Adaptation or random change? The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: *Chrysoperla*)

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Green lacewings of the *carnea* group of *Chrysoperla* constitute a large complex of cryptic species that differ principally in their substrate-borne, vibrational mating signals. Sexual selection has very likely contributed to their recent and rapid divergence. Some theoretical models require that evolutionary environmental adaptation assist sexual selection during speciation, whereas others do not. Here, we looked for evidence of adaptation having shaped the song phenotypes of lacewing species that have different habitat preferences. First, we looked for a pattern of convergent song evolution, predicted under a hypothesis of substrate adaptation during speciation. Focusing on species confined to conifers versus those on herbaceous vegetation, we found several examples where song phenotype showed a correlation with substrate that was independent of phylogeny. We also present results of experimental tests of environmental adaptation using *C. downesi* and *C. plorabunda*, which are conifer and meadow species, respectively. Experiments addressing purely biomechanical aspects of substrate transmission showed that the songs of *C. downesi* and *C. plorabunda* propagated equally well in either grass stems or conifer branches. Behavioural experiments showed, furthermore, that individuals did not discriminate between conspecific songs played through the 'correct' versus the 'incorrect' substrate. We conclude that the pattern suggestive of environmental adaptation of lacewing songs to their substrates was not supported by experiments that explicitly tested for adaptation. The *carnea* group of cryptic species may be one of the few examples where sexual selection has acted on arbitrary differences in premating signals, resulting in speciation.

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Mating signals comprise the specific mate recognition systems of animals. As such, they contribute significantly to reproductive isolation between closely related taxa (Mayr 1963). Changes to mating signals often accompany or precede species divergence, and may even play a causal role in the process of speciation. For example, recent theoretical and empirical work strongly suggests that sexual selection is capable of driving the divergence of mating signals between populations. When those signals become sufficiently different, assortative mating will cut off gene flow completely, resulting in speciation (Lande 1981, 1982; West-Eberhard 1983; Pomiankowski & Iwasa 1993; Ryan & Rand 1993; Turner & Burrows 1995; Kondrashov & Shpak 1998; Higashi et al. 1999; Seehausen

& van Alphen 1999; Gavrilets 2000; Gray & Cade 2000; Gavrilets et al. 2001; Panhuis et al. 2001).

As first noted by Darwin (1871), sexual selection should be capable of acting on any arbitrary differences in mating signals between populations. Fisher (1958) and others then showed that runaway sexual selection can rapidly augment those differences, provided that a strong genetic correlation exists between the signal (usually produced by the male) and the response to that signal (by the female). Indeed, recent theoretical work suggests that speciation can result from sexual selection acting on arbitrary differences in mating signals and on correlated preferences for those signals (Wu 1985; Turner & Burrows 1995; Higashi et al. 1999; Takimoto et al. 2000; Danley & Kocher 2001; Gavrilets et al. 2001; Takimoto 2002). However, mating signals are often tailored by evolution to the physical characteristics of the environments through which they travel (Endler 1993; Marchetti 1993; Boughman 2002). For example, display colours of fish may be optimized for

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transmission through water of different depths or turbidity (Seehausen et al. 1997, 1999). Similarly, vibrational signals of insects usually include a broad range of frequencies, to ensure that at least some information survives the filtering effect of a plant stem (Bradbury & Vehrencamp 1998). In such cases, the differences between the mating signals of closely related sibling species are not neutral, but instead represent environmental adaptations. Furthermore, there is little doubt that adaptations associated with improving the efficacy of communication (defined by Endler 2000, page 12) have contributed importantly to species divergence in some animal groups (e.g. sexually selected cichlid fish of Africa and Central America: Kondrashov & Kondrashov 1999; Seehausen & van Alphen 1999; Wilson et al. 2000; Schliewen et al. 2001; stickleback fish: Boughman 2001; bats: Jones 1997; Kingston et al. 2001; some birds: Price 1998). Therefore, according to some evolutionary biologists, sexual selection can drive speciation only when it is accompanied by traditional natural selection and environmental adaptation; in other words, when it is a part of 'ecological speciation' (Hatfield & Schluter 1999; Sætre 2000).

The substrate-borne, vibrational songs of green lacewings provide an excellent opportunity to study environmental evolutionary adaptation of mating signals in a group where sexual selection has contributed to speciation. The globally distributed *carnea* species group of *Chrysoperla* comprises at least 15 morphologically similar, closely related species that are reproductively isolated from one another principally by genetically encoded differences in their tremulation songs (Henry et al. 1999b, and references therein). In each cryptic species, both males and females sing the same song, and must duet with one another (which requires that their songs be nearly identical) before copulation will occur. Members of the complex have been shown to meet the prerequisites of sexually selected speciation, and to show all patterns expected to result from such a process (Gray & Cade 2000; Panhuis et al. 2001; Henry et al. 2002b). The numerous cryptic species across Eurasia and North America also show a broad diversity of song phenotypes and habitat preferences, such that there is considerable potential for many distinctly different types of biomechanical interactions between songs and substrates. One clear example is the strong association of several song species of the *carnea* group with conifers, while others are confined to herbaceous vegetation and grasses (Fig. 1).

To show that adaptation has occurred in a feature, one can confirm the existence of a pattern predicted from a hypothesis of adaptation, or perform experiments to demonstrate increases in efficacy (efficiency plus effectiveness) that result from changes in that feature. A pattern commonly resulting from environmental adaptation is evolutionary convergence. In lacewings, if songs indeed have adaptive properties, one would expect to find convergence of song phenotypes between species that share similar habitat preferences (substrate types) regardless of whether they inherited their similar songs from common ancestors (Henry et al. 1999b). Experiments testing for environmental adaptation can take either a biomechanical or behavioural approach. A strictly

biomechanical experiment might document losses of fidelity (efficiency) of a lacewing song as it travels predetermined distances through different substrates from the point of origin. Losses of fidelity would include overall amplitude attenuation, changes in amplitude (temporal) structure, and changes in frequency (spectral) characteristics. On the other hand, a behavioural experiment (testing effectiveness) might count the number of songs given by individual lacewings in response to playbacks of conspecific songs that had been recorded after passing through alternative substrates.

In this study, we used both patterns and experiments to test for the presence of environmental adaptation in the evolution of songs and origin of song species within the *carnea* group of green lacewings. First, we examined the patterns of association between song phenotype and substrate properties, which we interpreted within an existing phylogenetic framework for 15 described members of the complex. We predicted that adaptive responses of signals to substrate properties should produce more similar songs between species sharing one substrate type (e.g. only conifers) than between species found on different substrates (conifers versus grasses). Second, we analysed and compared the degradation of signal fidelity during substrate propagation in two species that showed distinct habitat preferences, meadow-associated *C. plorabunda* Henry, Wells & Pupedis, and conifer-associated *C. downesi* (Smith). We predicted that adaptive responses of songs to specific habitats should result in higher fidelity of a given song type in its 'correct' (native) substrate, compared to its fidelity in some 'incorrect' (non-native) substrate. Lastly, using playback of substrate-modified songs to individuals of *C. plorabunda* and *C. downesi*, we tested the behavioural preferences of each of the two species for songs propagated in native versus non-native substrates. We predicted that adaptive responses of signals to substrate properties should result in more responses by lacewings to songs transmitted through their own substrates than to songs transmitted through other substrates.

METHODS

Survey of Song Phenotypes versus Substrate Properties

The songs of 15 song species of the *carnea* group from around the world have been partially or fully characterized (Henry et al. 2001, and references therein). Taxa of special significance to the study, from central and eastern Asia, were collected by Peter Duelli (Swiss Federal Research Institute WSL, Birmensdorf, Switzerland) in Kyrgyzstan (May–June 1995) and near Beijing, China (September 1995).

Habitat preferences have been ascertained for most of the song species. We compared songs of species associated with conifers to those associated with herbaceous vegetation, under the assumption that conifers and leafy plants provide very different acoustical substrates to singing lacewings (Fig. 1). We assessed similarity of song phenotypes between taxa qualitatively, as well as by a principal
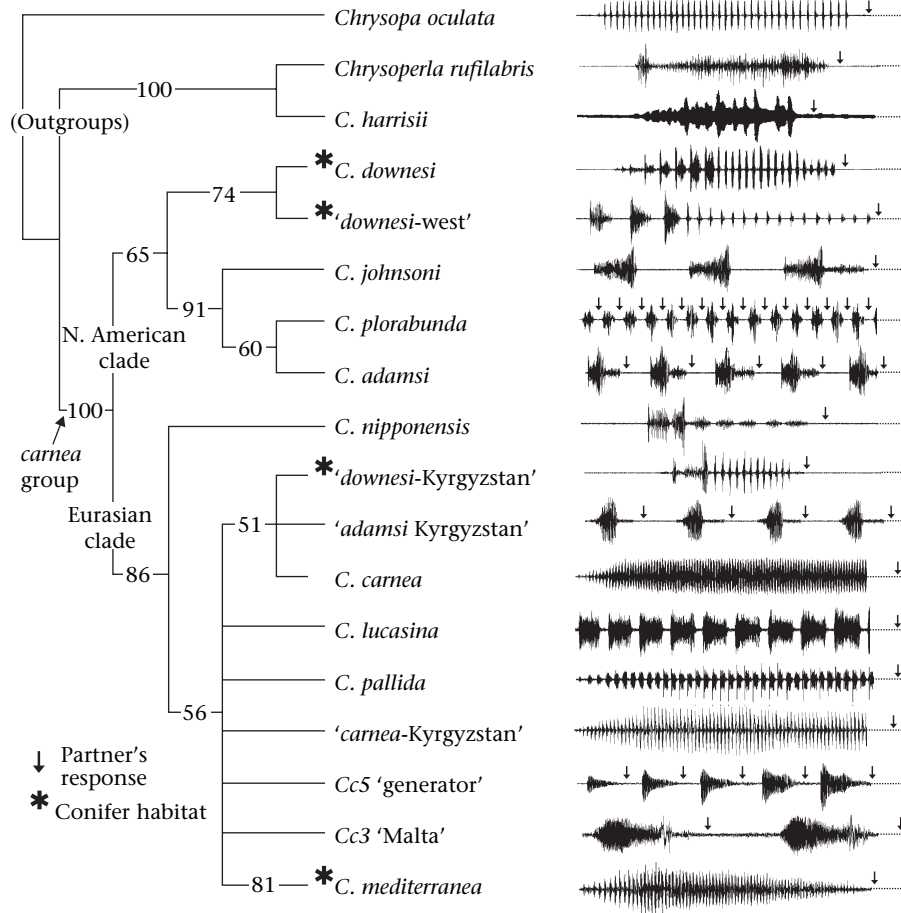
Figure 1. Maximum parsimony bootstrap phylogeny (50% majority rule) of 15 song species of green lacewings of the *carnea* group of *Chrysoperla* plus three outgroup taxa, inferred from combined DNA nucleotide data from sections of the ND2 (552 bp) and COII (516 bp) mitochondrial genes. Numbers at the nodes are bootstrap values. A 12-s oscillograph is shown for the song of each species; vertical arrows indicate where the partner would sing during a duet. *Species within the *carnea* group that are associated with coniferous evergreens.

components analysis of 18 song features measured in 12 song species. The contribution of evolutionary history to song phenotype was determined from an existing phylogenetic tree based on mitochondrial DNA sequences (Wells & Henry 1998; Henry et al. 1999b).

## Comparative Song Fidelity during Substrate Transmission

We selected two sympatric but ecologically distinct North American lacewing species, *C. downesi* and *C. plorabunda*, for comparison of song transmission through different substrates. All measurements described below were taken from songs recorded at $25 \pm 1°C$.

Chrysoperla downesi lives among the branches of coniferous evergreen trees such as hemlock, spruce, fir and pine, often in dense forests, and sings on terminal twigs and needles. Its mating signal is long ($\overline{X} \pm SD = 6.1 \pm 1.6$ s, $N = 13$) and relatively complex, consisting of several long volleys of abdominal vibration followed by a series of closely spaced short volleys (Fig. 2; Henry 1980b). This multivolley signal constitutes the 'shortest repeated unit' or SRU of the species, meaning that it is the shortest unit

of exchange between dueting individuals (arrows in Figs 1, 2). Carrier frequency of the SRU, representing the actual rate of oscillation of the insect's abdomen, is upwardly modulated within each long volley from approximately 68 Hz to 95 Hz, while the short volleys average 71 Hz (Fig. 5).

In contrast, *C. plorabunda* occupies herbaceous vegetation in meadows and at the edges of fields, and commonly sings on the long stems of grasses (Henry & Wells 1990b). The SRU of its song consists of just one short ($\overline{X} \pm SD = 0.63 \pm 0.08$ s, $N = 23$) volley of abdominal vibration, repeated approximately once every 1.2 s (the dueting partner times its SRUs to fall in the short silent spaces; see Fig. 2; Henry 1983). Carrier frequency is downwardly modulated, from about 87 Hz to 41 Hz (Fig. 6).

We recorded one song from two individuals of each species at 25°C. These four recordings were used as 'source' signals in all experiments. Two exemplars from each species were considered sufficient because song phenotype shows little intraspecific variation in either species (e.g. Henry 1980b; Henry & Wells 1990a). Recordings were made on cassette tape (Dolby off) from a plastic membrane stretched loosely over the top of a paper cup
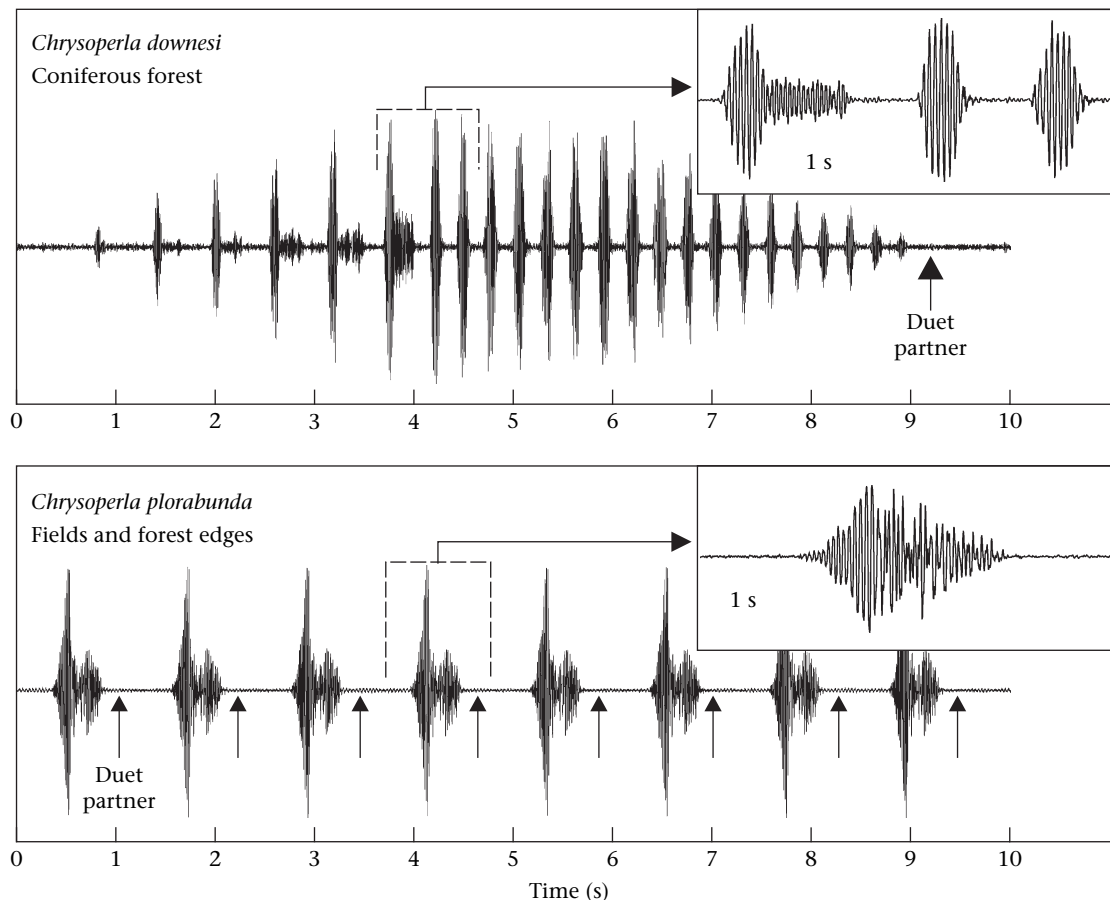
Figure 2. Oscillographs of the songs of *Chrysoperla downesi* and *C. plorabunda*, with 1-s details of each 10-s song segment magnified in the boxes. Vertical arrows indicate where the partner would sing during a duet. The SRU (shortest repeated unit) includes the signal between two arrows.

arena, using a compliant piezoelectric transducer touching the membrane (for details see Henry 1979, 1980a). All source signals were passed through an analogue filter, such that frequencies above 150 Hz were attenuated at 6 dB per octave. This effectively created a 'pure' stimulus, by removing any spurious harmonics that might have been added to the signal by the plastic membrane. Filtered signals were then digitized with 16-bit resolution at 20 kHz and normalized to a standard amplitude of ± 5 V (peak to peak) for playback from a computer. For *C. downesi*, the two source signals were recorded from two individuals, D1 and D2, collected in August 1987 at the Huyck Nature Preserve, Rensselaerville, New York, U.S.A. For *C. plorabunda*, source signals came from one individual (P1) collected in September 1987 in Storrs, Connecticut, U.S.A., and another (P2) collected in October 1989 at the Huyck Nature Preserve. Measured features of the four source signals are shown in Table 1.

Table 1. Measurements of major features of songs chosen as source (control) signals in bioacoustic and behavioural experiments

	Frequency measures (Hz)				Time measures (ms)				SRU features		
	Long (or only) volleys			Short volleys	Long volleys		Short volleys			Number of volleys	
	Start	Middle	End	Middle	Duration	Period	Duration	Period	Total duration (s)	Long	Short
D1	64.05	78.96	92.83	73.92	423.88	615.73	136.53	293.13	7.87	6	15
D2	71.72	82.52	95.37	75.55	383.33	549.09	123.12	240.85	8.29	7	20
P1	88.58	65.18	39.55	—	540.50	—	—	—	0.541	1	—
P2	77.49	59.87	38.13	—	589.20	—	—	—	0.589	1	—

SRU: shortest repeated unit.
D1 and D2 are different individuals of *Chrysoperla downesi*; P1 and P2 are different individuals of *C. plorabunda* (see text).

We selected two samples of each substrate type, grass versus conifer, for the experiments. Each was a freshly cut plant fragment 50–60 cm long, a length chosen because it approximated the average transmission range of lacewing signals under natural conditions (Henry & Wells 1990b). Grass substrates, Gr1 and Gr2, were green stems and attached leaf blades of a local perennial meadow grass (undetermined species). Conifer substrates, Hm1 and Hm2, were terminal portions of fresh lateral branchlets from eastern hemlock, *Tsuga canadensis*. Each substrate was anchored at its base and oriented horizontally from the point of attachment. Using an electronic shaker (model 132-203, Labworks Inc., Costa Mesa, California, U.S.A.), digitized source signals were introduced at constant reference amplitude into the substrate at one point (the origin) and then re-recorded using an accelerometer (mass = 2.3 g, model 352B65, PCB Piezotronics, Depew, New York, U.S.A.) at three sites away from the origin (Fig. 3). The three sites were defined as the 'midpoint' (30 cm from the origin for grass, 20 cm from the origin for hemlock), the 'tip' (50 cm for grass, 40 cm for hemlock), and the 'side leaf or branch' (5 cm from the base of a lateral leaf or branch emerging 10 cm from the origin). Changes at those positions to both the amplitude and spectral properties of the song waveform were then measured or described (Figs 4–6; Table 2) using Spike2 software (Smith 2001). Prior to analysis, all recordings were digitized at a high sampling rate (20 kHz, 16-bit resolution) without filtering, to retain all spectral information between 1 Hz and 9.5 kHz. Advertised frequency response of the shaker was 0–11 kHz $\pm$ 3 dB; that of the accelerometer was 0.5 Hz–10 kHz $\pm$ 3 dB.

We determined the effect of substrate on the amplitude structure of song waveforms from signals recorded without electronic manipulation. We calculated amplitude range, expressed as an alternating ($\pm$) voltage, from the largest peak-to-peak voltage excursion found between any two adjacent cycles within the signal (Table 2). This method eliminates spurious effects of low-frequency noise on global maxima and minima, and discounts 'spikes' within single waveform cycles. We documented changes to spectral (frequency) characteristics from sonagraphs of recordings that had been normalized to an amplitude range of $\pm$5 V using a simple Spike2 script. For analytical purposes, a sonagraph was preferred over a power spectrum applied to the same time period, because sonagraphs resolve transient and potentially meaningful changes in frequency that are simply averaged by power spectra.

Behavioural Responses to Substrate-modified Songs

We tested the behavioural responses of females of *C. downesi* and *C. plorabunda* to playbacks of control songs paired with songs modified by transmission through both types of substrates. The goal was to determine whether lacewings with species-specific substrate preferences would respond better to their own songs propagated through their own substrate than to their own songs propagated through the other substrate.

Trials were conducted on 12 individuals of *C. downesi* and 10 of *C. plorabunda*. In every case, we verified species identity using both morphology and song phenotype (Bram & Bickley 1963; Henry 1979, 1993). All *C. downesi* were from a hemlock forest near Meriden, Connecticut and included four females collected on 6 July 1999, and eight offspring reared from gravid females collected on 10 May and 6 July 1999. Eight of the *C. plorabunda* test insects were purchased as larvae from The Green Spot Ltd, Nottingham, New Hampshire, U.S.A., and eclosed as adults in early July 1999. Their geographical origin was not known. The remaining two individuals of *C. plorabunda* were collected at lights on 5 July and 8 July 1999 in Storrs, Connecticut. We used the same recordings of
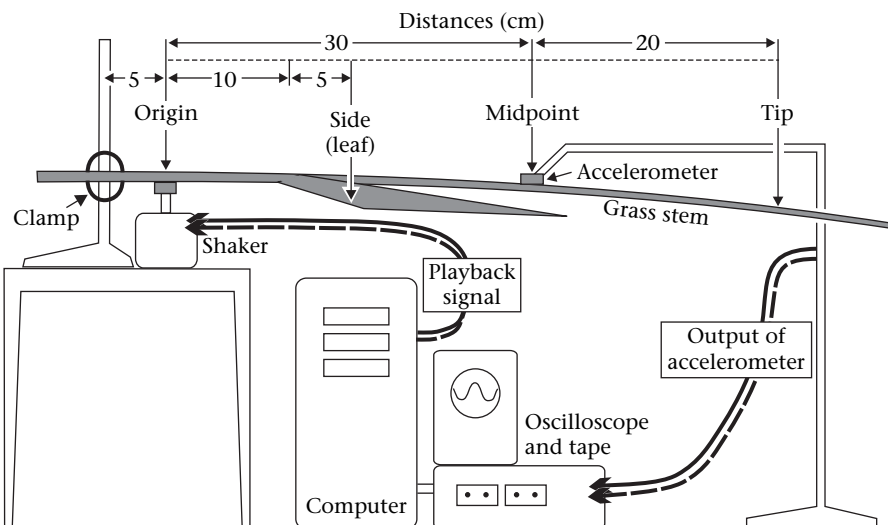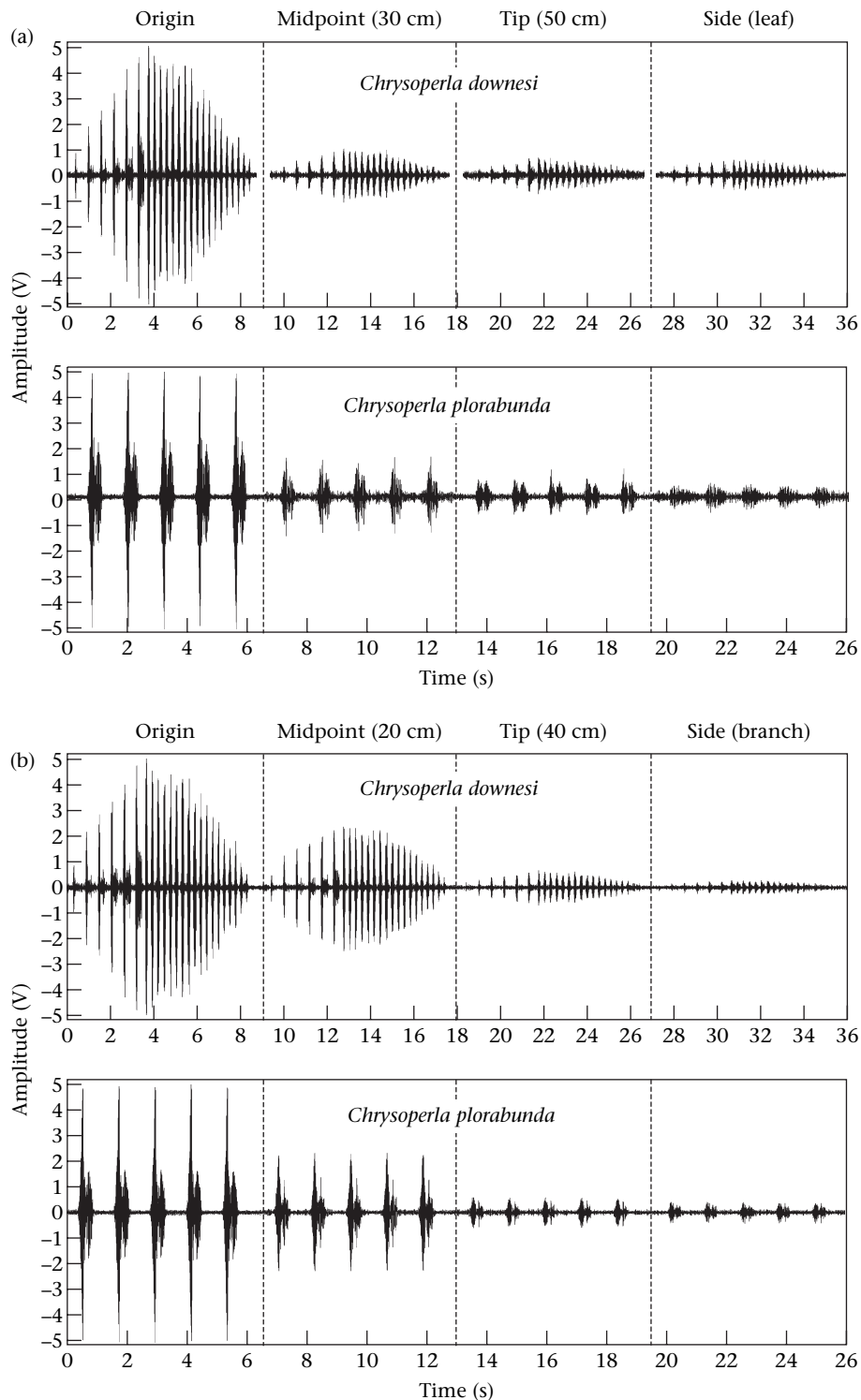

Figure 3. Diagrammatic view of the experimental set-up, illustrating a grass blade as the substrate for vibrational signals. A hemlock branchlet was configured similarly for other experiments.
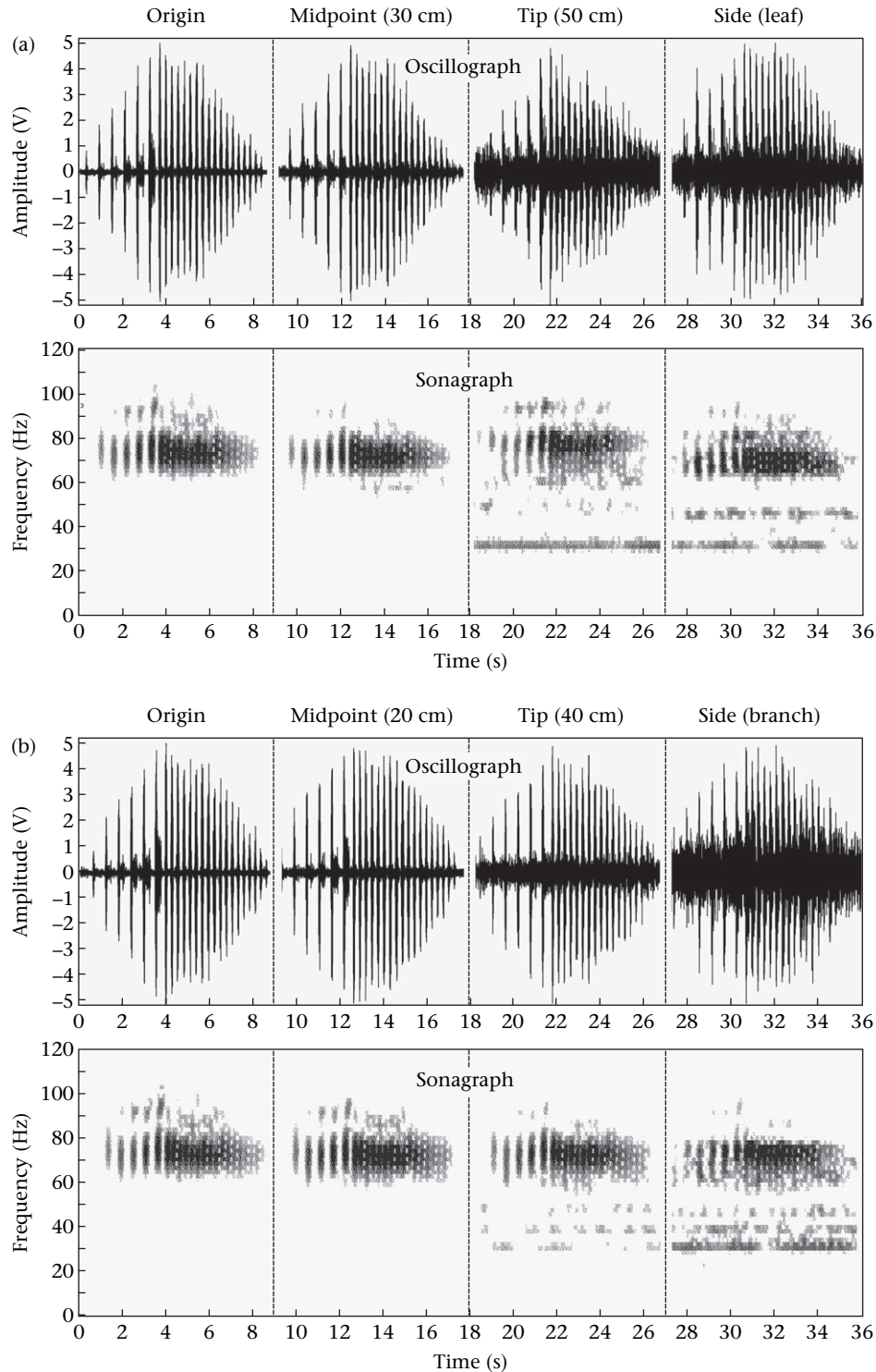
Figure 4. Oscillographs illustrating amplitude attenuation of the mating signals of *Chrysoperla downesi* and *C. plorabunda* in grass (a) and conifer (b) substrates (see text and Tables 1, 2 for details). All source signals (origin) were standardized to ± 5 V. Shown are the results for individuals D1 (*downesi*) and P1 (*plorabunda*) in substrates Gr1 (grass) and Hm1 (hemlock). Note that the 'midpoint' and 'tip' were 10 cm further from the origin in grass compared with hemlock.

source signals and substrate-modified signals that had been used in the song fidelity experiments (see above). Most trials were conducted on substrate samples Gr1 (grass) and Hm1 (hemlock), also from the song fidelity experiments.

Each experimental animal was placed atop the electronic shaker in a 7-dram (7 cm long, 2-cm diameter) cotton-stopped glass vial, at 25 ± 1 °C. It was then exposed to playback of a control signal and a stimulus signal in a paired design (Wells & Henry 1992). The

Figure 5. Noise and spectral changes typical of the mating signals of *Chrysoperla downesi* in grass (a) and conifer (b) substrates (see text and Tables 1, 2 for details). All source signals (origin) were standardized to ±5 V; all re-recordings of those signals from substrates were normalized to ±5 V to show the changes clearly. Upper and lower graphs for each substrate are oscillographs and sonagraphs, respectively. Shown are the results for individual D1 in substrates Gr1 (grass) and Hm1 (hemlock).

stimulus signal was the substrate-modified recording, whereas the control signal always consisted of the unmodified source signal used to produce that particular stimulus. All playback signals were conspecific with

respect to the insect being tested and consisted of a single, complete SRU. The tendency of the test animal to duet with the playback song was assessed by counting the number of correct responses (SRUs) the insect made
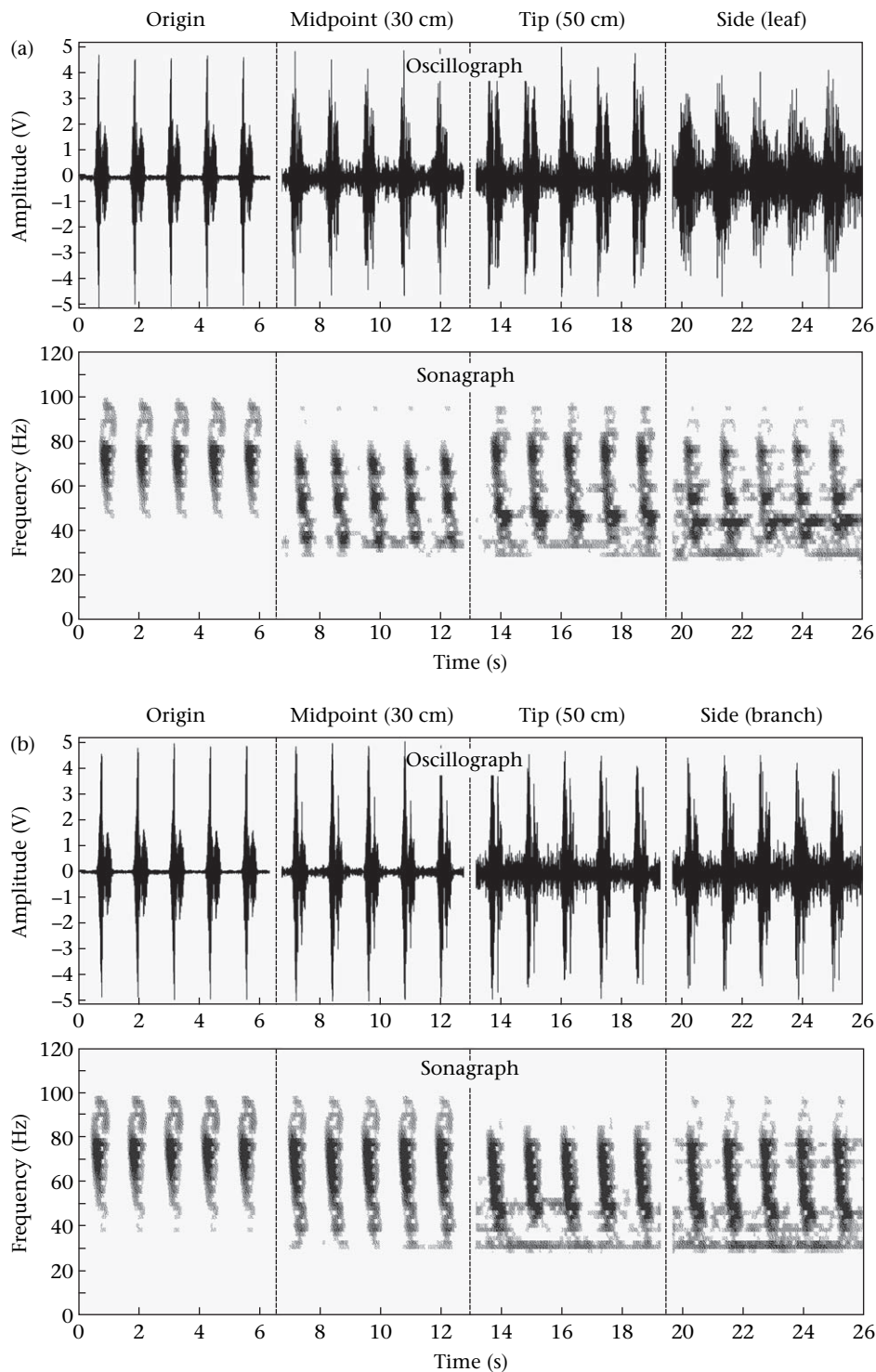
Figure 6. Noise and spectral changes typical of the mating signals of *Chrysoperla plorabunda* in grass (a) and conifer (b) substrates (see text and Tables 1, 2 for details). All source signals (origin) were standardized to ±5 V; all re-recordings of those signals from substrates were normalized to ±5 V to show the changes clearly. Upper and lower graphs for each substrate are oscillographs and sonagraphs, respectively. Shown are the results for individual P1 in substrates Gr1 (grass) and Hm1 (hemlock).

to 10 (*C. downesi*) or 20 (*C. plorabunda*) replicates of each signal (stimulus or control). Each replicate was presented either immediately after a positive response by an insect, or after a 5-s pause if the insect gave no response. We were careful to wait until an individual stopped tremulating before exposing it to a new signal. To separate the effect of the substrate on sound 'quality' from its simple attenuating effect on amplitude, all signals were played

Table 2. Amplitude of a reference signal introduced at ± 5 V into a substrate at one point and re-recorded at different distances from that point (see text)

		C. downesi		C. plorabunda	
		D1	D2	P1	P2
Grass substrate					
Midpoint (30 cm)	Gr1	± 1.013	± 1.226	± 1.498	± 3.740
	Gr2	± 2.245	± 2.090	± 2.180	± 4.750
Tip (50 cm)	Gr1	± 0.625	± 0.689	± 0.664	± 1.950
Side (leaf)	Gr1	± 0.557	± 0.314	± 0.558	± 1.128
Conifer substrate					
Midpoint (20 cm)	Hm1	± 2.389	± 2.356	± 2.258	± 3.215
	Hm2	± 4.630	± 4.000	± 4.200	± 4.260
Tip (40 cm)	Hm1	± 0.642	± 0.545	± 0.584	± 1.503
Side (branch)	Hm1	± 0.277	± 0.313	± 0.371	± 1.122

The vibrational song of two individuals from each of two lacewing species, *Chrysoperla downesi* (individuals D1 and D2) and *C. plorabunda* (P1 and P2), was tested on two samples of each of two different substrates, a grass stem (samples Gr1 and Gr2) and a conifer branchlet (Hm1 and Hm2).

back at a standard reference volume, achieved by normalizing the waveform to ± 5 V (as described above) and maintaining one volume setting on the integrated amplifier. Amplification level was chosen to guarantee that a lacewing could always 'hear' the playback signals. Therefore, if an insect did not answer, we were sure that it did not like the signal being played. Evidence that insects were responding to signal quality rather than loudness comes from our unpublished observations showing that increasing the playback volume does not increase the responsiveness of lacewings to incorrect (e.g. nonconspecific) or undesirable signals. We were not able to investigate the quantitative effect of progressive amplitude reduction on lacewing responses.

For each species, we prepared four three-part series of tests (Table 3, Fig. 7). Each test consisted of two pairs of trials, administered in close succession. In the first pair of trials, the stimulus and control were presented in the order dictated by a coin toss. The order of stimulus and control was then reversed in the second pair of trials. Each series consisted of three such tests, associated with the stimulus signals recorded at the (1) midpoint, (2) tip and (3) side leaf/branch of one substrate sample (see above). Order of presentation of the three tests in each series was randomized, but the tests were administered in close succession. A different series of tests was created from each of the two source signals and for each of the two different substrate types, resulting in four series per species. A 5-min pause was enforced between series. With four trials per test, three tests per series, and four series per species, the target for each individual was a minimum of 48 trials (Table 3, Fig. 7).

We used the mean number of responses to recordings from each substrate type given by each individual to a specific type of test as a single data point in our analysis. For all experiments, main effects and factorial ANOVAs were performed on the response variable to analyse the effect and interactions of the two categorical variables 'substrate' and 'site'. Statistica v. 6.0 StatSoft (2001) served as the platform for all analyses.

RESULTS

Survey of Song Phenotypes versus Substrate Properties

We placed oscillographs of the vibrational songs of 15 members of the *carnea* group on a cladogram of phylogenetic relationships (Fig. 1, based on data in Henry et al. 1999b). We distinguished conifer-associated species (e.g. *C. downesi*, *C. 'downesi*-west', *C. 'downesi*-Kyrgyzstan') from those that occupied meadows, shrubs, or deciduous woodlands by their dark green coloration, which makes them inconspicuous against evergreen foliage (Tauber & Tauber 1981). Another dark green lacewing, *C. mediterranea* (Hölzel), from southern Europe, was also associated with coniferous habitats (Henry et al. 1999a).

There were qualitative phenotypic similarities between the songs of the three conifer-associated '*downesi*'-type species (Figs 1, 8). All produced long, multivolley SRUs, consisting of one or several long volleys followed by a series of closely spaced short (≤ 0.30 s) volleys. They also shared basic spectral characteristics (e.g. relatively high carrier frequencies of 70–100 Hz) that either remained constant or increased during each volley (Fig. 8; C. S. Henry, unpublished data). Phylogenetically, the two '*C. downesi*' species from North America were sister species, but '*downesi*-Kyrgyzstan' belonged to a different continental clade (Eurasian rather than North American, see Henry et al. 1999b).

The songs of *C. downesi* (North America) and *C. mediterranea* (Europe) also resembled one another, as indicated by close clustering of those two species in the principal components analysis of song features in 12 taxa (Fig. 9). Unlike *C. downesi*, however, *C. mediterranea* produced only short, closely spaced volleys (Fig. 8). In that respect, it more closely resembled *C. carnea* (Stephens), *C. pallida* Henry, Wells & Pupedis and '*carnea*-Kyrgyzstan', none of which was found in association with conifers.

One other east Asian species, tentatively identified as *C. nipponensis* (Okamoto), had a song consisting of two

Table 3. Number of responses of individuals of *Chrysoperla downesi* and *C. plorabunda* to 10 (*downesi*) or 20 (*plorabunda*) playbacks of songs of two different conspecific individuals recorded after transmission to different sites in grass versus conifer substrates

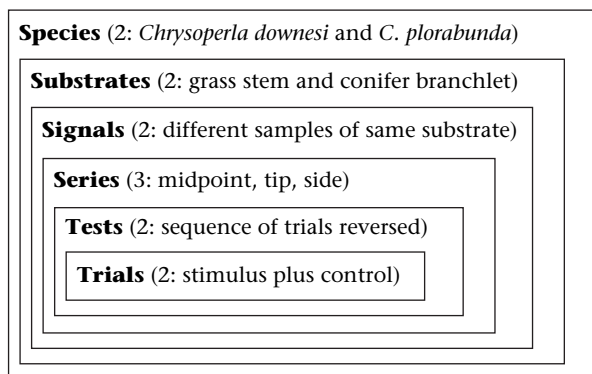| Individual	Origin (0 cm) Grass	Origin (0 cm) Conifer	Midpoint (30 or 20 cm) Grass	Midpoint (30 or 20 cm) Conifer	Tip (50 or 40 cm) Grass	Tip (50 or 40 cm) Conifer	Side (leaf or branch) Grass	Side (leaf or branch) Conifer
C. downesi*								
1	9.00	10.00	10.00	6.25	9.75	9.00	2.75	5.00
2	9.25	9.33	9.25	9.00	9.25	9.00	7.00	2.50
3	9.75	10.00	9.00	9.00	9.75	9.00	10.00	8.50
4	9.50	7.83	8.50	9.00	7.50	4.50	4.50	6.25
5	9.79	9.75	10.00	10.00	9.50	10.00	7.75	10.00
6	10.00	5.50	10.00	9.50	9.75	7.50	5.00	4.00
7	9.33	9.25	6.25	10.00	10.00	7.75	5.00	8.00
8	10.00	8.00	8.50	10.00	10.00	5.00	8.00	10.00
9	10.00	10.00	10.00	10.00	10.00	10.00	10.00	9.00
10	10.00	9.50	10.00	10.00	10.00	10.00	10.00	10.00
11	10.00	10.00	10.00	—	—	10.00	—	—
12	—	10.00	10.00	—	—	—	—	—
N	11	12	12	10	10	11	10	10
$\overline{X}$	9.69	9.10	9.29	9.28	9.55	8.34	7.00	7.33
C. plorabunda†								
1	18.67	16.33	19.50	18.50	17.50	18.00	18.50	12.50
2	16.33	17.83	15.00	17.50	16.00	17.00	16.00	17.00
3	12.88	9.40	18.00	18.00	17.00	5.50	15.75	15.50
4	13.00	17.00	13.00	15.50	13.50	9.50	1.00	6.00
5	16.50	13.50	17.00	18.50	15.00	12.33	7.50	1.50
6	20.00	16.50	14.00	4.67	16.50	7.00	10.67	9.50
7	17.00	18.50	17.00	8.00	17.00	16.00	15.00	12.00
8	18.00	17.50	16.00	14.50	9.00	15.00	4.00	5.50
9	18.00	18.00	14.50	17.50	9.50	11.50	15.50	9.67
10	—	14.50	—	12.00	—	—	—	—
N	9	10	9	10	9	9	9	9
$\overline{X}$	16.71	15.91	16.00	14.47	14.56	12.43	11.55	9.91

Values are averages of at least four trials per individual per site per substrate (two songs, two replicates).
*Source signals D1 and D2, substrates Gr1 and Hm1.
†Source signals P1 and P2, substrates Gr1 and Hm1.

distinct volley types, as in the three '*downesi*'-type species (Figs 1, 8). However, this species was collected in meadows and croplands, not coniferous forests (P. Duelli, unpublished data). Compared with the conifer-associated

species, the 'short' volleys of *C. nipponensis* were actually rather long, with a correspondingly slower repetition rate. A more striking difference was the presence of carrier frequencies that were both much lower (second long volley, Fig. 8) and significantly higher (all short volleys) than those found in the songs of the conifer dwellers.

Comparative Song Fidelity during Substrate Transmission

Amplitude attenuation

Measured amplitude attenuation varied substantially between the two source signals (from different individuals) selected to represent each species, and between the two samples of each substrate. Although such variation could not be treated statistically, certain patterns were evident. In general, source signals showed a progressive loss of amplitude with increasing distance from the origin (Figs 4, 10). However, even at the midpoint of either type of substrate, signals of both species could remain nearly as 'loud' as they were at the origin (e.g. P1 in Hm2, P2 in Gr2 and Hm2, D1 and D2 in Hm1; see Table 2). Transmission
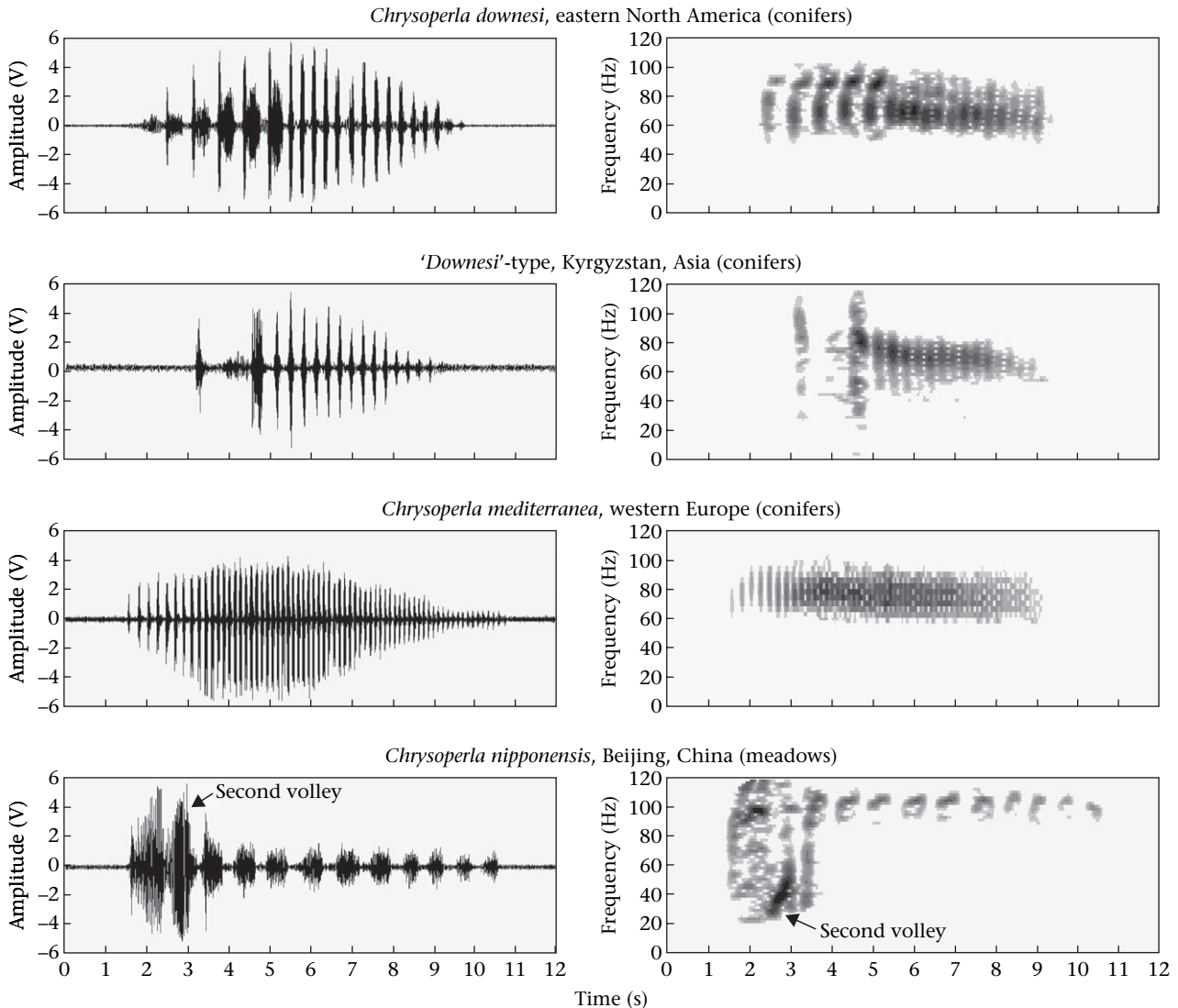

Figure 7. Diagrammatic summary of the protocol used in the behaviour experiments testing responsiveness of individuals of *C. downesi* and *C. plorabunda* to their own songs propagated through grass versus conifer substrates.

Species (2: *Chrysoperla downesi* and *C. plorabunda*)
Substrates (2: grass stem and conifer branchlet)
Signals (2: different samples of same substrate)
Series (3: midpoint, tip, side)
Tests (2: sequence of trials reversed)
Trials (2: stimulus plus control)

Figure 8. Oscillographs (left) and sonagraphs (right) of songs of selected lacewing species that showed a pattern of possible convergent evolution. The first three species (from top of figure) were associated with coniferous forests. The song of *C. nipponensis* (bottom of figure) showed some phenotypic similarity to those of the first three, but *C. nipponensis* was not found on coniferous vegetation. Note the unusually low carrier frequency of the second volley of abdominal vibration in the song of *C. nipponensis*.

was also good out to the tips of all substrates, such that all signals retained their distinctive amplitude structure 40–50 cm from the origin. Degree of attenuation with distance differed more between the two samples of *C. plorabunda* than between the two samples of *C. downesi* (Fig. 10). One song sample, that of individual P2, propagated more strongly in both grass and hemlock than the songs recorded from any other individual (Fig. 10). Signals of either species recorded from a side leaf (grass) or side branch (hemlock) generally showed as much attenuation as signals that had travelled all the way to the tip of the main stem or primary branch axis (Figs 4, 10, Table 2).

Spectral changes

Frequency spectra (sonagraphs) of recorded signals did not change as distance from the source signal increased (Figs 5, 6). This was true of both species in either substrate. All frequencies present in the original signals remained present at all recording sites in all substrates; in other words, the basic acoustical signature of lacewing signals remained unchanged by substrate transmission. Level and type of background noise remained approximately constant across all recordings, such that signal-to-noise ratio progressively decreased as song amplitude decreased.

Behavioural Responses to Substrate-modified Songs

Individuals of both *C. downesi* and *C. plorabunda* were highly responsive to substrate-modified signals (Table 3, Figs 11, 12). In *C. downesi*, responsiveness relative to the source signal (excluding 'side' leaf/branch sites) declined
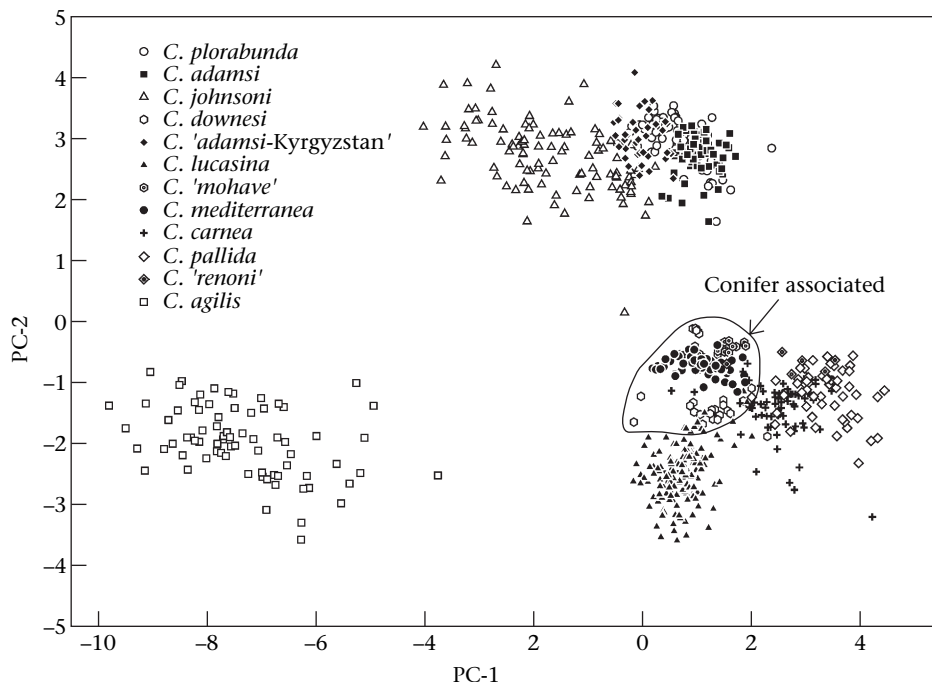
Figure 9. Scatterplot of the first two principal components (PC-1 and PC-2) of a principal components analysis of 18 song features measured in 12 distinct song species of the *carnea* group. Each data point represents a single individual, coded by species. Together, PC-1 and PC-2 accounted for 74.64% of total variance. A polygon is drawn to surround the values plotted for three song taxa typically associated with conifers: *Chrysoperla downesi*, *C.* '*mohave*' (an ecotype of *C. downesi* from southwestern U.S.A.) and *C. mediterranea*.

by maxima of only 4% for grass and 8% for conifer. Corresponding declines in *C. plorabunda* measured 13% (grass) and 22% (conifer). Both species were least responsive to signals recorded from 'side' leaf/branch sites; even so, responsiveness relative to the source declined by only 28% and 19% in *C. downesi* (grass and conifer, respectively) and by 31% and 38% in *C. plorabunda* (also grass and conifer). Those results suggested that (1) both species usually preferred signals that had been transmitted through grass rather than conifer substrates, and (2) *C. plorabunda* was generally less responsive than *C. downesi* to all substrate-modified signals. Despite such qualitative tendencies in the data, none of the observed differences in lacewing responsiveness to different types of substrates was statistically significant (Table 4).

DISCUSSION

Within the 15 cryptic song species of the *carnea* group, there are several examples of convergent song evolution where substrate preference correlates well with song phenotype. This pattern would suggest that environmental adaptation plays an important role in shaping signal evolution and driving species divergence within this complex. However, experimental tests for environmental adaptation of songs did not support the hypothesis. First, study of the biomechanics of the signals of lacewings associated with meadows versus conifers indicated that transmission of a signal in the correct substrate was no more efficient than in the incorrect substrate. Second, behavioural experiments showed that lacewing species

with different substrate preferences did not discriminate between songs that had travelled through correct versus incorrect media. These findings are discussed in more detail below, together with their bearing on sexual selection and speciation in the species complex.

Patterns Predicted from a Hypothesis of Adaptation

Four of 15 song species were associated with coniferous evergreen trees (Figs 1, 8). Three of these had generally similar '*downesi*'-type songs distinguished by long SRUs and two volley types, while the fourth, *C. mediterranea*, clustered closely with *C. downesi* in a principal component analysis that used all song features. Two of the '*downesi*'-type species, *C. downesi* and '*downesi*-west', are the closest relatives in North America and must have inherited their similar songs from a common ancestor (Fig. 1). However, '*downesi*-Kyrgyzstan' is from Asia, belongs to a different major clade of the *carnea* group, and seems to have acquired its similar song through convergent evolution. Because it is also associated with conifers, one could reasonably interpret the *downesi*-like song of '*downesi*-Kyrgyzstan' as an adaptive response to the bioacoustic properties of conifer substrates. The same could also be said of European *C. mediterranea*: the song characteristics that it shares with North American *C. downesi* are more likely due to evolutionary convergence than common ancestry, and coniferous substrates may be the selective agent.
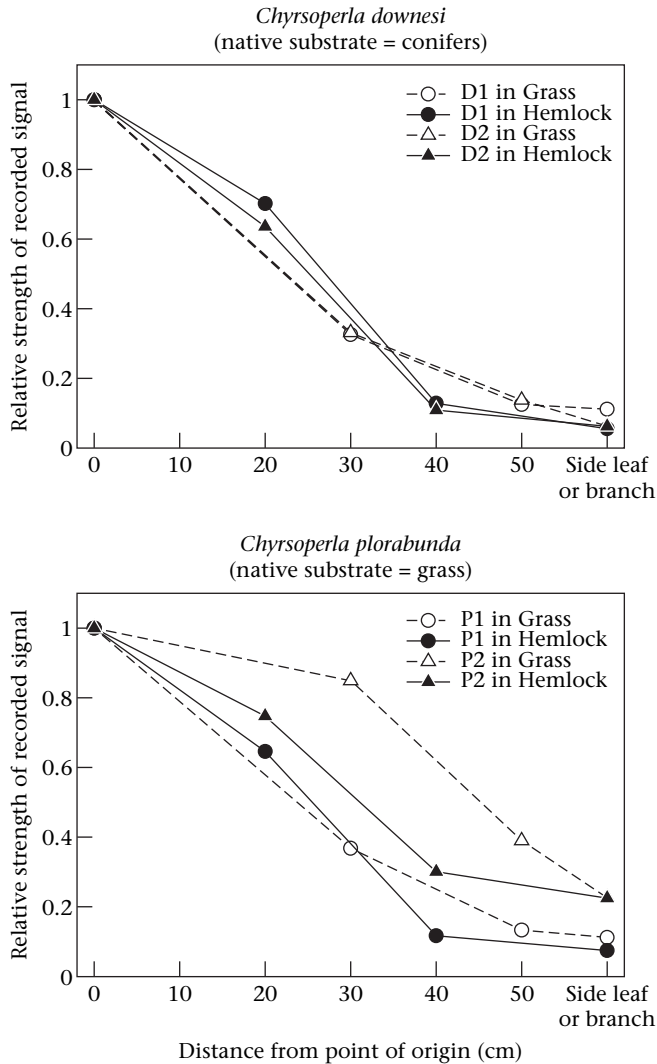
Figure 10. Plots of relative amplitude versus propagation distance for recorded signals of *Chrysoperla downesi* (individuals D1 and D2) and *C. plorabunda* (individuals P1 and P2) propagated through grass and hemlock substrates. Each data point in a plot is the average of measurements taken from two different samples of the given substrate (Gr1 and Gr2 for grass, Hm1 and Hm2 for hemlock). Measurements were taken at the origin (0 cm), midpoint (30 cm and 20 cm from origin for grass and conifer, respectively), tip (50 cm and 40 cm from origin for grass and conifer, respectively), and side leaf or branch (5 cm from the base of a lateral structure attached 10 cm from origin).

All four of these conifer-associated species shared long songs made up in part or completely by series of short, closely spaced volleys of relatively high carrier frequency. However, this suite of features was also found in *C. carnea*, *C. pallida* and 'carnea-Kyrgyzstan', and so cannot be considered unique to conifer dwellers (Fig. 1). In addition, *C. nipponensis* had a song resembling those of conifer-associated *C. downesi* and 'downesi-Kyrgyzstan', but occupied meadows and croplands (Figs 1, 8). Consequently, it is not clear that a train of short volleys represents an adaptation to conifer substrates. The phylogenetic and ecological distributions of song phenotypes in the *carnea*

group suggest substrate adaptation as the cause of song convergence, but they do not fully support that hypothesis.

Experimental Tests of Adaptation: Signal Fidelity

The vibrational songs of *C. plorabunda* and *C. downesi*, like those of all other cryptic species in the *carnea* group, are oscillatory rather than percussive although there are exceptions (e.g. spikes in the songs: *C. pallida*: Henry et al. 2002a; *C. agilis*: Henry et al. 2003). The insect shakes the substrate but does not strike it, resulting in the production of bending waves (Michelsen et al. 1982). The propagation of bending waves in plant stems is particularly complex, because (1) bending waves of different frequencies travel at different velocities within the plant, and (2) each abrupt change in mechanical impedance along the plant's axis (e.g. the root or top of the plant, or the site of a side leaf or branch) will reflect the signal back towards the source. Use of an accelerometer to detect the signal, as we did here, places an unnatural mechanical load on the plant. Such loading will alter the acoustic filtering properties of the plant, affecting the temporal and spectral characteristics of vibrational signals that pass through it. Although it is impossible to predict all such changes, mechanical loading is likely to affect lightweight media more severely than massive media (e.g. the grass stem more than the hemlock branch). In principle, a laser vibrometer should give more realistic results, but exploratory studies using an optical transducer (OM-1 by J. Schwenk, Schwenk Industries, Willimantic, Connecticut) revealed that undamped plant stems were subject to periods of intense, irregular vibration triggered by weak air currents present in the laboratory, making that approach impossible. Therefore, we chose to use the contact accelerometer, reasoning that any bias introduced by mass loading would be consistent across tests and should therefore not affect the significance of our results.

Much of our understanding of the transmission of insect vibrational signals in plants has come from the work of Axel Michelsen and his collaborators. In a classic study, Michelsen et al. (1982) determined that structurally different plants propagate and filter vibrational signals in similar ways, such that any signal spanning a relatively broad range of frequencies around 100 Hz is reasonably well matched to the transmission properties of most plant substrates (see also Miklas et al. 2001). Although the vibrational songs of several diverse Hemiptera show general adaptations that promote efficient propagation in all plant stems, none is specifically adapted to the biomechanical properties of a particular host plant (Michelsen et al. 1982).

Signal transmission in lacewings is consistent with those findings. Although very different from one another in many respects, the tremulation songs of *C. downesi* and *C. plorabunda* shared two attributes: very low carrier frequency, and significant modulation of that frequency. Both attributes contributed to efficient propagation in all plants. Yet in neither species has evolution fine-tuned the signalling system to the specific properties of the native
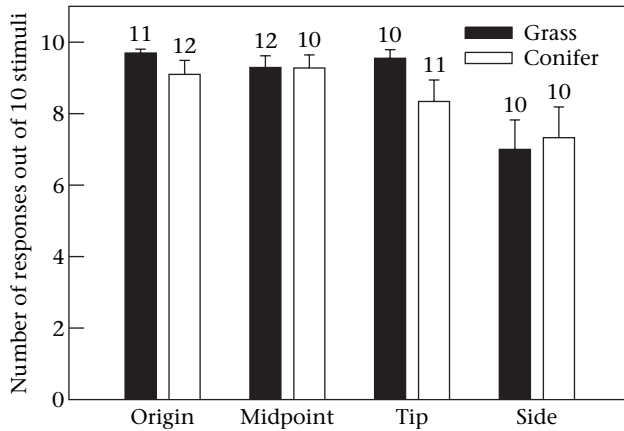
Figure 11. Responsiveness of *Chrysoperla downesi* individuals to playbacks of 10 of their own songs (shortest repeated units, SRUs), after propagation through grass versus conifer substrates to four sites along those substrates. All playback signals were normalized to an amplitude of ± 5 V. Sites included the origin (unmodified signal), midpoint (signal recorded 30 cm and 20 cm from origin for grass and conifer, respectively), tip (50 cm and 40 cm from origin for grass and conifer, respectively), and side leaf or branch (5 cm from the base of a lateral structure attached 10 cm from origin). Each value on the Y axis is the mean of the means for several individuals. The number of individuals is specified above each bar. See Table 3 for details.

plant substrate. Songs of *C. downesi* and *C. plorabunda* propagated equally well in the same grass substrate, and equally well in the same conifer substrate (Table 2). Furthermore, there were no consistent differences in the fidelity of signals measured between species on a given substrate, or between substrates in a given species (Figs 5, 6, 10).
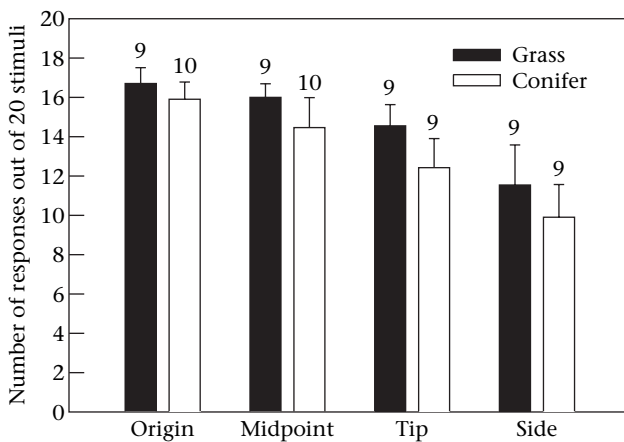
Figure 12. Responsiveness of *Chrysoperla plorabunda* individuals to playbacks of 20 of their own songs (shortest repeated units, SRUs), after propagation through grass versus conifer substrates to four sites along those substrates. All playback signals were normalized to an amplitude of ± 5 V. Sites included the origin (unmodified signal), midpoint (signal recorded 30 cm and 20 cm from origin for grass and conifer, respectively), tip (50 cm and 40 cm from origin for grass and conifer, respectively), and side leaf or branch (5 cm from the base of a lateral structure attached 10 cm from origin). Each value on the Y axis is the mean of the means for several individuals. The number of individuals is specified above each bar. See Table 3 for details.

Signals containing a broad range of low frequencies, such as those of lacewings, should show major spectral anomalies at different recording sites along a plant stem, due to reinforcement and cancellation of frequency-dependent standing waves (Michelsen et al. 1982). For example, a particular narrow range of frequencies might be missing at one recording site, but amplified at another, and this anomaly is thought to be the selective force favouring broadband rather than narrow bandwidth acoustic signals in animals that use plants as channels for communication. Oddly, in our experiments, we found little evidence of frequency-specific acoustic filtering at any recording site. At every site on each of the two substrates, every song retained its original range of frequencies, and those frequencies retained their original relative amplitudes (Figs 5, 6). Possibly, the footprint of the accelerometer was too large (8-mm diameter) to demonstrate the effect of frequency filtering at single points along the stem. However, the six-legged sound-sensing 'footprint' of a lacewing is also fairly large, suggesting that an insect is no more likely than the accelerometer to detect the signal at a point source.

In summary, our findings tentatively reject the notion that fine-tuned adaptation to specific substrate properties has shaped the evolution of song phenotype in the cryptic song species of the *carnea* group. Because we did not observe higher fidelity of a given song type in its correct (native) substrate, the hypothesis of environmental adaptation was not supported.

Experimental Tests of Adaptation: Behavioural Preferences

Substrate-borne vibrational signals of insects have been reported to travel several metres in plant stems, while retaining sufficient amplitude to be 'heard' by other individuals (Michelsen et al. 1982). Although our experiments were compromised by the damping effect of the relatively massive accelerometer, they nevertheless demonstrate moderately long-distance transmission of lacewing signals. Songs elicited nearly normal levels of responsiveness even when re-recorded 40–50 cm from the point of origin. In plant stems free of mass loading, biologically useful signals from lacewings have been shown to travel considerably further (Henry & Wells 1990b).

Substrate makes little difference to a lacewing in its responsiveness to playbacks of its own song. In fact, both species prefer grass over conifers for signal transmission. Even conifer-associated *C. downesi* shows this preference, although not as strongly as *C. plorabunda* (Table 3). Because we did not observe significantly more responses by lacewings to songs transmitted through their native substrates, the hypothesis of fine-tuned adaptation to specific plant substrates was not supported. These results are consistent with the general conclusions of Michelsen et al. (1982) discussed earlier, and suggest that the biomechanics of grass stems versus hemlock branches are too similar to have provoked a specific adaptive response in lacewings of the *carnea* group.

Table 4. Results of a univariate, factorial ANOVA performed on the dependent variable 'mean number of responses' for the two categorical variables 'substrate' and 'site', for two lacewing species listening to playbacks of their own songs

	Chrysoperla downesi			*Chrysoperla plorabunda*		
	df	F	P	df	F	P
Intercept	1	2289.030	0.0000	1	862.270	0.0000
Substrate	1	1.059	0.0367	1	2.583	0.1128
Site	3	7.898	0.0001	3	6.554	0.0006
Substrate∗site	3	0.834	0.479	3	0.084	0.9787

Substrate: grass stem or conifer branchlet; site: song recorded at origin, midpoint, tip, and side leaf or branch of the substrate (see text).

Alternative Hypotheses

Our experiments were not so exhaustive that they can altogether reject a significant role for natural selection in shaping the signals of lacewings. For example, the efficiency of signals might depend importantly on the disparate branching patterns of grasses and conifers and not so much on differences in substrate physics. Alternatively, the fitness value of a signal could be a function of how far it is able ultimately to travel rather than of its performance within a circumscribed range. Or, there may exist substrate differences in performance only when multiple signallers are present on the same plant. Additional experiments are needed to test whether there are more subtle ways in which songs have been matched by natural selection to specific substrates. Particularly useful will be experiments that can reliably measure the behavioural responses of lacewings to signals of progressively reduced amplitude. However, these caveats aside, current evidence suggests that differences between lacewing songs are selectively neutral.

Adaptation and Speciation

Molecular markers support rapid and recent speciation in the *carnea* group (Henry et al. 1999b), and evidence is accumulating that its diversification has been driven by sexual selection acting on song phenotype (Henry et al. 2002b). However, the contribution of natural selection and adaptation to the process of species divergence via sexual selection in lacewings appears to be small. The present study confirms that lacewing songs show basic spectral adaptations to the general biomechanical properties of plant stems, but casts doubt on a role for environmental adaptation in speciation. Our choice of environmental substrates and their associated species, grass stems for *C. plorabunda* and conifer branchlets for *C. downesi*, was intended to maximize the effect of substrate differences on the waveforms of songs, yet physical analyses and behavioural experiments were insufficient to detect environmental adaptation. The only evidence of natural selection gleaned from this study was the apparent pattern of convergent evolution of song phenotype in taxa associated with coniferous substrates.

In the absence of natural selection, reproductively isolating differences in lacewing mating signals could develop through sexual selection (mate choice) alone. The theoretical likelihood of nonadaptive speciation resulting from 'pure' sexual selection has been established (see Introduction), but very few examples have been put forward as evidence (e.g. two *Gryllus* cricket species, Gray & Cade 2000). In lacewings, such a process may be facilitated by simple genetic architecture underlying song phenotype. Crossing experiments between closely related pairs of species within the *carnea* group (including the taxa used in this study, *C. downesi* and *C. plorabunda*) have shown that the inheritance of song phenotype is consistent with control by a few genes of major effect, rather than many genes of incremental effect (Henry 1985; Henry et al. 2002b). Consequently, single allelic substitutions could create new species nearly instantaneously, without requiring natural selection to fine-tune the new mating signals to the biomechanical properties of their substrates. The gradual process of environmental adaptation typically requires strong, continuous disruptive selection acting on quantitative (polygenic) traits, and we have found neither the song properties nor the genetic architecture to support such adaptation. Darwin's early insight, that sexual selection might cause speciation through its powerful influence on arbitrary traits, receives cautious support from this study.

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