Evolution, 53(4), 1999, pp. 1165–1179

CONVERGENT EVOLUTION OF COURTSHIP SONGS AMONG CRYPTIC SPECIES OF THE *CARNEA* GROUP OF GREEN LACEWINGS (NEUROPTERA: CHRYSOPIDAE: *CHRYSOPERLA*)

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Abstract.—Although traits of related species are likely to be similar due to common ancestry, mating signals are an exception. In singing insects, for example, song similarity has been documented only for allopatric or allochronic species pairs, and even then, not often. Where song similarity does occur, it has been logically attributed to the inheritance of ancestral traits rather than convergence. It is quite common for related, sympatric insect species to differ dramatically in calling song, which is predicted by evolutionary theory to maximize intraspecific mating success. Given that there are a limited number of ways to make sounds on anatomically similar organs and given that there would be no selective pressure for songs to differ in widely separated geographic areas, convergence in songs among related species living on different continents might be expected. Here we present the first well-documented case of such convergence, in a group of sibling, cryptic species characterized by substrate-borne vibrational mating songs. In this example from green lacewings of the *carnea* group of the genus *Chrysoperla*, a variety of statistical tests shows that one species in North America and another in Asia possess songs that are strikingly similar to each other. DNA data demonstrate that the species involved belong to divergent speciose lineages, and behavioral data demonstrate that the convergent songs are readily accepted by members of both species.

Key words.—*Chrysoperla*, Chrysopidae, evolution, homoplasy, insect, mtDNA, Neuroptera, phylogeny, songs, speciation, systematics.

Received May 13, 1998. Accepted March 12, 1999.

Similarity between different taxa results when evolutionary change is variously constrained in its scope. Constraints are commonly set in place by environmental (or other) selection, physical or physiological limitations, ontogenetic pathways, or genetic architecture (Schwenk 1994; Brooks 1996; Wake 1996). For example, many organisms have been shown to evolve convergent morphological, physiological, or ecological adaptations to shared, usually stressful, environmental conditions, including high altitude (Landmann and Winding 1995), excessive dryness or heat (Mares 1993; Sinha and Kellogg 1996; Felger and Henrickson 1997), existence below ground (Nevo 1995), or pronounced seasonality (Cowling et al. 1994). Functional, biomechanical, or biochemical requirements can direct unrelated species toward nearly identical solutions, as seen in the pointed wings of migrant birds (Monkkonen 1995), high-crowned teeth in different orders of grazing mammals (MacFadden 1997), sequestered defensive chemicals of different moth families (Wink and Von Nickisch-Rosenegk 1997), or electrophysiological adaptations of weakly electric fishes in South America and Africa (Winemiller and Adite 1997). Limited developmental design options often impose internal constraints on adult phenotypes, resulting in repeated appearance of the same morphology in different parts of a lineage (Wake 1991; Hufford 1997). Likewise, a genetic anomaly such as haplodiploidy can predispose a clade (e.g., the insect order Hymenoptera) to repeated independent evolutionary origins of a single condition, eusociality (Bourke and Franks 1995).

Apparent sameness that results from independent evolution, as in the examples above, is known as homoplasy (Wake 1996). However, resemblance between two taxa in some characteristic is most simply explained by evolutionary descent from a common ancestor because no evolutionary change is required (Fitch and Margoliash 1967). Thus, before alternative explanations invoking evolutionary convergence, parallelism, or reversal are accepted, one must first reject the hypothesis that the taxa share plesiomorphic traits. Such phylogenetic constraints can bias the detection of adaptive or other evolutionary responses in comparative studies of any group of organisms, unless the constraints are recognized as sources of similarity and factored out (Felsenstein 1985; Harvey and Pagel 1991; Garland et al. 1992). Similarity due to common ancestry can occur in three ways. First, the similar forms may simply be members of the same species. Second, similarity can result from their monophyly, where all members of a group are the only descendants of a single recent progenitor possessing that trait (Wiley 1981). Finally, certain members of a more heterogeneous clade may retain a relictual trait possessed by a more distant common ancestor.

Mating signals in animals are used by individuals to assess possible sexual partners for information about species identity, location, physical condition, or even genetic quality (e.g., Moore 1994; Butlin 1995; Kirkpatrick 1996; Wells and Henry 1998). As phenotypic traits, mating signals should be subject to the factors favoring resemblance described above. Paradoxically, however, mating signals are seldom similar among related taxa (Foster et al. 1996), probably due to their roles in species recognition and reproductive isolation. Because of the need to recognize appropriate, conspecific mates, mating signals within a population or species are normally under strong stabilizing selection (Paterson 1986). However, sympatric co-occurrence of closely related taxa with similar

signals might precipitate directional selection, leading to a period of rapid signal divergence (i.e., reproductive character displacement) until new, different mate recognition systems are stabilized in each species (Butlin 1987). Thus, similarity or identity of signals is nearly unknown in areas of sympatry between closely related species that breed at the same time, regardless of potential constraints on those signals imposed by environmental selection, functionality, development, genetics, or phylogenetic history. In singing insects, for example, song similarity has been documented only for allopatric or allochronic species pairs, and even then, not often. Where song similarity has been found (Alexander 1957; Alexander and Moore 1962; Walker 1963, 1974; Walker and Rentz 1967; Shaw 1996a,b; Alexander et al. 1997), it has generally been attributed to the passive inheritance of plesiomorphic traits from ancestral to descendant species rather than to convergence resulting from repeated evolutionary origin of particular song types.

Green lacewings of the genus *Chrysoperla* Steinmann use abdominal oscillation to produce substrate-borne vibrational songs (Smith 1922; Henry 1979). The abdomen does not strike the substrate, but instead shakes the stem or leaf upon which the individual is standing. During this process of tremulation, vibrational signals are picked up by subgenual organs in the tibiae of the legs of potential mates (Michelsen et al. 1982; Devetak and Pabst 1994; Devetak and Amon 1997). Tremulation signals have a powerful effect on species recognition in *Chrysoperla* (Henry 1994). In one section of the genus, the common and widespread *carnea* group (Brooks 1994), songs have become quite elaborate and equally expressed in both sexes; mating will only occur after the matching of songs has produced a prolonged and precise duet between partners (see Fig. 3, arrows). Songs serve as the principal barriers to interspecific mating; postzygotic isolation of species is secondary and less important (Wells and Henry 1994). The species of the *carnea* group closely physically resemble each other and, in fact, are best (or solely) identified by their songs (Henry et al. 1993, 1996, 1999). To date, we have described the songs of five cryptic song species in North America, six cryptic song species in Europe, and four more in central and eastern Asia. All are sufficiently alike morphologically to be traditionally classified as a single taxonomic species, *Chrysoperla carnea* (Stephens), but each actually represents a valid, reproductively isolated, biological species (Wells and Henry 1998).

Within any single region, the songs of different cryptic species of the *carnea* group are very distinctive, as is typical of acoustic mating signals in other insect taxa with overlapping geographic ranges (Jansson 1979; Otte 1994; Tomaru and Oguma 1994; Den Hollander 1995; Ingrisch 1995). However, in pairs of species from North America and Eurasia, several examples of remarkably similar mating songs have been found. One of these, a *Chrysoperla adamsi*–type mating signal in western North America and central Asia, is the focus of this paper. Although similarity can be a subjective property, here we judge its authenticity using two approaches. First, we evaluate phenotypic resemblance of songs by statistically comparing measured song features of the two *C.* "*adamsi*" populations to one another, as well as to several other song-defined species of the *carnea* group. Second, we utilize an objective and natural test of song similarity in green lacewings, the dueting responses of sexual partners to each others' signals (Wells and Henry 1992b, 1994). By quantifying dueting responses of *C.* "*adamsi*" from North America to recorded songs of *C.* "*adamsi*-K" from central Asia, we assess the functional resemblance of their songs.

To understand the evolutionary basis of similarity in lacewing mating signals, it is necessary to develop a phylogenetic hypothesis of the relationships among sibling species in the *carnea* group. Only with phylogenetic information can it be determined whether similar songs in Asia and North America are indicative of the conspecific status of these populations, recent monophyly, retention of the ancestral condition, or truly independent origin of identical phenotypes (convergent evolution). Morphology is of little use in developing such a hypothesis because lacewings of the *carnea* group appear very similar (Henry et al. 1993). Therefore, in this complex of cryptic species rapidly evolving mitochondrial DNA sequence data are more likely to be useful as indicators of evolutionary relationships. The results of a phylogenetic analysis based on such mtDNA molecular characters are reported here for 18 species of green lacewings. We chose to examine partial nucleotide sequences from two mitochondrial genes, cytochrome oxidase II (COII) and NADH dehydrogenase subunit 2 (ND2), based on the proven utility of those genes for resolving relationships at the species level (Beckenbach et al. 1993; Brower 1994; Simon et al. 1994). The ingroup included 15 song-defined cryptic species within the *carnea* group of *Chrysoperla*. The outgroup for the analyses were two species of the *pudica* group (sensu Brooks 1994) of *Chrysoperla*. One member of a more distant genus, *Chrysopa* Leach, was sequenced, but not included in all phylogenetic analyses.

In summary, we use acoustic and behavioral analyses to document the phenotypic and functional similarity between songs of two geographically distant taxa of the *carnea* group. Those results are interpreted in a phylogenetic framework provided by mtDNA sequence data to evaluate whether resemblance of songs is due to common ancestry or convergent evolution. If convergence can be demonstrated, it will be the first such case in songs among related species living on different continents.

Materials and Methods

Collecting, Rearing, and Identification

Live individuals of *Chrysoperla* and *Chrysopa* were collected by the authors or their associates from 1977 to 1996 across the width of North America and Europe, portions of central and eastern Asia and northern Africa, the British Isles, Fennoscandia, and several islands in the Mediterranean Sea. In particular, *C. adamsi* Henry, Wells and Pupedis was collected over the course of many years at numerous localities in western North America from northern Idaho to southern California (Henry et al. 1993). Its song twin from Asia was discovered by Peter Duelli at several sites in the Republic of Kyrgyzstan from 1350 m to 2000 m elevation in late May and June 1995, during an expedition organized by Horst Aspöck. Individuals of Nearctic *C. adamsi* used in the song preference experiments were captured in May 1996 from a

TABLE 1. Collecting information for the specimens of green lacewings used in the molecular phylogenetic analyses. Names in quotation marks are informal epithets for the song species, based either on descriptive qualities of the song or similarity of the song to that of a valid, named species. Species with the prefix "*C.c.*" conform to the nomenclature proposed by Duelli et al. (1996) for the undescribed members of the *Chrysoperla carnea* complex. Initially, two specimens of each song species from each site were used, with the exception of *C. mediterranea,* in which each of the two specimens came from different localities.

Song species	Collecting locality	Collecting date
C. plorabunda	Storrs, Connecticut	September 1993
C. adamsi	Smartville, central California	July 1996
C. johnsoni	Moscow, northern Idaho	September 1992
C. downesi-sp. 1	Rensselaerville, eastern New York	September 1991
C. downesi-sp. 2	Moscow, northern Idaho	September 1992
C. "*adamsi*-K"	Kyrgyzstan, central Asia	May–June 1995
C. "*downesi*-K"	Kyrgyzstan, central Asia	May–June 1995
C. "*downesi*-CH"	Beijing, eastern China	October 1995
C.c.2 "slow motorboat"	Traffiume, northern Italy	May 1994
C.c.3 "maltese"	Brissago, Ticino, Switzerland	July 1993
C.c.4 "motorboat"	St. Didier, Aøsta, northern Italy	July 1993
"motorboat-K"	Kyrgyzstan, central Asia	May–June 1995
C.c.5 "generator"	Al Buraymi, Oman, southern Asia	October 1994
C. lucasina	Brissago, Ticino, Switzerland	July 1993
C. mediterranea	Megara, Greece	June 1994
C. mediterranea	Duchonka, central Slovakia	August 1994
C. rufilabris	Houston, Texas	December 1993
C. harrisii	New Haven, Connecticut	September 1991
Chrysopa oculata	Storrs, Connecticut	July 1992

population 8.8 km south of Smartville in central California and sent to us by James B. Johnson (see Acknowledgments for additional information).

All insects were shipped or hand-carried to Storrs, Connecticut. They were segregated by song, locality, and sex; placed in groups of 10–14 individuals in low-profile clear plastic champagne cups inverted over 10-cm petri dish lids; and supplied with water and a Wheast™-based diet (Hagen and Tassan 1970). Long-day laboratory photoperiods (17:7 L:D) generally prevented or terminated reproductive diapause in field-collected individuals and induced sexual receptivity and spontaneous singing after zero to six weeks (Tauber and Tauber 1982). Gravid females laying fertile eggs were typically not receptive (Henry and Busher 1988); in those cases, progeny were reared to adulthood using established methods (Henry 1991, 1993) and then tested for courtship songs.

Individuals were identified to species using playback of a series of previously recorded song types from a computer (see below) through an amplifier and loudspeaker. Morphology was used to confirm species determinations in those few species of the *carnea* group possessing distinctive physical features, for example, *C. downesi* (Smith), *C. lucasina* (Lacroix), and *C. mediterranea* (Hölzel) (Brooks 1994; Henry et al. 1996, 1999). Only specimens verified as to species using song phenotype were deep frozen at −70–100°C for molecular systematic studies. These specimens were obtained from subsets of the sites yielding specimens for song analysis (Table 1).

Several adult males and females of each lacewing population (pinned or in preserving fluid) were deposited as voucher specimens in (1) the personal collection of Charles S. Henry, Storrs, Connecticut; (2) the Connecticut State Museum of Natural History (CSMNH) at the University of Connecticut, Storrs; (3) The Natural History Museum, London, England; (4) the Yale Peabody Museum, New Haven, Connecticut; (5) the personal collection of Peter Duelli, Birmensdorf, Switzerland; and (6) the W. F. Barr Museum, University of Idaho, Moscow.

Recording and Analysis of Songs

In the laboratory, lacewings will tremulate inside a small cardboard coffee cup covered with plastic wrap (the arena). Their vibrational signals were detected by a piezoelectric transducer touching the plastic wrap and recorded on cassette tape (for details, see Henry 1979; 1980b). The same arena was used for playback experiments. Recorded songs from tape or digitized on computer disk were played through a speaker placed just above the arena, causing the plastic wrap to reproduce the low frequencies faithfully in the speaker's near-field.

At least five complete courtship songs (i.e., shortest repeated units or SRUs) of 10–71 individuals of each lacewing species were recorded on a high-quality cassette tape recorder, with Dolby™ signal processing disabled. To avoid temperature effects, all songs were recorded at 25 ± 1°C. They were then digitized on a personal computer, using either a Cambridge Electronic Design (Cambridge, England) 1401plus 12-bit Intelligent Laboratory Interface or a Data Translation (Marlboro, MA) DT2821 12-bit digitizer board coupled with a 32-bit digital signal processing (DSP) board. Song analysis software included Cambridge Electronic Design's Spike2 version 2.01 for Windows (Smith 1995) and Engineering Design's (Belmont, MA) Signal/RTS Sound Analysis System version 3.0 for MS-DOS (Beeman 1996). Males and females were induced to sing by playing recorded songs of conspecifics.

The substrate-borne courtship songs of *Chrysoperla* green lacewings are low frequency, between 30 Hz and 120 Hz. They consist of volleys of abdominal vibration repeated with a regular period, each of which can also exhibit carrier frequency modulation. Some taxa, such as Nearctic *C. plora-*

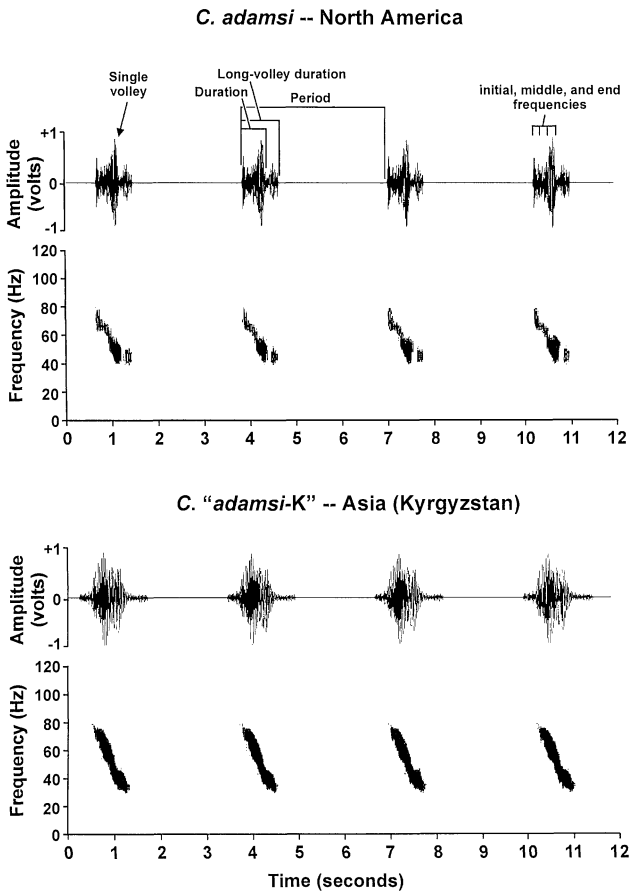

FIG. 1. Oscillographs and sonographs (below each oscillograph) of typical songs of *Chrysoperla adamsi* from North America and *C.* "*adamsi*-K" from Kyrgyzstan, central Asia. In both song species, partners duet by interdigitating their songs, so that each volley produced by one individual is followed shortly afterward by a volley from its partner and vice versa.

bunda (Fitch) and *C. adamsi* (Fig. 1), have relatively simple songs, composed of single-volley (monosyllabic) SRUs repeated many times (Henry et al. 1993). Other species, including Nearctic *C. downesi* and Palearctic *C. mediterranea* (see Fig. 3), produce complex songs that consist of much longer, multisyllabic SRUs, repeated only in response to other such songs (Henry 1980a). To characterize and analyze the full range of song variation found across the study taxa, 17 song features were measured for each song species. Those included the seven features shown for *C. adamsi* in Table 2, plus 10 more that were applicable to species with more complex songs.

Analysis of song differences among the cryptic species of the *carnea* group was limited to seven (of 15) ingroup taxa for which complete acoustic data for 20 or more individuals existed. To visualize and clarify differences, a discriminant function analysis (DFA) and a principal components analysis (PCA) were applied to song features. In both DFA and PCA, strong correlations between variables will bias the analysis, so we eliminated one of each pair of variables exhibiting high correlation coefficients ($r \geq 0.80$). The feature retained was chosen for its lower correlations, on average, with the other

TABLE 2. Values at 25 ± 1°C of the song characteristics (see Fig. 1) of *Chrysoperla adamsi* from North America and *C.* "*adamsi*-K" from Kyrgyzstan, central Asia. Data from both males and females are pooled. Each value is the mean of the means of *N* individuals in the population subsample (± 1SD). Pairwise comparisons were tested for statistical significance using *t*-tests for independent samples. SRU, shortest repeated unit exchanged between individuals while dueting.

	Frequency measures of volleys (Hz)			Volley duration (msec)	Long-volley duration (msec)	Volley period (msec)	Number of volleys per SRU
	Start	Middle	End				
C. adamsi (North America) ($N = 63$)	73.34 ± 3.22	55.98 ± 2.42	38.00 ± 4.04	794.74 ± 79.46	1318.12 ± 291.33	3243.65 ± 381.17	1
C. "*adamsi*-K" (Kyrgyzstan) ($N = 39$)	84.63 ± 4.29	62.89 ± 3.06	42.69 ± 3.86	891.42 ± 116.75	1073.90 ± 372.58	3718.26 ± 282.81	1
t-value	−15.11	−12.66	−5.80	−4.98	3.69	−6.71	–
Degrees of freedom	100	100	100	100	100	100	–
P	< 0.000001	< 0.000001	< 0.000001	0.000003	0.000362	< 0.000001	–

TABLE 3. Results of a discriminant function analysis of the seven least-correlated song measurements of seven song species of the *carnea* group of green lacewings, showing squared Mahalanobis distances (above the diagonal) and *F*-values (below the diagonal). All *F*-values were statistically significant at $P < 0.0001$. Degrees of freedom = 7461. Highlighted in bold are the values comparing the two *C. "adamsi"* populations.

	plorabunda	*adamsi* (North America)	"*adamsi*-K" (Asia)	*johnsoni*	*mediterranea*	*lucasina*	*downesi* (*mohave*)
plorabunda	—	33.71	42.54	43.14	140.80	200.00	129.47
adamsi (North America)	156.32	—	**6.82**	27.55	198.87	229.66	183.16
"*adamsi*-K" (Asia)	147.75	**22.67**	—	38.30	199.68	249.26	195.04
johnsoni	247.36	146.97	147.47	—	163.38	161.02	140.22
mediterranea	617.73	825.17	637.49	817.98	—	76.50	22.99
lucasina	1272.76	1349.30	1028.10	1248.83	419.27	—	34.40
downesi (*mohave*)	249.73	334.61	323.05	286.02	42.28	72.74	—

variables. In the present analyses, 17 song variables yielded seven least-correlated features, which were then used to extract either seven multidimensional roots (DFA) or the first two principal components (PCA). Statistical differences in songs among the seven lacewing taxa were determined from the matrix of squared Mahalanobis distances generated by the DFA, assuming a priori classification probabilities proportional to group sizes (Table 3). For the PCA, ANOVAs and Scheffé's contrast tests (Scheffé 1953) were applied to the scores of the first two factors.

When comparisons were limited to the two similar geographical forms of *C. "adamsi,"* six features (plus one invariable trait, volleys/SRU) were sufficient to describe the songs completely (Table 2, Fig. 1). For each of 10 series of SRUs per individual, we calculated the mean value of each song feature and then took the mean ($n = 10$) of those means as the representative value of the feature for each individual. A paired *t*-test (Snedecor and Cochran 1980) was performed on those individual averages, comparing North American to Asian "*adamsi*" populations with respect to statistically significant differences in the population means of each feature. All statistical analyses were performed using Statistica/Win versions 5 and 5.1h.

Behavioral Tests

To test the responsiveness of North American *C. adamsi* individuals to recorded songs of Asian *C. "adamsi*-K," each insect was presented with its own song type and the alternative song type in a paired design (Wells and Henry 1992b). For each of the two populations, to minimize pseudoreplication three different stimulus tapes were prepared (Kroodsma 1989). Each consisted of a typical series of 20 volleys of abdominal vibration recorded from a different individual at 25 ± 1°C. Each recording was digitized and recorded again to create signals of equal amplitude. As has been found in all species of the *carnea* group (Henry et al. 1996 and references therein), sexual differences in song phenotype were slight or absent, so both males and females were used to make the stimulus tapes. Temporal and frequency characteristics of those songs are given in Table 4.

Three females and two males of *C. adamsi* (North America), which were field collected and sexually receptive, were each tested against at least two independently selected pairs of stimulus tapes consisting of one 20-volley song from North America and the other from Asia. Each pair of tapes was presented twice, randomized by coin toss as to order of presentation and in both possible orders, yielding a minimum of four paired tests for each test animal (Table 5). The tendency of the test animal to duet with the recorded song was assessed by counting the number of correct responses (single volleys) the insect made to the 20 volleys of the stimulus tape. After a tape of one song type had been presented, we waited until the individual stopped tremulating before playing the tape of the second type of song. Once this first pair (set) of song presentations was completed, each song type was presented a second time, with the order of presentation reversed. The entire experiment was then repeated using a second and different set of stimulus tapes, chosen at random from among the six recordings. In addition, stimulus tapes of prerecorded *C. plorabunda* songs were played back to the same five individuals of *C. adamsi*, using an identical experimental protocol. Unfortunately, live individuals of Asian *C. "adamsi*-K" were not available to perform reciprocal tests of song acceptability.

TABLE 4. Values of the song characteristics (see Fig. 1) recorded on the stimulus tapes of *Chrysoperla adamsi* from North America and *C. "adamsi*-K" from Kyrgyzstan, central Asia. Tapes were used in the behavioral tests described in the text.

	Frequency measures of volleys (Hz)			Volley duration (msec)	Long-volley duration (msec)	Volley period (msec)
	Start	Middle	End			
C. adamsi male 1 (North America)	70.31	54.69	42.97	592.00	833.00	3251.00
C. adamsi female 1 (North America)	70.31	50.78	35.16	721.00	992.00	3250.00
C. adamsi female 2 (North America)	74.22	54.69	35.16	850.00	1230.00	3452.00
C. "adamsi-K" male 1 (Kyrgyzstan, Asia)	78.13	58.59	39.06	939.00	1037.00	3402.00
C. "adamsi-K" male 2 (Kyrgyzstan, Asia)	78.13	62.50	35.16	1148.00	1537.00	3290.00
C. "adamsi-K" male 3 (Kyrgyzstan, Asia)	78.13	62.50	42.97	974.00	1234.00	3408.00

TABLE 5. Mean number of dueting responses (volleys) given by five *Chrysoperla adamsi* from North America to stimulus tapes (n = independent trials). Each tape consisted of 20 naturally spaced volleys recorded from an individual of North American *C. adamsi* or Asian (Kyrgyzstan) *C.* "*adamsi*-K." Each tape set was a different combination of two recordings randomly selected from a total of six stimulus tapes (Table 4). A volley was counted as a response only if it was produced immediately after a volley on the stimulus tape. Responses to North American versus Asian songs were compared using a paired t-test and a two-tailed test for significance.

	Mean number of responses to *C. adamsi* song	Mean number of responses to *C.* "*adamsi*-K" song
Female 1, tape set 1 (n = 2)	20.00	20.00
Female 1, tape set 2 (n = 2)	11.50	12.50
Female 1, tape set 3 (n = 2)	12.50	14.00
Female 1, tape set 4 (n = 2)	19.00	20.00
Female 2, tape set 3 (n = 2)	19.00	15.00
Female 2, tape set 5 (n = 2)	19.00	15.50
Female 3, tape set 1 (n = 2)	16.50	16.50
Female 3, tape set 3 (n = 2)	19.00	18.00
Female 3, tape set 5 (n = 3)	16.33	20.00
Male 1, tape set 6 (n = 3)	20.00	19.67
Male 2, tape set 6 (n = 2)	20.00	19.00
Mean of means	17.53	17.29
Variance	9.11	7.39
t-value	0.3693	
Degrees of freedom	10	
P	0.7196	

We used the mean number of responses to each song type given by each individual to a specific pair of stimulus tapes as a single data point in our analysis. For all tests, paired t-tests were performed to compare means.

Molecular Methods

DNA extraction methods closely followed the DTAB-CTAB protocol of Gustincich et al. (1991), with modifications described below. To obtain DNA, whole fresh or deep-frozen lacewing specimens with wings removed were ground in microcentrifuge tubes containing 300 μl 1 × TE grinding buffer (pH 8.8). Cells were lysed in 600 μl of DTAB solution, mixed, and incubated for 15 min at 65°C, followed by two washes with equal volumes of chloroform. The final aqueous upper layer was added to 600 μl dH_2O and 100 μl CTAB solution for precipitation of DNA. The pellet was resuspended in 300 μl 1.2-M NaCl, washed sequentially with 750 μl 100% EtOH and 300 μl 70% EtOH, dried in a SpeedVac for 10–15 min, dissolved in 50 μl dH_2O, and stored at −70–100°C.

The polymerase chain reaction (PCR; Mullis et al. 1986) was used to amplify two segments of lacewing mitochondrial DNA (mtDNA). Primers for this procedure were selected and modified from Simon et al. (1994) or designed by the authors (Table 6). The first amplified section was a 516-bp fragment spanning most of the COII gene. The second amplified section was a 552-bp fragment located within the ND2 gene, which required a more complicated amplification procedure. First, we amplified an oversize fragment. Then, we sequenced (see below) the PCR product using C1-N-1560 and using Oligo version 4.0 for MS-DOS designed an internal primer, TW-N-1303, which is specific to that lacewing sequence. The PCR procedure was then repeated using TM-J-206 and TW-N-1303 as the new flanking primers, which thus amplified the smaller ND2 fragment of interest. Primer cycle conditions for COII included a denaturing step of 60 sec at 94°C, an annealing step of 60 sec at 55°C, and an extension step of 70 sec at 72°C repeated for 30 complete cycles. ND2 primer cycle conditions were identical, except that the extension step was lengthened to 120 sec and the number of PCR cycles was increased to 45.

Fragments were cleaned in preparation for sequencing using a simplified SAP (shrimp alkaline phosphatase) procedure. One microliter of exonuclease I and 1 μl of SAP were added to 5 μl of double-stranded PCR product and the resulting solution incubated, first at 37°C for 15–20 min and then at 80°C for 15–20 min. The purified PCR products were then sequenced manually, using a double-stranded method, as well as automatically, using an ABI 377 automated se-

TABLE 6. Descriptive information about the flanking and internal primers used in the study, with site positions aligned with the published sequence of *Drosophila yakuba* (Clary and Wolstenholme 1985). Refer to text (molecular methods) and Simon et al. (1994) for additional details.

Primer name	Type	Design	Nucleotide sequence (5′ . . . 3′)
		NADH dehydrogenase subunit 2 (ND2)	
TM-J-206	Flanking	*D. yakuba*	GCTAAATAAAGCTAACAGGTTCAT
C1-N-1560	Flanking	*D. yakuba*	TGTTCCTACTATTCCGGCTCA
TW-N-1303	Both[1]	lacewing specific	GCTTTGAAGGCTATTAGTTTTA
N2-N-1184	Internal	lacewing specific	GTTAATTAAAGTATTTCATTTC
N2-N-985	Internal	lacewing specific	CCC[2]AATGATAAAAAATTAC
		Cytochrome oxidase II (COII)	
TL2-J-3037 (mod)	Flanking	*D. yakuba*	AATATGGCAGATTAGTGCA
TK-N-3785 (mod)	Flanking	*D. yakuba*	GTTTAAGAGACCAGTACTT
C2-N-3661 mod 1	Internal	*D. yakuba*; lacewing ingroup taxa	CCACACATTTCTGAACATTGACC
C2-N-3661 mod 2	Internal	*D. yakuba*; lacewing outgroup taxa	CCACACATTTCTGAACATTGTCC
C2-N-3494 mod 1	Internal	*D. yakuba*; lacewing ingroup taxa	GGTAATACAGTTCGGTTATCTAC
C2-N-3494 mod 2	Internal	*D. yakuba*; lacewing outgroup taxa	GGTAATACAGTTCGGTTATCAAC
C2-N-3308	Internal	lacewing specific	ACGTAAAGAAGGTAAG[3]GCAA

[1] See text (molecular methods) for explanation.
[2] Degenerative base: C or T.
[3] Degenerative base: G, T, or A. See text (molecular methods) for details.

TABLE 7. Characteristics of COII, ND2, and COII + ND2 (combined) molecular data used in phylogenetic analyses of 17 lacewing taxa (*Chrysopa oculata* has been excluded). Each of the three datasets is broken down by codon position.

		Nucleotide sites							
		Total	Variable	Parsimony informative	α-value (GTR + Γ)[1]	Relative rates (GTR + rates)	Percent sites variable per codon position	Percent AT	Ti/Tv[2]
COII	ALL	516	41 (8%)	31	< 0.000001	—	—	76.2	1.87
	1	172	3 (2%)	2	0.00022	0.23	7.0	63.5	∞
	2	172	1 (0.5%)	0	0.003	0.04	2.0	70.0	∞
	3	172	37 (21.5%)	29	0.474	2.73	90.0	97.1	7.29
ND2	ALL	552	61 (11%)	38	0.13	—	—	83.4	2.86
	1	184	16 (8.7%)	8	0.16	0.61	21.0	84.3	3.34
	2	184	2 (1.1%)	2	0.005	0.06	5.3	73.9	0.33
	3	184	43 (23.4%)	28	0.36	2.33	73.7	92.1	6.16
COII + ND2	ALL	1068	102 (9.6%)	69	0.08	—	—	79.9	1.92
	1	356	19 (5.3%)	10	0.04	0.44	14.5	74.2	2.50
	2	356	3 (0.8%)	2	0.0003	0.06	2.9	71.0	0.25
	3	356	80 (22.5%)	57	0.406	2.49	82.6	94.4	4.71

[1] GTR + Γ, discrete approximation with eight rate classes specified (Yang 1994).
[2] Adjusted for nucleotide bias and ASRV with α equal to values in column 4.

quencer (with XL upgrade) and Sequencher version 3.0 software to verify the results. Internal, reverse primers specific for lacewing mtDNA sequences were designed by the authors and used to sequence the COII and ND2 gene fragments obtained by PCR. We used a "walking" method to design primers and gather sequence data. At each step, several primers were usually designed before finding one that worked well. For *yakuba* primers C2-N-3661 and 3494, two slight variants of each were needed to obtain COII sequence from all lacewing ingroup and outgroup taxa (Table 6).

Sequence Analysis

Sequence data were collected from individuals of 18 distinct morphological or song species of Chrysopidae in North America, Europe, and Asia. At least two individuals of each species were sequenced, usually from the same collecting locality (Table 1). In all cases, conspecific individuals shared identical or nearly identical sequences and grouped together, so these duplicates were collapsed into a single operational taxonomic unit (OTU) in our final analyses. An exception was made for two individuals of *C. mediterranea* because each was obtained from a different site outside the known range of the species (Henry et al. 1999) and differed from its conspecific at four (of 1068) nucleotide positions. Two North American members of the *pudica* species group, *C. rufilabris* (Burmeister) and *C. harrisii* (Fitch), were specified as outgroups for the 15 cryptic species of the *carnea* group. *Chrysopa oculata* Say (North America) was included as the overall outgroup to provide support for the phylogenetic positions of the two specified outgroups and to calculate mean pairwise sequence divergences (see below), but it was excluded from final analyses to speed maximum-likelihood tree reconstruction.

Initial alignments of multiple sequences were performed in Clustal W (Thompson et al. 1994). However, final alignment of all sequence data was done by eye with Eric Cabot's ESEE version 3.0s and 3.2s (Eyeball SEquence Editor for MS-DOS). Sequences were aligned with the published sequences of *Drosophila yakuba* (Clary and Wolstenholme 1985). Mean pairwise sequence divergences were calculated using PAUP* 4.0b1 (Swofford 1996) from the uncorrected distance matrix and the maximum-likelihood distance matrix under the general-time-reversible model with rate heterogeneity (GTR + rates; Yang 1994).

Phylogenetic analyses of the COII and ND2 gene segments, and the two segments combined were performed using PAUP* (18 OTUs; 516, 552, and 1068 characters respectively). Optimization assumptions included maximum parsimony (MP), minimum evolution (ME), and maximum likelihood (ML). The two genes were first analyzed separately to determine if they were similar enough in their characteristics to be combined, which was the case (Table 7).

An MP tree was generated from each dataset and from the combined data using a branch-and-bound search that retained all equally parsimonious trees (MULPARS option). Gaps were treated as missing and all characters were uniformly weighted. A 50% majority rule bootstrap of 500 replicates was then performed to produce each final cladogram. Decay indices (DI) were calculated by instructing PAUP* consecutively to save all trees one to six steps longer than the best MP tree and in each case to compute the strict consensus of those trees: A loss of phylogenetic resolution at a given node for a certain number of extra tree steps gave the decay index of that node.

The data were then evaluated according to the ML criterion under an array of substitution models, including Jukes-Cantor (JC; Jukes and Cantor 1969), Kimura two-parameter (K2P; Kimura 1980), Hasegawa-Kishino-Yano (HKY85; Hasegawa et al. 1985), and general-time-reversible (GTR; Yang 1994). Each ML model was implemented using each dataset and the combined data under four assumptions of among-site rate variation: (1) no variation; (2) variation fitting the gamma distribution; (3) variation accommodated by estimating the proportion of invariant sites; and (4) a mixture of invariant sites and gamma-distributed sites. After discovering extreme among-site variation in our data (Table 7), we then applied each ML model with (5) codon position-specific rate heterogeneity (+ rates). Based on log-likelihood scores obtained

for each run on the two separate datasets, we chose GTR with rate heterogeneity as the model of evolution providing the best compromise between goodness of fit and parameter economization (Frati et al. 1997).

Assuming the GTR + rates model, an ME tree was produced for COII, ND2, and combined data. A full heuristic search, stepwise addition of 100 random-addition sequences, and tree-bisection-reconnection branch rearrangement (TBR) were specified in each run. Branch lengths were constrained to be nonnegative. The final consensus trees were generated from 500 bootstrapped replicates (50% majority rule).

An ML analysis was also performed for separate and combined datasets. Each run employed a heuristic search, stepwise addition using one random-addition sequence, and TBR; assumptions included nonnegative branch lengths and a GTR + rates model of evolution. The final consensus trees were generated from 500 bootstrapped replicates (50% majority rule).

Alternative hypotheses of relationships among several taxa were tested by forcing PAUP* to place those taxa in different phylogenetic positions on the tree and then making note of changes in the lengths of the shortest trees generated under the assumption of MP. Statistical significance of tree length changes under forced topologies was calculated using Templeton's (1983) nonparametric procedure, which utilizes a Wilcoxon signed-rank test to compare the distribution of character changes between two topologies. We also assessed significant differences between constrained and unconstrained trees using the Kishino-Hasegawa test as implemented in PAUP*.

Drosophila yakuba alignments of lacewing nucleotide sequences have been deposited in the EMBL Nucleotide Sequence Database under accession numbers AF064125–AF064143.

Mapping Song Characters on the Molecular Phylogeny

The computer program MacClade for the Macintosh was used to trace the evolution of discrete mating signal characters of lacewings on cladograms generated from sequence data (Maddison and Maddison 1992; Cunningham et al. 1998; Wells and Henry 1998). An MP procedure was used, which assumed an equal probability of gains and losses, no delayed or accelerated transformations, and hard polytomies. Analyses were performed on minimum-length trees based on combined data from the COII and ND2 genes. Seven characters were coded and mapped (Table 8): number of volley types per song, direction of frequency change in a volley, direction of frequency change during an SRU, presence of a discrete "calling song" in males, pattern of volley production in the SRU during dueting, length of interval between short volleys (two discrete size categories), and duration of long volleys (two discrete size categories).

TABLE 8. Coding of character states for seven song features of the lacewing species used in the phylogenetic analyses. For song species explanation, see Table 1. A, number of types of volleys produced: 0 = 1, 1 = 2; B, direction of frequency modulation in a volley: 0 = rising, 1 = falling, 2 = no change; C, direction of frequency change during SRU: 0 = rising, 1 = falling, 2 = rising then constant, 3 = falling then constant, 4 = rising then falling, 5 = no change; D, presence of a distinct "calling song" in males: 0 = yes, 1 = no; E, pattern of volley production in the SRU during dueting: 0 = train of short volleys, 1 = multiple long volleys, 2 = single volley; F, short-volley interval: 0 = < 50 msec, 1 = > 50 msec; G, long-volley duration: 0 = < 1.5 sec, 1 = > 1.5 sec.

Song species	Song characters						
	A	B	C	D	E	F	G
C. plorabunda	0	1	1	1	2	1	0
C. adamsi	0	1	1	1	2	1	1
C. johnsoni	0	1	5	1	1	1	1
C. downesi-sp.2	1	0	4	1	0	1	0
C. downesi-sp.1	1	0	4	1	0	0	0
C. "adamsi-K"	0	1	1	1	2	1	1
C. "downesi-K"	1	0	4	1	0	1	0
"motorboat-K"	0	2	3	1	0	0	0
C.c.2 "slow motorboat"	0	2	4	1	0	0	0
C.c.4 "motorboat"	0	2	2	0	0	0	0
C.c.3 "maltese"	0	0	0	1	2	1	1
C. lucasina	0	0	1	0	1	1	0
C. mediterranea	0	2	4	1	0	0	0
C.c.5 "generator"	0	1	1	1	2	1	1
C. "downesi-CH"	1	0	4	1	0	1	0
C. rufilabris	0	2	5	1	0	0	0
C. harrisii	0	2	5	1	0	0	0
Chrysopa oculata	0	1	5	1	0	0	0

RESULTS

Song Phenotype and Similarity

Time-domain (oscillograph) and frequency-domain (sonograph) plots of a 12-sec series of song volleys of *C. adamsi* from North America and *C. "adamsi*-K" from Kyrgyzstan are shown in Fig. 1. There is a strong overall resemblance in song phenotype between the two populations, and the amplified songs are nearly indistinguishable to the human ear. Songs of both types consisted of volleys of approximately the same duration, period, carrier frequency, and frequency modulation, and both possessed a short "rumble" appended to most volleys (constituting part of the "long volley").

Despite qualitative similarity of the song types, *t*-tests of means revealed significant differences between the two populations in all six diagnostic song features (Table 2). The largest differences, as indicated by the absolute value of *t*, were found in measures of frequency from the start and middle of a volley. Smallest differences were found in two temporal measures, volley duration and long-volley duration.

When the songs of American and Asian *C. "adamsi"* were compared to other cryptic song species of the *carnea* group, the magnitude of the differences between the *"adamsi"* populations was relatively small. DFA showed the two *"adamsi"* populations clustering much more closely to one another than to any other taxon (Fig. 2). Although the *"adamsi"* populations remained significantly different from one another in the DFA, the Mahalanobis distance between them measured only 6.82 (vs. values of 22.99–249.25 for other species-pair comparisons; Table 3). The scores of the first two factors of a PCA were then analyzed for species differences using an ANOVA and Scheffé's contrasts tests (Table 9). For factor 1, all species except American and Asian *C. "adamsi"* differed from one another significantly. The second principal component was less discriminating: Although most pairwise

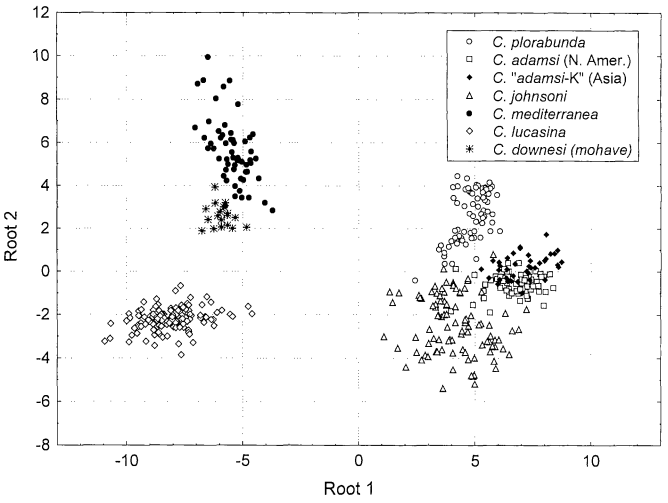

FIG. 2. Scatterplot of the first two roots of a discriminant function analysis of seven least-correlated measurements of the songs of seven distinct song species of the *carnea* group. Each datapoint represents a single individual coded by species.

TABLE 9. Comparison of seven song species of the *carnea* group of green lacewings, using the scores of the first two factors of a principal components analysis of seven least-correlated 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 within a column ($P \leq 0.05$).

Song species (*N*)	Factor 1, Mean ± SD	Factor 2, Mean ± SD
C. plorabunda (71)	0.005 ± 0.229	−1.302 ± 0.485^{A}
C. adamsi North America (63)	−0.721 ± 0.125^{A}	−0.551 ± 0.178^{BC}
C. 'adamsi-K' Asia (39)	−0.591 ± 0.136^{A}	−1.050 ± 0.241^{A}
C. johnsoni (98)	−1.376 ± 0.557	0.576 ± 0.502
C. mediterranea (57)	1.451 ± 0.132	−0.677 ± 0.151^{B}
C. lucasina (128)	0.789 ± 0.200	1.217 ± 0.311
C. downesi (mohave) (18)	1.068 ± 0.133	−0.308 ± 0.133^{C}

species comparisons showed significant differences, *C. adamsi* (North America) proved to be indistinguishable from both *C. downesi (mohave)* and *C. mediterranea*, whereas *C. "adamsi*-K" (Kyrgyzstan) was not significantly different from *C. plorabunda*.

Behavioral Responses to Song Playback

Responses of North American *C. adamsi* individuals to songs of their own type versus songs of *C. "adamsi*-K" from Asia are shown in Table 5. Every stimulus tape, regardless of its song type or acoustic characteristics (Table 4), elicited the same response. Each individual answered nearly every volley on the stimulus tape with an appropriately placed response volley, indicating that a true duet was established by the insect with the playback signal. A paired *t*-test showed no significant difference between the mean number of responses given by the five insects to the two types of songs. It is evident that North American *C. adamsi* were completely unable to discriminate between their own type of song and that of *C. "adamsi*-K" from Kyrgyzstan. We also ran the same individuals of *C. adamsi* through several trials of identical design testing their response to recorded songs of another species, *C. plorabunda* (results not shown). *Chrysoperla adamsi* individuals rejected the songs of this nonconspecific, confirming the results of a previous study (Wells and Henry 1992b). Although live *C. "adamsi*-K" from Kyrgyzstan were not available for reciprocal experiments, there is no a priori reason to expect significantly different results.

Molecular Phylogeny

The phylogenetic relationships of 19 lacewing OTUs (18 song species) are depicted in Fig. 3, as inferred from combined DNA sequence data for the COII and ND2 mitochondrial genes. (Although *C. oculata* was not included in all analyses, it is shown in its correct position as basal to the taxa we examined and was used to determine bootstrap support for monophyly of *C. rufilabris* + *C. harrisii*.) The single cladogram drawn is a 50% majority-rule bootstrap tree, which exhibited the same topology regardless of the method (MP, ME, or ML) employed to generate it. Bootstrap proportions resulting from the three tree-building procedures are placed above each node; below the node is the decay index calculated under MP. Minimum tree length under branch-and-bound MP measured 147 steps, found in two trees with retention indices of 0.777 and rescaled consistency indices of 0.582. Of 1068 total characters, 102 were variable and 69 of those were parsimony informative (see Table 7 for complete descriptive statistics of separate and combined data). The majority of variable sites were in third-codon positions, and analysis of third positions alone produced a topology identical to that produced using all positions.

Deeper branches of this phylogeny were well supported by bootstrap. The *pudica* group of *Chrysoperla*, represented here by *C. rufilabris* and *C. harrisii*, appeared with 100% confidence at its expected basal position in the phylogeny, as sister-taxon to the *carnea* group (see Brooks 1994). Uncorrected average pairwise sequence divergence between the *pudica* and *carnea* groups measured 5.2% (0.09 expected substitutions per site, corrected divergence under GTR + rates model). Bootstrap support for monophyly of the *carnea* group measured 100% under all methods (MP, ME, and ML). The decay index for the *carnea* group was calculated to be equal to or greater than six tree steps.

Within the *carnea* group, haplotypes from North American and Eurasian species always formed separate, well-supported clades, again regardless of method of analysis. Uncorrected sequence divergence between the two continental clades averaged 2.4% (0.04 expected substitutions per site, corrected divergence). Bootstrap support for monophyly of the North American clade measured 70% under ML and 96–97% under ME or MP, with a decay index of 5. The North American clade was further resolved into two monophyletic clades, one including *C. plorabunda, C. adamsi*, and *C. johnsoni* Henry, Wells and Pupedis (the *C. plorabunda* complex) and the other consisting of two taxa exhibiting the basic diagnostic characteristics of *C. downesi* (the *C. downesi* complex). Those two species complexes, although clearly distinct, differed in their sequences by an average of only 1.8% (0.03 expected

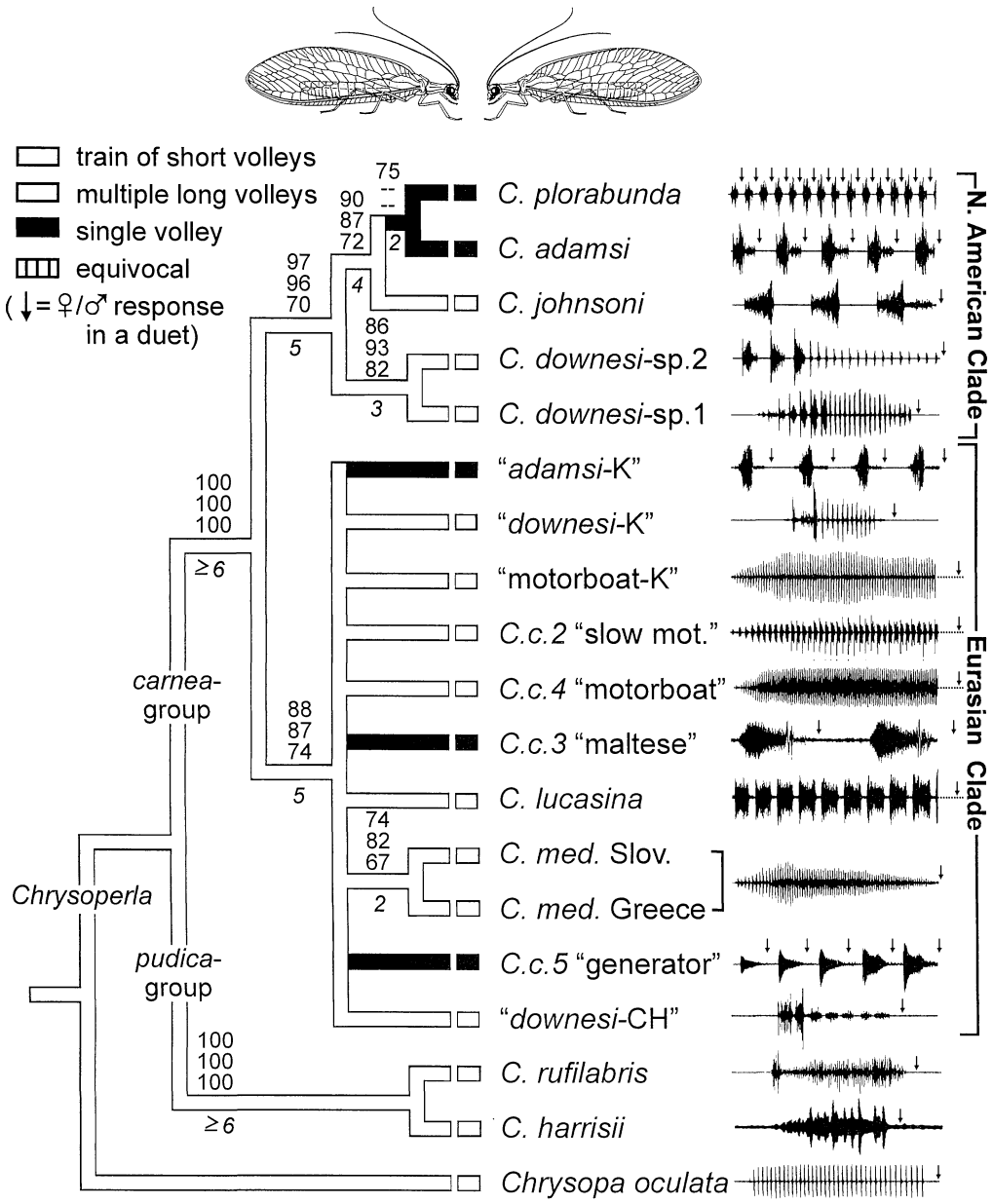

FIG. 3. Bootstrap phylogeny (50% majority rule) of 15 song species of green lacewings of the *carnea* group plus three outgroup taxa, inferred from DNA nucleotide sequence data from sections of the ND2 and COII mitochondrial genes using maximum parsimony (MP), minimum evolution (ME), and maximum likelihood (ML). Numbers above the nodes are bootstrap proportions for each of the three procedures, stacked in the order MP/ME/ML. Italicized numbers below the nodes are decay indices. A 12-sec oscillograph is shown for the song of each species; vertical arrows indicate where the partner would sing during a duet. The song character, pattern of volley production in duets, is traced on the cladogram. Production of a train of short volleys is ancestral for the genus *Chrysoperla*. Production of multiple long volleys and single volleys are derived character states (see text).

substitutions per site, corrected divergence). Relationships among the species of the Eurasian clade (the *C. carnea* complex) were unresolved, despite a high level of confidence in the monophyly of the group (74% under ML and 87–88% under ME or MP). The two specimens of *C. mediterranea* emerged together in all analyses (decay index = 2), despite their geographic origins in widely separated parts of Europe.

Based on the analyses above, *C. adamsi* from North America and *C.* "*adamsi*-K" from Kyrgyzstan, Asia, belong to separate clades within the *carnea* group. When the topologies of the two shortest trees obtained by branch-and-bound search were constrained such that the two populations of *C.* "*adamsi*" became their own closest relatives, tree length increased by 19 steps (to 166), a difference that was highly significant using either Templeton's method ($P < 0.001$, Wilcoxon signed-rank test) or the Kishino-Hasegawa test ($P < 0.0001$).

Song Character Evolution

Four song features (Table 8), describing frequency modulation and temporal attributes of volleys (B, F, G) and fre-

quency changes in SRUs (C), were too variable among species to map successfully onto the molecular cladogram. For these traits, terminal character states exhibited excessive homoplasy and character states in ancestral branches were often equivocal (reconstructions not shown). Character states of some other features were best interpreted as convergent autapomorphies, including two-volley types (A), confined to the four *C. downesi*-like forms from North America and Asia, and presence of a special male calling song (D), found only in *C. lucasina* and *C.c.4* "motorboat" of Eurasia. The remaining trait, pattern of volley production in duets (E), contained appropriate levels of interspecific variation to be informative about character evolution in *Chrysoperla*, permitting a test of the hypothesis of symplesiomorphy of the *C. "adamsi"* song type. Dueting lacewings exhibit three very distinct patterns of volley production (Fig. 3; Wells and Henry 1998). Some species, exemplified by the three "motorboats," the four "*downesis*," and *C. mediterranea*, produce a long train of very short volleys, given as a unit before the partner responds. Others, like *C. lucasina* and *C. johnsoni*, produce several longer (> 0.3 sec), temporally more discrete volleys as a repeating unit, followed by a similar response from the partner. Finally, *C. plorabunda, C.c.3* "maltese," and *C.c.5* "generator" duet by answering after each volley, in which case volleys are also relatively long. The *C. "adamsi"* populations are in this last category, which proved to be the most derived state for the trait when analyzed by MacClade (Fig. 3), falsifying the hypothesis of symplesiomorphy. The ancestral condition in *Chrysoperla*, which characterizes both outgroups and *C. oculata*, appeared to be trains of very short volleys.

Discussion

Acoustic Similarity of Songs

The striking resemblance in the songs of the two allopatric *C. "adamsi"* taxa is apparent both from listening to them and inspecting the sonograph displays (Fig. 1). However, quantitative analyses of specific song components demonstrate the presence of significant differences between the taxa. Those differences are generally larger than has been measured as intraspecific variation across the full geographical range of other song species of the *carnea* group, for example, *C. plorabunda* (Henry and Wells 1990), *C. adamsi* (Henry 1991), *C. lucasina* (Henry et al. 1996), and *C. mediterranea* (Henry et al. 1999). For the songs of Asian and North American *C. "adamsi"* to be judged truly alike, the importance of their differences needs to be evaluated.

Vibrational signals, whether confined to a substrate or propagated through the air, have components in both time and frequency domains (Ewing 1989). Studies of mating signals in frogs and insects have shown that most acoustic-orienting animals are more sensitive and responsive to small changes in the temporal (phrasing) properties of signals than they are to equivalent changes in carrier frequency (Pollack 1979; Doherty and Hoy 1985; Schwartz 1987; Bailey 1991; Allan and Simmons 1994; Ritchie and Gleason 1995). In other words, significant frequency (tonal) differences in courtship and mating songs between closely related species will have less effect on the ability of individuals to discriminate conspecific from heterospecific partners than will differences in temporal qualities, such as pulse or phrase duration, interval, and period. Lacewings also follow these rules. In a study of the responses of female North American *C. plorabunda* to computer-synthesized songs, it was found that decreasing or increasing overall carrier frequency by as much as 27% had no significant effect on behavior, whereas eliminating frequency modulation or altering volley period effectively prevented normal responses (Wells and Henry 1992a). In the present study, *t*-tests on means show that all song features, in both time and frequency domains, differ between North American *C. adamsi* and Asian *C. "adamsi*-K" (Table 2). However, the two populations differ significantly from one another more in tonal than temporal qualities, as indicated by *t*-values of very different magnitudes for those classes of features. Basically, North American *C. adamsi* possesses a song approximately 12% lower in pitch than its Asian analogue, but with the same direction and degree of frequency modulation and very similar volley phrasing. Because absolute frequency has been shown to be much less important to singing lacewings (and many other acoustic animals) than temporal characteristics and frequency modulation, we argue that the small tonal differences should be discounted and that the two songs are functionally identical.

Song differences between North American *C. adamsi* and Asian *C. "adamsi*-K" appear relatively minor when these taxa are compared to other song species of the *carnea* group (Fig. 2). Mahalanobis distance between them is smaller by nearly a factor of four, compared to the next-most-similar pair of taxa, *C. mediterranea* and *C. downesi* (*mohave*) (Table 3). Additionally, in the PCA, the two *C. "adamsi"* populations are indistinguishable from one another along the factor 1 axis, whereas all of the other species are clearly separable (Table 9). Again, we conclude that the apparent similarity of the songs of North American and Asian versions of *C. "adamsi"* is real.

Functional Similarity of Songs

Another way to approach the question of resemblance is to ask the insects themselves to decide, as we have done in the behavioral experiments. The results (Table 5) show clearly that individuals of North American *C. adamsi* judge the songs of Asian *C. "adamsi*-K" to be identical to their own. Most important, live insects establish normal duets with taped signals and maintain the kind of prolonged duet that is a prerequisite for successful courtship leading to copulation in species of the *carnea* group (Wells and Henry 1992b). Thus, the songs of the two taxa are not only phenotypically similar, but also functionally (biologically) interchangeable. A previous study (Wells and Henry 1992b) demonstrated that North American *C. adamsi* discriminates strongly against the songs of both *C. plorabunda* and *C. johnsoni*, which are the most similar to *C. adamsi* based on DFA (Fig. 2). Those results reinforce the significance of our behavioral findings here.

Phylogenetic Relationships

With the acoustic and functional similarity of the two geographical song types established, causal factors can be con-

sidered. Phylogenetic relationship is the most parsimonious explanation of similarity. The results of all phylogenetic analyses of DNA sequences from the COII and ND2 mitochondrial genes indicate that *C. adamsi* from North America and *C.* "*adamsi*-K" from Kyrgyzstan are not the same species nor are they even sister taxa (Fig. 3). Nineteen tree steps must be added to bring the two taxa into a sister-species relationship. In fact, the two taxa are members of different clades, each of which is confined to a different continent and supported as monophyletic at a high level of statistical confidence. Thus it is highly unlikely that the markedly similar features of the songs of North American and Asian *C.* "*adamsi*" are due to close phylogenetic relationship.

More generally, the cladogram tells us that the *carnea* group is experiencing rapid evolution and that speciation events within it have been recent. For other complexes of closely related species, COII and ND2 have proven effective (Simon et al. 1994), yet we were unable to resolve the relationships among most of our species (Fig. 3). Sequence divergence (uncorrected) among terminal taxa is very low, averaging only 1.1% for 30 pairs of possible sister species (0.02 expected substitutions per site, corrected divergence). These data are consistent with the results of two earlier allozyme studies, which demonstrated extreme genetic similarity among three song species of the North American clade (Nei's index of genetic distance = 0.000–0.010; Wells 1994) and moderate similarity among five "morphospecies" of the *C. carnea* complex of Europe (Nei's index = 0.02–0.13; Cianchi and Bullini 1992). The authors of the latter study went so far as to place the time of origin of the European species in the Pleistocene (100,000–500,000 years ago) based on a molecular clock (Cianchi and Bullini 1992). Although precise calculations based on molecular clocks rely on a large number of assumptions and often have large confidence intervals (Brower 1994; Hillis et al. 1996), the times of origin of the cryptic species of the *carnea* group are very recent, by any method of estimation.

Reconstructing Song Evolution

If *C. adamsi* and *C.* "*adamsi*-K" are not the same species and do not share a most recent common ancestor, then their striking similarity might instead be the result of both taxa retaining the plesiomorphic state for song phenotype from a distant ancestor. Mapping an important song character—pattern of volley production in duets—onto the chrysopid molecular phylogeny shows single-volley SRUs to be independently derived in the *C.* "*adamsi*" populations on the two continents (Fig. 3). The basal chrysoperlan multiple-short-volley-train SRU has been retained in the North American *C. downesi* complex, but progressively modified in its sister clade, *C. johnsoni* + *C. adamsi* + *C. plorabunda.*

Constraint and the Convergent Evolution of Songs

In the absence of phylogenetic constraints, there are two plausible explanations for evolutionary convergence of signals between North American and Asian *C.* "*adamsi.*" The first is adaptation (environmental selection), and the second is chance (random mutation) acting on limited options.

Classic examples of convergence involve similar evolutionary solutions to similar environmental challenges, through an adaptive response on the part of two "unrelated" organisms, that is, the occurrence of homoplasy due to selective constraints. In lacewings, it is reasonable to assume that certain variants of vibrational mating signals will propagate more efficiently through one type of substrate than another. For example, the optimal signal for transmission through conifer needles will probably have different biomechanical properties than a signal that is ideally suited to travel through grass blades or broad leaves (Michelsen et al. 1982; Markl 1983). Thus, selection is likely to favor convergence of tremulation songs in two (or more) lacewing song species that share the same type of special habitat. However, the cryptic species of the *carnea* group show very little ecological differentiation, at least any that is consistent over an entire species range. For example, only two species, *C. downesi* (North America) and *C. mediterranea* (Europe), are known to be associated as adults with conifers, yet careful study has shown that even those presumably definitive plant-insect associations break down in certain local populations of each species (Henry 1993; Henry et al. 1999). Furthermore, none of the many other song species that we have discovered appears to be linked to any particular type of vegetation (C. S. Henry, unpubl. data). *Chrysoperla adamsi* from North America, like most *Chrysoperla* species, is an ecological generalist that sings and mates on almost any substrate (Henry et al. 1993). Its song phenotype is not likely to have been shaped by strong environment-specific selection. Consequently, an explanation of convergence between the two *C.* "*adamsi*" song species that presumes such adaptation is unsupported and will remain so until habitat preferences and the transmission qualities of songs in different substrates are examined in more detail.

Random mutation can produce convergence if the necessary changes are simple and therefore reasonably likely to occur independently in separate lineages. Thus, in lacewings, simple genetic architecture could constrain the number of possible song phenotypes, causing homoplasy. Although the precise genetic basis of song phenotype in lacewings is not known, laboratory studies of forced hybridization between *C. plorabunda* and *C. downesi* have shown that inheritance of song traits in F_1 and F_2 progeny and parental backcrosses is consistent with a Mendelian model that assumes two loci and two alleles per locus (Henry 1985). Similar results have been obtained in crosses between *C. plorabunda* and *C. johnsoni* (C. S. Henry, unpubl. data; Wells and Henry 1994). Those findings suggest that the genetic basis of song differences in the cryptic species of the *carnea* group is indeed simple, thus strengthening the likelihood that chance alone accounts for the convergence observed between *C. adamsi* and *C.* "*adamsi*-K." Furthermore, rapid song evolution and frequent speciation within the *carnea* group increase the probability of recurrence of a particular song phenotype in the clade, especially in different geographical areas where two species with similar songs will never interact.

Although the present work has focused on North American and Asian *C.* "*adamsi*," there exist in the *carnea* group additional examples of song similarity that are probably also cases of evolutionary convergence. Two of these are song analogues of North American *C. downesi*, shown in Fig. 3

as *C.* "*downesi*-K" (Kyrgyzstan) and *C.* "*downesi*-CH" (China). Unfortunately, insufficient live individuals prevented inclusion of Asian *C.* "*downesi*" taxa in the present analysis. Song convergence has also been found between lacewing genera, for example, *Chrysopiella minora* Banks and *C. plorabunda* possess strikingly similar vibrational mating signals (Henry and Johnson 1989).

Multiplicity of song convergence in lacewings is remarkable, considering how rarely one finds convergent mating signals in other acoustic animals. Furthermore, as a type of display behavior, mating signals should (and generally do) exhibit lower levels of homoplasy than other morphological or behavioral features (de Queiroz and Wimberger 1993; Foster et al. 1996). Even in the absence of a compelling biomechanical (environmental selection) explanation, the existence of multiple homoplasies in the *carnea* group suggests that song evolution has been constrained in important ways. We have summarized evidence for one plausible constraint, simplicity of the genetic architecture underlying song phenotype, but other developmental or genetic factors may also be at work. Genetic (or other) constraints on such a crucial component of the mating system predispose the clade to repeated evolution of functionally identical songs, having the effect of limiting ultimate large-scale diversity by the origin of song types that are too similar to achieve reproductive isolation. We predict that as additional cryptic song species are discovered and described in the *carnea* group, other cases of evolutionary convergence will emerge.

Acknowledgments

This study was supported in part by National Science Foundation Award DEB-9220579 to C. S. Henry and by several grants from the Research Foundation of University of Connecticut to the authors and to C. M. Simon. We thank P. Duelli (Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf); J. B. Johnson (University of Idaho, Moscow); S. J. Brooks (The Natural History Museum, London); H. Aspöck (Universität Wien, Austria); M. Canard (Université Paul-Sabatier, Toulouse); and L. Wilson (University of Idaho, Moscow) for help in collecting and maintaining live lacewings from many geographic locations. Special thanks go to D. Funk (University of Arizona, Tucson) and C. S. Jones (University of Connecticut) for their thoughtful suggestions for improving the manuscript. The efforts of two anonymous reviewers are also much appreciated.

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Corresponding Editor: J. Mallet