coussac (Bordeaux)	W France	50 m	44°50'	24 Jul. 1995
Terrasson and Tulle (Brive-la-Gaillarde)	W France	380 m	45°10'	25 Jul. 1995
St Jean-d'Angely	W France	80 m	45°56'	26 Jul. 1995
Tours	NW France	190 m	47°23'	26 Jul. 1995
Col de Peyreson (Pyrenées)	SW France	1400 m	42°35'	20 Dec. 1988
Bise-Minervois (Béziers)	S France	68 m	43°21'	16 Jul. 1995
Les Baux (Avignon)	S France	150 m	43°55'	15 Jul. 1995
Carcès	S France	180 m	43°20'	1994
Estérel (Cannes)	SE France	300 m	43°35'	17 Oct. 1988
Cannes	SE France	230 m	43°35'	14. Dec. 1988
Lanslebourg	E France	1400 m	45°40'	1993
Ste. Menehould	NE France	270 m	48°55'	27 Jul. 1995
New Forest, Hampshire (heathland)	S England	< 100 m	51°01'	1994
York University (heathland)	C England	< 100 m	53°58'	1 Sept. 1994
Follaterre (Valais region)	SW Switzerland	450 m	45°40'	1993
Sierre (Pfywald region, N slope of Alps)	SW Switzerland	600 m	46°30'	1984, '89, '93
Basel (N slope of Alps)	NW Switzerland	350 m	47°32'	1983
Monte Caslano (Ticino region, S slope of Alps)	S Switzerland	275 m	46°30'	1981–94
Biasca (Ticino region, S slope of Alps)	S Switzerland	300 m	46°30'	1993
Bedretto (Ticino region, S slope of Alps)	S Switzerland	1400 m	46°30'	1993
Brissago (Ticino region, S slope of Alps)	S Switzerland	820 m	46°30'	1989–93
Piodina (Ticino region, S slope of Alps)	S Switzerland	400 m	46°30'	1989–94
Arlenheim (S slope of Alps)	S Switzerland	450 m	46°30'	1994
Zürich (N slope of Alps)	N Switzerland	500 m	47°22'	1983–94
Bözberg (N slope of Alps)	N Switzerland	500 m	47°21'	1994
Berchtesgaden (N slope of Alps)	S Germany	600 m	47°35'	9 Aug. 1994
Tirano (Valtellina), Sernio & Ragno	N Italy	500 m	46°	1994
Roledo, Toce (S slope of Alps)	N Italy	300 m	46°	1990
Allain, Aosta (S slope of Alps)	NW Italy	800 m	45°40'	1989
Melia, Calabria	S Italy	350 m	38°25'	1992
Cecita, Calabria	S Italy	1150 m	38°25'	1993
Sila Mountains, Calabria	S Italy	1500 m	38°25'	1990, '93
Desulo, Gennargentu Mountains	Sardinia (Italy)	900 m	40°28'	1991
Agrustos	Sardinia (Italy)	sea level	40°28'	1991
Femmina Morta Pass	NE Sicily (Italy)	1500 m	37°48'	1993
Mt. Etna (on pine)	NE Sicily (Italy)	1416 m	37°48'	1993
Solomos (Corinth)	Greece	210 m	38°	1994
Kastraki (Nemea)	Greece	NA	38°10'	1994
Megara (on pine)	Greece	50 m	38°	1994
Dimitsana (lights)	Greece	1000 m	37°30'	1994
Kamara (N Taygetos)	Greece	800 m	36°50'	1994
Taygetos (top of mountain pass)	Greece	1400 m	36°40'	1994
Lemnos (Aegean Sea)	Greece	sea level	39°58'	1990
Crete (SE of Greece)	E Mediterranean	sea level	35°15'	Sept. 1985
Paphos, W Cyprus	E Mediterranean	sea level	35°	1992–93
Famagusta, E Cyprus	E Mediterranean	sea level	35°08'	1992–93
Bugacz (S of Budapest)	Hungary	150 m	46°40'	1992
Banovce (Czech border; alfalfa meadow)	Slovak Republic	300 m	48°40'	8 Jun. 1994

low-profile clear plastic champagne cups inverted over 10-cm Petri dish lids, and supplied with water and a Wheat™-based diet (Hagen & Tassan, 1970). In the laboratory, long-day photoperiods (17 : 7 h L:D) terminated reproductive diapause in field-collected individuals and brought on sexual receptivity and spontaneous singing after 1–6 weeks. Gravid females laying fertilized eggs were usually not receptive; in those cases, progeny were reared to adulthood using established methods (Henry, 1991, 1993b) and then tested for their courtship songs.

Individuals of *C. lucasina* were identified initially by their courtship songs, using computer playback of a complete array of previously recorded European song types to each insect from a computer (see below) through an amplifier and loudspeaker. 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 'lucasina' songs were also determined to be *C. lucasina* based on morphological criteria. Insects with non-*lucasina* songs did not display *C. lucasina* morphology.

#### Analysis of songs

At least five complete courtship songs (= shortest repeated units or SRUs) of each presumed member of *C. lucasina* were recorded on cassette tape (Dolby™ off) and then digitized and analysed with hardware and software on a personal computer. Hardware included a Cambridge Electronic Design 1401plus 12-bit Intelligent Laboratory Interface and a Data Translation DT2821 12-bit digitizer board. Specialized software included Cambridge Electronic Design's *Spike2* for MS-DOS and *Windows*, and Engineering Design's *Signal/RTS* Sound Analysis System for MS-DOS. Males and females were induced to sing by playing back to them recorded songs of conspecifics.

The songs of *Chrysoperla* green lacewings consist of frequency-modulated volleys of abdominal vibration repeated with a regular period. Some taxa (e.g. Nearctic *C. plorabunda*) have relatively simple songs, composed of single-volley (monosyllabic) SRUs repeated many times (Henry et al., 1993). Other species (e.g. Nearctic *C. downesi*) produce more complex songs that consist of much longer, multisyllabic SRUs (Henry, 1980). *C. lucasina* analysed here has a complex song, in which the SRU consists of many similar volleys and can be up to several minutes in duration. Also, slightly but distinctly different 'short' (courtship) vs. 'long' (calling) songs are produced, at least by males. We defined and measured seven song features, chosen for maximum compatibility with the song measurements published for other species (Table 2; Fig. 1). The following were tabulated for both short and long songs: (i) duration of each volley, (ii) volley period (from the start of one volley to the start of the next; this is incorrectly called 'interval' in previous publications), (iii) volley interval (end of one volley to start of the next), (iv) number of volleys per SRU, and (v–vii) frequency or pitch of initial, middle, and end portions, respectively, of each volley.

In total, analyses were performed on the songs of 126 *C. lucasina* individuals. To avoid temperature effects, all songs were recorded at  $25 \pm 1$  °C. Although many populations

throughout Europe and western Asia were sampled (Table 1), we grouped those into eight regional areas (Fig. 2): England (8 individuals), the Alps (36), southern France (8), southern Spain (6), northern Spain (8), southern Italy (33), Greece (12) and Cyprus (15). All statistical analyses were performed on these larger geographic units. For each of five–fifteen songs (SRUs) per individual, we calculated the mean value of each song feature and then took the mean (i.e.  $n = 5$ –15) of those means as the representative value of the feature for each individual. An ANOVA was then performed on those individual averages using geographic region as the independent variable, and Scheffé's contrast test (Scheffé, 1953) was used to compare population means for significant differences. Statistical analyses were performed using *Statistica/Win* 5.0.

#### Analysis of adult morphology

One-hundred and eight individuals identified acoustically as *C. lucasina* were examined for external morphological features that might vary among geographic populations. An additional 132 museum specimens were identified morphologically as *C. lucasina* and studied similarly. Genitalia of preserved males were dissected, stained and mounted using established techniques (Bram & Bickley, 1963; Brooks, 1994). Localities ranged from the Canary Islands to Turkey and from northern Africa to Scotland.

Adults were examined for the states of 10 characters. These included (i) extent of markings on the stipes; (ii) extent and colour of the genal markings; (iii) relative abundance and distribution of black and blonde setae on the pronotum; (iv) relative size of the basal dilation of the claw expressed as a ratio of the length of the basal dilation of the claw to the length of the claw tooth; (v) extent to which the fore-wing is rounded or tapered at the apex; (vi) presence or absence of black markings on the wing veins; (vii) length of the costal setae; (viii) presence or absence of black setae on the abdominal sternites; (ix) length of the medial lobe (acumen) of the tignum in the male genitalia, in relation to the length of the arms of the tignum; and (x) presence or absence of a dark brown stripe on the pleural membrane of the second abdominal segment.

#### Analysis of larval morphology

We examined third-instar larvae from twelve acoustically identified populations, distributed from France and Germany through Switzerland and Italy to Greece and Cyprus ( $n = 3$ –25 per population). 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.

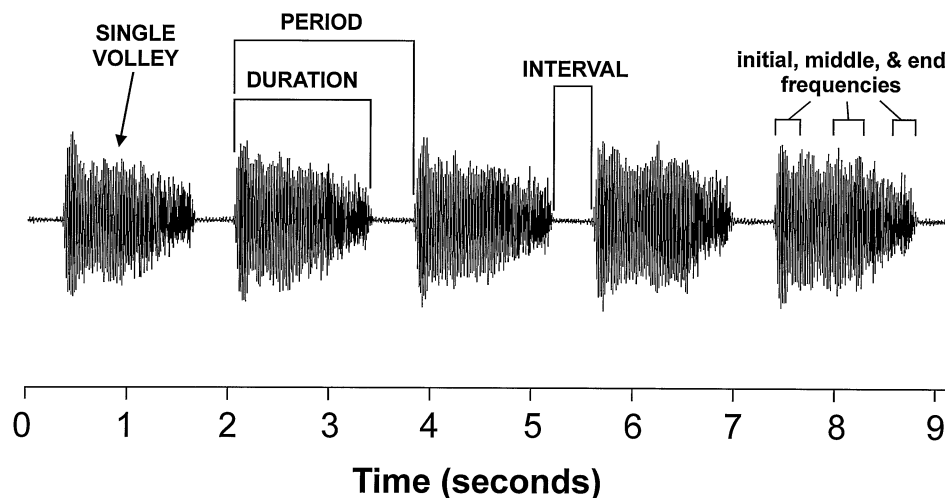
#### Voucher specimens

Adults and larvae of *C. lucasina*, pinned or in preserving fluid, have been deposited in the personal collection of Charles

**Table 2.** Values at  $25 \pm 1^\circ\text{C}$  of the song characteristics of *Chrysoperla lucasina* from eight geographic areas in Europe, comparing short songs to long songs and male long songs to female long songs.

	Abdominal vibration frequency, Hz			Volley duration (ms)	Volley period (ms)	Volley interval (ms)	No. volleys per SRU
	Initial	Middle	End				
Short songs, all ( $n = 57$ )	56.79*** $\pm 3.66$	69.97 $\pm 4.39$	87.20*** $\pm 5.73$	961.40*** $\pm 152.11$	1214.71*** $\pm 195.08$	283.40*** $\pm 118.25$	4.14*** $\pm 1.47$
Long songs, all ( $n = 71$ )	59.10*** $\pm 3.68$	68.94 $\pm 4.01$	94.78*** $\pm 5.49$	1084.34*** $\pm 136.53$	1528.57*** $\pm 144.56$	442.89*** $\pm 78.09$	(> 15)***
Long songs, Male ( $n = 22$ )	58.23 $\pm 3.64$	67.25* $\pm 3.51$	94.27 $\pm 5.15$	1144.31* $\pm 142.94$	1615.48*** $\pm 123.89$	468.81 $\pm 99.49$	(> 15)
Long songs, Female ( $n = 49$ )	59.50 $\pm 3.67$	69.71* $\pm 4.02$	95.00 $\pm 5.67$	1057.41* $\pm 125.92$	1489.55*** $\pm 136.91$	431.24 $\pm 64.15$	(> 15)

Each value is the mean of the means of  $n$  individuals in the population subsample,  $\pm$  SD (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: \* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; \*\*\* =  $P \leq 0.001$ .

**Fig. 1.** Oscillograph of a 9-s song fragment of *Chrysoperla lucasina*, showing five volleys of abdominal vibration. Song features discussed in the text are labelled.

S. Henry, Storrs, Connecticut; in the Connecticut State Museum of Natural History (CSMNH) at the University of Connecticut, Storrs; in The Natural History Museum, London; and in the W. F. Barr Museum, University of Idaho, Moscow. Additional verified specimens of the species were deep frozen at  $-70^\circ\text{C}$  for future molecular systematics studies.

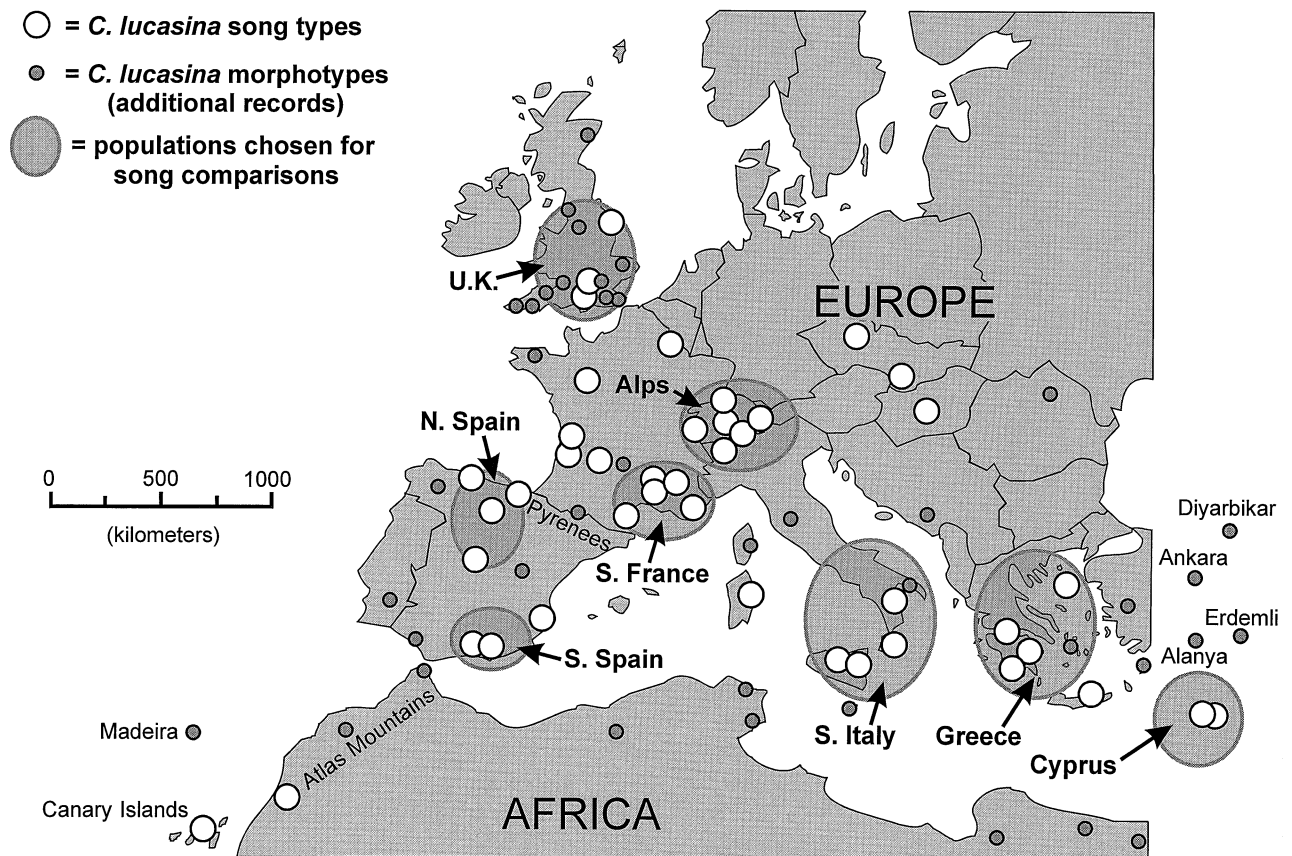
## Results

As mentioned earlier, two distinct types of songs characterize *C. lucasina*, although only males can produce both types. We interpret the shorter of the two as a courtship song (Ewing, 1989; Bailey, 1991), used by males during duets with females, or even with other males. The long song is a calling song (Ewing, 1989) or possibly an advertisement song (Wells, 1977; Gerhardt, 1994), produced by both males and females when alone. When a female establishes a duet with a male, she

continues to produce a song that is essentially identical to the calling song, but of much shorter duration because of regular interruption by the male after a few volleys (see Figs 3 and 4). Both song types share the same suite of features, shown and labelled on an oscilloscope trace in Fig. 1.

### Long song (calling song)

Solitary males and females spontaneously produce long trains of at least fifteen nearly identical volleys, which can last a minute or more (Fig. 3B). Volleys average slightly over 1 s in length and are produced with a period of about 1.5 s (Table 2). Males have slightly but statistically significantly longer volley durations, periods and intervals than females. Each volley has an upwardly modulated frequency spectrum, starting at  $\approx 60$  Hz and rising sharply toward the end of the volley to  $\approx 95$  Hz at  $25^\circ\text{C}$  (Fig. 3B). Sex differences in volley



**Fig. 2.** Collecting sites of *Chrysoperla lucasina* in Europe, with ellipses drawn around the discrete geographical areas which were used for song comparisons.

frequency structure are slight and predominantly insignificant (Table 2).

#### Short song (male courtship song)

The male courtship song averages four volleys (range = 2–8) and differs significantly from long songs of either males or females in nearly every feature (Fig. 3A, Table 2). Temporal features, particularly volley interval, are shorter in short songs; in fact, the interval between volleys becomes progressively smaller during the course of each SRU (Fig. 3A). The frequency spectrum of the short song is consistently lower by several Hz than that of the long song, and frequency modulation is more gradual (less sharp) than is found in the long song, giving the sonographs of the two song types similar but distinctive shapes (Fig. 3A).

#### Duetting songs

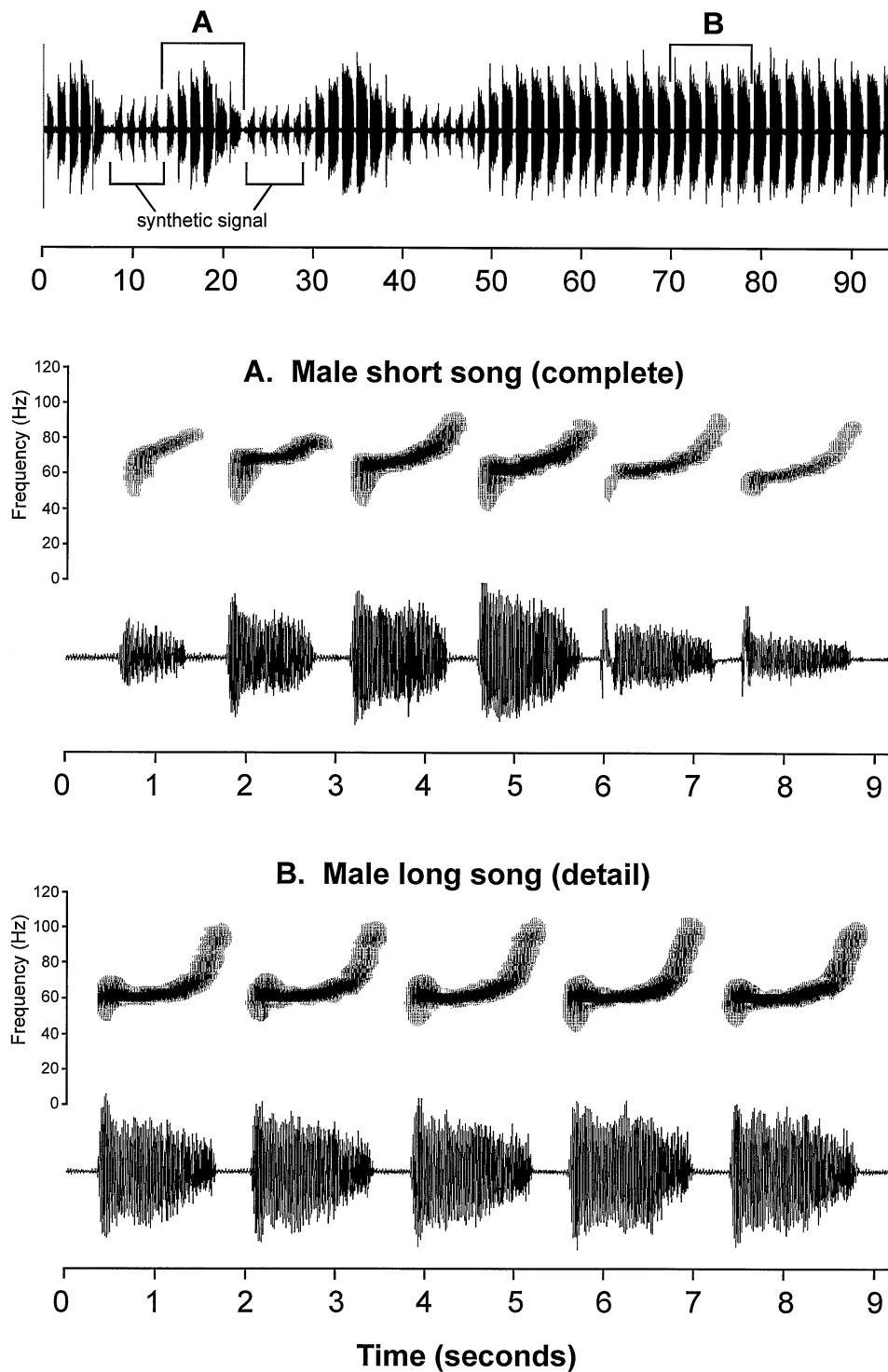
Either sex of *C. lucasina* can initiate a duet with another individual (Fig. 4, top trace), usually by interrupting or answering a long calling song. A duet is characterized by the alternate production by each partner of 2–8 (mean = 4) volleys

of abdominal vibration. It can continue for several minutes, until copulation or separation occurs. An individual does not seem to 'know' when to begin its contribution to the duet. Instead, it simply starts singing, often before its partner has stopped; the partner immediately stops singing, but then interrupts after remaining silent for several volleys. This pattern may persist until copulation, or sometimes the two insects will briefly switch to single-volley songs, alternately produced by each partner just before copulation (Fig. 4, bottom trace).

Males and females differ in the nature of their songs produced during a duet (Fig. 4, top trace). The male produces a courtship song, with temporal and frequency traits characteristic of that type of short song. The female, however, produces an 'interrupted calling' song, which retains all traits characteristic of a typical female or male long song, except for length. For females, then, the long song performs as both a calling and a courtship song.

#### Geographic variation in songs

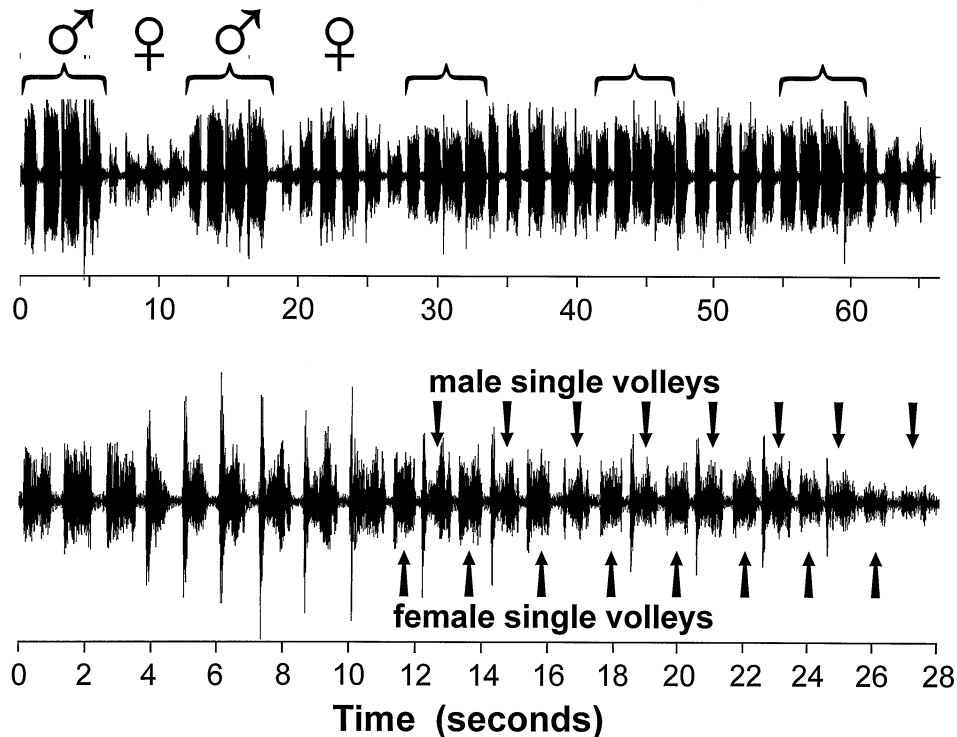
Populations of *C. lucasina* from eight geographic areas of Europe were compared with respect to measurements of song features (Figs 5 and 6). Long and short songs were treated



**Fig. 3.** Oscillographs and sonographs of songs and song fragments of *Chrysoperla lucasina*. Top: 100 s of short (A) and long (B) songs of a single male, showing the male duetting with a synthetic signal generated by a computer. Middle: a magnified view of the male's complete short (courtship) song, showing its spectral properties. Bottom: magnified view of a section of the male's long (calling) song, showing its spectral properties.

separately, because of their distinctive characteristics. For six features of interest, only volley duration and volley period in long songs showed geographic differences, and even then for

only one comparison (southern France vs. Greece,  $P \leq 0.05$ ; see Figs 5 and 6). The seventh song feature, number of volleys per SRU, was too variable to permit meaningful geographical



**Fig. 4.** Oscillographs of a heterosexual duet in *Chrysoperla lucasina*. Top: 67 s taken from the middle of the duet. Bottom: 28 s from the end of the duet, showing the transition from a multisyllabic to a monosyllabic interaction.

comparisons, although preliminary data indicate that this number is less variable and more informative in the context of heterosexual duets.

#### Adult morphology

The only character attribute that reliably segregated *C. lucasina* from other song species was the dark brown stripe on the pleural membrane of the second abdominal segment, which was uniquely and consistently present in all individuals identified as *C. lucasina* by their songs. However, three other character attributes were useful in distinguishing *C. lucasina*, although they were not unique to the taxon and/or were not consistently present. In most specimens identified as *C. lucasina* by their songs, the posterior margin of the fore-wing was straight, making the wing appear to taper apically. In addition, the setae on the costal margin of the fore-wing were usually short (< 1.0 mm). Finally, basal dilation of the claw in *C. lucasina* was intermediate compared to other song species (ratio in *C. lucasina* = 2.31–3.10; mean = 2.71, SD = 0.24,  $n = 55$ , localities = 17; see Fig. 7).

#### Larval morphology

*Chrysoperla lucasina* larvae have head markings dominated by a pair of longitudinal, dorso-lateral brown stripes, each with a baso-lateral expansion and a darker area mesad of the eyes

(Fig. 8). There was substantial variation within each population sample in width and colour intensity of the stripes, although the latter could have been influenced by preservation. In several populations, some or all individuals also had one or three spots placed anteriorly and laterally on the frons (Fig. 8A, C). The most striking variation was seen in a population from Gennargentu, Sardinia, Italy, where the posterior third of the dorso-lateral stripe was absent in two of six third-instar larvae examined.

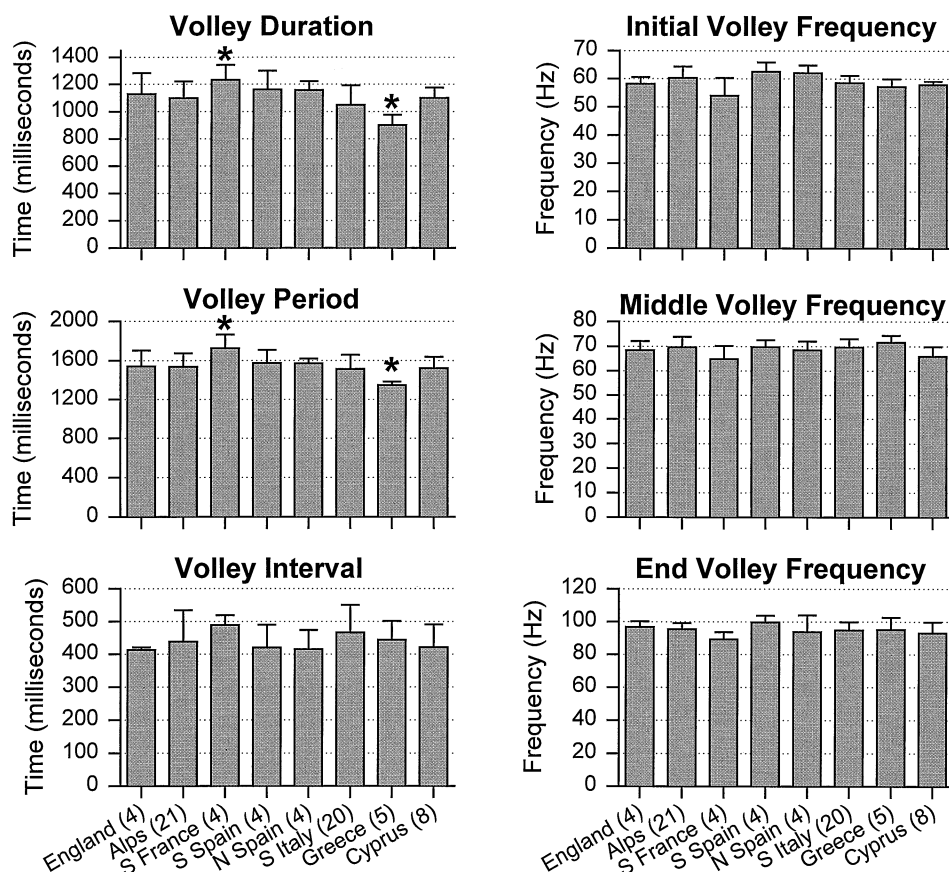
Larval head markings also varied significantly between populations. The greatest differences were seen in the width and colour intensity of the dorso-lateral stripe, the prominence of the darker area, and the addition of one or three frontal spots. The dorso-lateral stripe tended to be wider and darker in northern or high-elevation populations, e.g. Berchtesgaden, Germany and Femmina Morta Pass, Sicily (Fig. 8C, G).

## Discussion

#### Song variation

Results of ANOVAs (not shown) indicate that approximately equal amounts of variation exist among the songs of a single individual, and among the individual averages of a given geographic population of individuals. In addition, those measures of variation are low: song features are highly





**Fig. 5.** Bargraphs comparing eight distinct geographic populations of *Chrysoperla lucasina* with respect to the means and standard deviations of six song features of the long (calling) song. Both sexes were included in the calculations. Levels of significance are shown by an asterisk: \*  $P \leq 0.05$ .

constrained, both within an individual and within a population. Therefore, we felt that it was valid to compare geographic populations using individual averages, as has been done in previous studies of song variation in lacewing species (Henry & Wells, 1990a).

Among populations selected from widely separated parts of Europe, little significant variation could be found in any song feature of *C. lucasina* (Table 2; Figs 5 and 6). Clearly, the taxon is geographically homogeneous with respect to its calling and courtship songs, and on this basis alone 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). The significant differences seen in volley duration and volley period (long songs) between southern France and Greece are minor and probably of no biological importance. In addition, those differences in both features probably stem from the same cause, because duration and period are highly correlated traits in *C. lucasina* and other *carnea*-group *Chrysoperla* species.

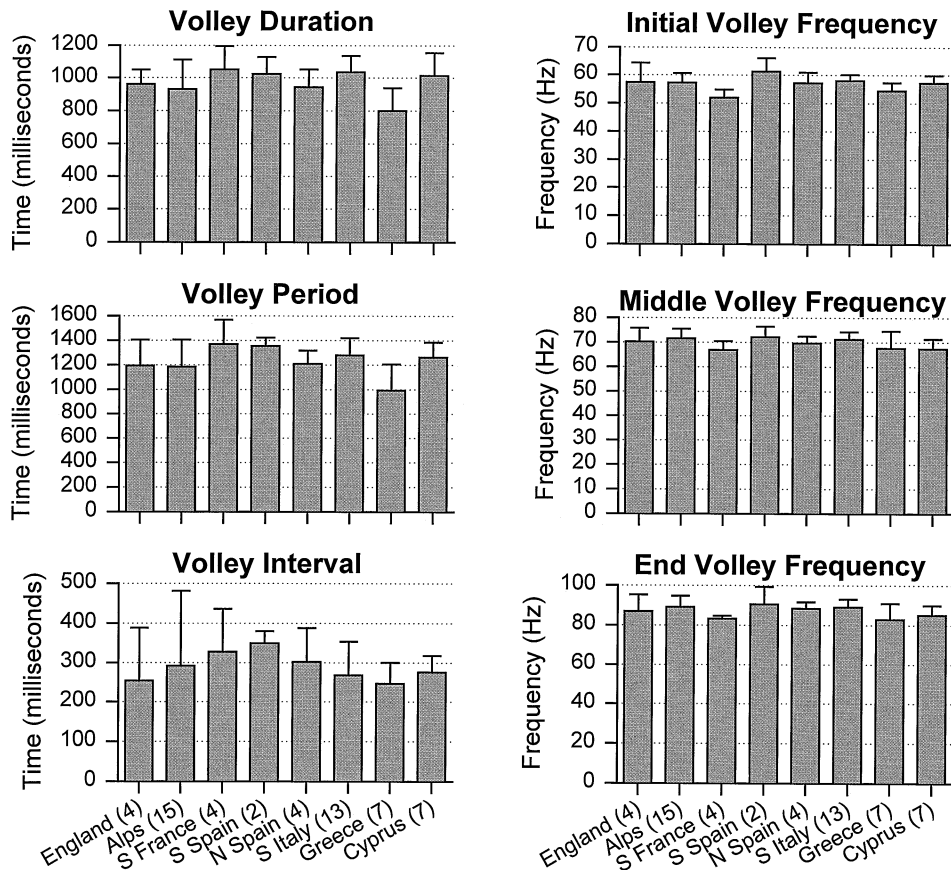
#### Song types and evolution

The existence of two distinctive types of songs in *C. lucasina* has not previously been reported in the *carnea*-group of

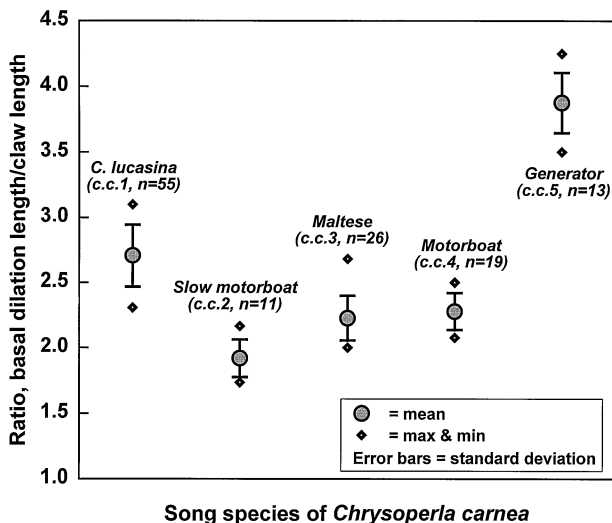
*Chrysoperla*. North American members are characterized by one song type per species (Henry *et al.*, 1993), as are several song species of the *carnea*-group in Eurasia (C. S. Henry, unpublished data).

Although the long song of *C. lucasina* most closely corresponds to a calling song (Ewing, 1989), it is unlike the calling song of most other acoustical insects and anurans in that it cannot attract conspecifics from very far: in lacewings, songs are low-intensity, substrate-borne signals, capable of traveling a metre at most (Henry & Wells, 1990b). Also, the long song's important presence in both males and females is an unusual if not unique trait for a calling song, because males alone usually sing to attract females or to interact with other males (Henry, 1994). However, its general effect is to elicit singing responses and orientated movement from other individuals, which is appropriate for a calling song.

The short song is produced by males in the context of courtship, and is used only during duets with females or other males. Duets between males are relatively brief and involve little if any aggression or other overt hostility, so it is not plausible that the short song evolved under a regime of intense intrasexual competition. This conclusion is consistent with the low levels of conflict that have been observed in the sexual behaviour of other species of the *carnea*-group (Henry & Busher, 1988), and suggests that the short song is best



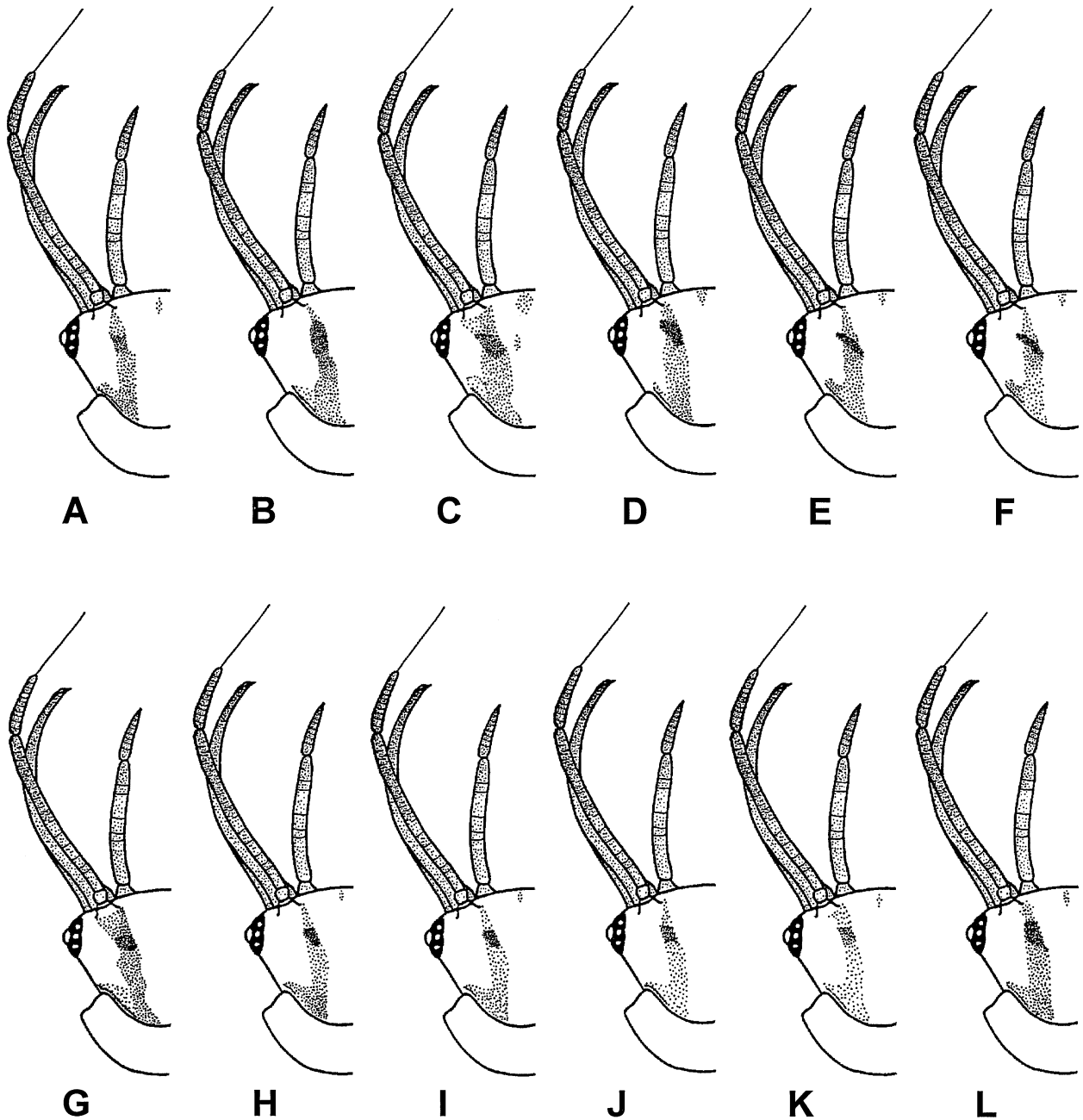
**Fig. 6.** Bargraphs comparing eight distinct geographic populations of *Chrysoperla lucasina* with respect to the means and standard deviations of six song features of the short (courtship) song. Only males produce such songs. There are no significant differences between the populations.



**Fig. 7.** Plot comparing the mean sizes and size ranges of the basal claw dilation in five sibling, cryptic song species of the *carnea*-group of European green lacewings.

interpreted as a courtship song (Bailey, 1991). Heterosexual duets are much longer than homosexual ones, and involve the reciprocal exchange of short songs that superficially appear to be identical in both sexes. However, as described above, the female's contribution to a duet is actually an interrupted long song, without the distinctive spectral and temporal features of the male's courtship (short) song. Properly designed behavioural experiments will be needed to determine whether the functions of both long and short songs are as hypothesized here, as well as to see if the female's single song type has the dual role (calling and courtship) suggested by these preliminary data.

Duets of *C. lucasina* are multisyllabic, which places the species in behavioural company with the North American species *C. downesi* and *C. johnsoni*, as well as at least three currently unnamed song morphs from central Asia (Henry, 1980; Henry, 1993a; C. S. Henry, unpublished data). However, the phylogenetic significance (and utility) of monosyllabic vs. multisyllabic duet modes is not so clear, because song features within the *carnea*-group are potentially quite labile and probably indicate relationship only within song taxa that have diverged very recently. For example, in the closely related species *C. plorabunda* and *C. downesi*, the radically different songs typical of each are controlled by a simple genetic system,



**Fig. 8.** *Chrysoperla lucasina*, third-instar larval head capsules. (A) FRANCE: Carcès; (B) FRANCE (Pyrenées): Col de Peyreson; (C) GERMANY: Berchtesgaden; (D) SWITZERLAND: Bözberg; (E) SWITZERLAND: Brissago; (F) ITALY (Aosta): Allain; (G) ITALY (Sicily): Femmina Morta Pass; (H) ITALY (Sardinia): Desulo; (I) ITALY (Sardinia): Gennargentu; (J) CYPRUS: Famagusta; (K) CYPRUS: Paphos; (L) GREECE: Taygetos Pass.

suggesting that a minor genetic change could produce a major change in song phenotype (Henry, 1985). Thus, rapid evolution of songs would soon mask deeper relationships among song-defined taxa, even within a closely knit taxon like the *carnea*-group of *Chrysoperla*. Complicating the picture in *C. lucasina* is the occasional switch to monosyllabic dueting in the few seconds just before copulation (Fig. 4), making it difficult to

assign character states definitively. Generating a robust molecular phylogeny of the emerging biological species of the *carnea*-group would solve many of these problems, allowing us to map song features on to an independently generated tree to determine directions and patterns of evolutionary change in lacewing songs, as has been done for some anuran Amphibia (Cocroft & Ryan, 1995; Ryan & Rand, 1995).

*Other song species in Europe*

This work is part of a larger study, during the course of which we have identified five other distinctive, partially sympatric song species in the *carnea*-group. We have assigned informal, descriptive names to those song species which have no clear morphological equivalents, but there is also merit to referring to them simply by letter-number codes. In this regard, *C.lucasina* is code-named *c.c.1* (standing for 'Chrysoperla carnea song-species one').

One of these has for some time been recognized formally as a distinct species, *C.mediterranea* (Hölzel). It is characterized by relatively dark green body colour, narrow wings, and very small basal dilation of the pretarsal claws (Hölzel, 1972; Aspöck *et al.*, 1980; Brooks, 1994). Found principally in the Mediterranean regions of Europe, *C.mediterranea* has also been collected as far inland as Switzerland, Austria and the Slovak Republic, based on morphology and song phenotype (Duelli, 1987; C. S. Henry, unpublished data). It has a multisyllabic song consisting of many short, closely spaced volleys (C. S. Henry, P. Duelli, S. J. Brooks & J. B. Johnson, unpublished data).

Another European song species with a more northern distribution was first described acoustically (but tentatively) as *C.carnea sensu stricto* (Henry, 1983). The volleys of its multisyllabic song are so short and closely spaced that the overall (amplified) effect is that of a single long, drawn-out, pulsating signal similar to the sound produced by a motorboat. There is accumulating evidence that this 'motorboat' song species (*c.c.4*) is indeed the original type species of *C.carnea* described by Stephens (1835) from England, although that hypothesis remains to be tested more rigorously (C. S. Henry, P. Duelli, S. J. Brooks and J. B. Johnson, unpublished data). It has also been referred to as *C.kolthoffi* by Thierry *et al.* (1992, 1995, 1996), but morphological studies do not strongly support that view (S. J. Brooks, unpublished data).

The remaining three song species have not previously been associated with existing morphological taxa. One, 'slow motorboat' (*c.c.2*), appears to be restricted to central and western portions of Europe, including Spain, France and the Alps; its song resembles that of *C.mediterranea*, but with several unique temporal and tonal characteristics. The second, 'Maltese' (*c.c.3*), is widespread across southern Europe, from Spain to Israel; it has a monosyllabic song with long, upwardly modulated volleys. The last species, 'generator' (*c.c.5*), seems to be an Asian element possibly referable to *C.sillemi* (Esben-Petersen) (Brooks, 1994), whose range just touches the eastern edge of the Mediterranean Sea. Its monosyllabic song is superficially like that of 'Maltese,' but with downwardly modulated volleys. Because the songs of all the other European song species are upwardly modulated, 'generator' would appear to be an outgroup with respect to song phenotype.

*Morphology*

*Chrysoperla lucasina* shares two character attributes, pointed fore-wing apex and short costal setae, with the song species

'generator' (*c.c.5*). In all the other European song species, the apex of the fore-wing is rounded and the costal setae are long.

The basal dilation of the claw in 'generator' (ratio = 3.63–4.25) is smaller than in *C.lucasina* (ratio = 2.31–3.10). The basal dilation ratio of *C.lucasina* overlaps that of both 'motorboat' (2.24–2.48) and 'Maltese' (2.20–2.68). However, the basal dilation in 'slow motorboat' (ratio = 1.79–2.09) is larger than in *C.lucasina* or any of the other song species. In summary, 'generator' and 'slow motorboat' can be identified unambiguously by their relatively extreme basal dilation ratios, while *C.lucasina*, 'motorboat' and 'Maltese' have intermediate basal dilation ratios which cannot be used to discriminate among those three song taxa (Fig. 7).

The brown pleural stripe is clearly visible on the second abdominal segment of all fresh specimens of *C.lucasina*. The stripe contrasts strongly with the green coloration of the abdomen. In old discoloured specimens with shriveled abdomens, the brown pleural stripe is harder to see. If the abdomen has discoloured to dark brown there will be insufficient contrast for the stripe to be visible. Similarly, in faded specimens the stripe may no longer be apparent. In shriveled specimens the pleural membrane may be concealed beneath the sclerites. However, even in such poor condition, most specimens of *C.lucasina* should still be identifiable.

The basic pattern of larval head markings described here for *C.lucasina* occurs throughout the *carnea*-group, although not all species are known to have the darker area in the dorso-lateral stripe (Tauber, 1974; C. S. Henry *et al.* unpublished data). *C.lucasina* larvae are especially similar to those of 'Maltese' (*c.c.3*), although confusion of some individuals with other song taxa can also be a problem. It appears that larval head capsule markings will not be taxonomically useful, except perhaps on a local geographic scale.

**Conclusion**

The taxonomically intractable complex of cryptic, sibling species of the *carnea*-group in Europe is responding favourably to song analysis. Species demarcations can now be recognized and defined more accurately using reliable acoustical criteria to supplement or even supersede morphological data. As a first step, we have shown here that the morphological species *C.lucasina* is characterized by a well-defined, geographically invariant song, which constitutes compelling evidence for recognizing the taxon as a valid biological species as well. That conclusion is strengthened by life-history traits, notably the absence of a colour change in overwintering adults of *C.lucasina* (Thierry *et al.*, 1995).

Song analysis and morphological study of museum specimens additionally show that *C.lucasina* has a southern and western Palaearctic distribution, ranging from the Canary Islands in the west to Turkey in the east and from North Africa to Scotland. Evidence from adult morphology suggests that *C.lucasina* and 'generator' are more closely related to each other than to the other three song species identified in this study, while larval morphology implicates 'Maltese' as the sister species of *C.lucasina*. Acoustically, however, *C.lucasina*

is more similar to the other song species of Europe than it is to 'generator;' furthermore, it displays no particular similarity of its songs to 'Maltese'. Therefore, it is premature to draw any firm phylogenetic conclusions.

Future studies will extend this approach to the other five song species of the *carnea*-group in Europe and attempt to resolve phylogenetic relationships among the many song species hidden within the *carnea*-group on a world-wide basis.

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