

Calling and Duetting Behavior in the Leafhopper *Balclutha incisa* (Hemiptera: Cicadellidae: Deltocephalinae): Opportunity for Female Choice?

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Male *Balclutha incisa* (Cicadellidae: Deltocephalinae) produces substrate-borne signals as well as sounds produced by wing fluttering. Males produce 6 signal types that are associated with different behavioral activities: (i) cleaning, (ii) calling, (iii) precopulatory, (iv) copulation, (v) postcopulatory behavior, and (vi) aggression. Within each category signals have a high degree of stereotypy in regard to their temporal structure. Females produce two kinds of signals: (i) a duetting signal in response to the male, and (ii) an introductory or preparatory signal before the main signal. The sexes duet, with the female placing its signal immediately after the first two components of the male's call, with an interval of some 136 ms. We discuss the role of the male calls in attracting females and the importance of the duet in maintaining a temporary pair-bond.

KEY WORDS: acoustic behavior; duet; substrate vibration; calling tactic; leafhopper.

INTRODUCTION

Small Auchenorrhyncha, including leafhoppers and planthoppers, communicate by vibrational signals transmitted through the substrate (Ichikawa and Ishii, 1974; Ichikawa, 1976, 1979; Inoue 1982; Claridge, 1985a,b)

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often as bending waves (Cocroft *et al.*, 2000; Greenfield, 2002). Although there may be an airborne component, vibrational information is more usually received as substrate-borne waves (Ichikawa, 1976). Tymbals are absent from females, and as in most leafhoppers and planthoppers signals are produced by dorsoventral vibrations of the abdomen (Ichikawa, 1976). However, little is known of acoustic/vibrational signal production and reception in this group of animals, and more surprisingly given their economic importance, there appears little published work on mating systems of leafhoppers and planthoppers (Ott, 1994), which is the context of this paper.

Most Auchenorrhyncha have a wide range of acoustic or vibrational signals associated with different behaviors (Claridge, 1985a), but the most commonly produced signals are those involved in pair formation. These signals are usually termed “calling signals” (Ichikawa, 1976; Claridge, 1983, 1985a). The general rule is that once the calling male receives an auditory/vibratory response from a responding female, the male continues to duet while searching for the stationary female. Such behavior is in contrast to many acoustical insects where stationary males call and the female tracks down the male signal (Bailey, 1991, 2003).

Balclutha incisa is a small (<5 mm) and widespread leafhopper that lives on various grass hosts (Knight, 1987). The insect is abundant in the southwest of Western Australia during midsummer until early winter where the primary host, which is an introduced crab grass *Digitaria sanguinalis*, senesces. The leafhopper uses the panicles of the host grass for feeding and at these sites may be found calling, mating, and laying eggs (Nuhardiyati and Bailey, in press). After mating, males continue to produce signals and often remate several times within days (see Ott, 1994, for review of mating systems of delphacid planthoppers). The substrate-borne signals are transmitted from one panicle stalk to another, or between plants by intervening vegetation, and so males search for their mates by walking or jumping from one panicle stalk to the other, stopping briefly to signal and orient to the responding female (Nuhardiyati, 1998).

In this paper we describe the calls of male *B. incisa* and the duetting response of the female. We examine within- and between-male variation in signal structure and relate this variation to age and male-mating status. We discuss the role of these calls in maintaining an acoustic pair bond between the sexes and, finally, by using the female’s acoustic response to playback experiment, we test which of the male calls are most effective in attracting a mate. By examining the levels of variation within each signal element we speculate on the role of female signal preference in the evolution of male calls.

MATERIAL AND METHODS

Acoustic Signal Recording

Techniques have been developed for recording the signals of small Auchenorrhyncha and some of these have been described by Claridge *et al.* (1985b). We employed a similar technique to that of Claridge *et al.* (1985b) using a piezoelectric stylus resting against the leaf within 2 cm of the calling insect. The output impedance of the stylus was matched to a preamplifier, allowing the signal to be recorded onto a Sony Professional Walkman cassette recorder (WMD6C). Acoustic-vibratory signals were monitored during the recording process simply by headphones and an oscilloscope. Once on tape, sections of the signal were then transcribed in digital form using an A-D converter (Sound FX[©], Silicon Soft, CA). The stimulus and the fidelity of recording was checked in two ways. First, the observed response of the insect was consistent with that of the natural signal and second, we recorded the form of the output of the signal delivered through one stylus, the stimulus, with a second recording stylus. While transmission and recording through this technique is at best crude, the similarities between stimulus and recorded signal were remarkably consistent.

As temperature greatly influences both planthopper (Booij, 1982; de Vrijer, 1984) and leafhopper (Gillham, 1992) acoustic behavior and particularly calling rate, a small digital thermometer was fixed as close to the recorded insect as possible. During recording the temperature around insect was maintained at $25 \pm 1^\circ\text{C}$ by adjusting the distance of a local radiant heat source. Recordings were carried out within the early hours of the photophase.

As far as possible we have used the terminology of Claridge (1985b) to describe the vibrational signals of *B. incisa*, where a signal is composed of repeated elements within which one or more pulses may be recognized. As with all acoustic systems the pulse, with its unique waveform, is the smallest quantifiable component. The term *chirp* represents the shortest coherent train of pulses, while the term *phrase* merely reflects a train of chirps separated by periods of silence. The intuitive terms *song*, *call*, and *signal* are interchangeable while the term *bout* or *bursts* of calling activity indicates the production of a series of calls often followed by silence.

Where there is a female reply, the distinction between an acoustic response to a male or the establishing a duet may be semantic, however, in the context of this paper a duet is defined as a predictable and stereotyped temporal association between initiating signal and the reply (Bailey, 2003). Finally, while substrate vibration is commonly used as a term to describe

the transmission of information along a substrate such as a plant stem, particularly by planthoppers and leafhoppers, the transmission of signals along plant stems are best described as bending waves (see discussion in Greenfield, 2002).

Age, Mating Status, and Male Signal Variation

To examine the influence of age and mating history on calling signals, 36 males and 20 females of known mating history were recorded. Males were placed into four groups: 4-day-old unmated (10 males), 4-day-old mated 3 times (10 males), 10-day-old unmated (8 males), and 16-day-old unmated (8 males). Recordings were made of males within 2 min of being placed on the plant or immediately following cleaning activity. All insects were allowed to produce calling signals without stimulation, and recordings were made as early as possible following the initial burst of calling. (Because the tape recorder was switched on only after the male commenced calling, the first few elements of the signal were inevitably missed.) The recording of less vigorous males was made after the male completed his initial pre-signal cleaning behavior. Nine single calling signals obtained from the first three calls of three randomly selected series of calls from each male were analyzed.

In order to estimate within- and between-male variation, we measured 10 elements of the male's call: the number of pulses and total lengths of components "a," "b," and "c" (Fig. 1); the interval between parts "b" and "c," between chirps and between complete calls or bouts of calling; the duration of the call. Independence of sampling was achieved by taking values of 10 samples from complete signals sampled at random from each recording.

Virgin females were grouped into two age classes, 4–5 days and 16 days old, with 10 females in each group. All females were stimulated using the male signal. The first 8–10 female signals recorded after stimulation were analyzed for temporal pattern. We only analyzed the length of the signal and the number of signals in 10 s following the start of the male prerecorded signal. Because of difficulties, such as the irregularity of the pulses, their very low intensity and the practical difficulty of eliminating background noise by digital filtering, pulse repetition rate within each call could not be measured.

While the transmission of signals via bending waves was clearly crucial in sexual communication, we recognize that vision may also have played a role however, we had no means of correcting for this in our experiments except to maintain light levels at a more-or-less constant level throughout the experiments.

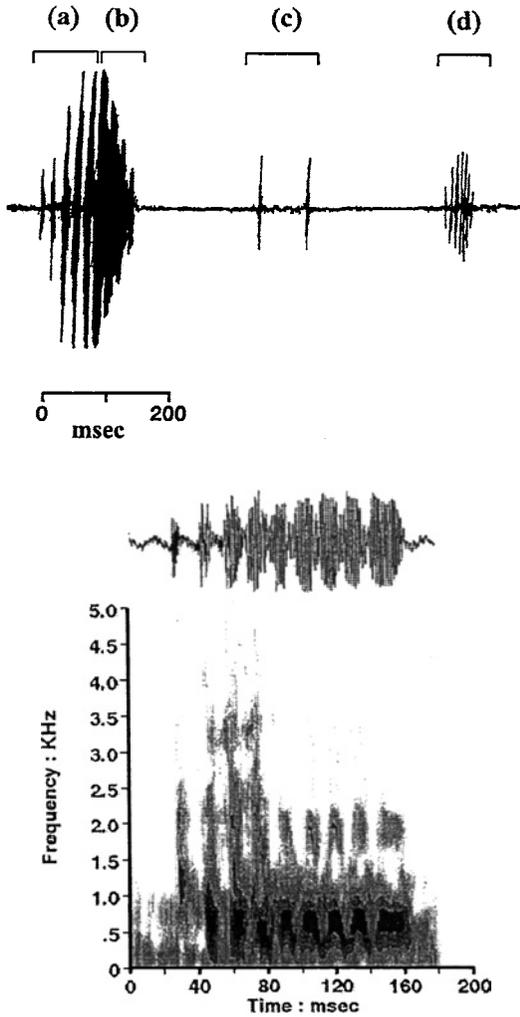


Fig. 1. Wing fluttering (a) and calling signal (b) of *Balclutha incisa* showing in "b" the temporal pattern with the main signal "a + b" and the quieter following calls "c" and "d." (c) A detail of parts "a + b" shown as a sonogram indicating the high frequencies produced by the transients in part "a" and the frequency modulation of part "b."

Statistical Analysis

Each variable was analyzed using ANOVA. Before calculating ANOVA, a Hartley's F -max test was calculated to test for homoscedasticity. Normality of residuals was tested with Kolmogorov–Smirnov goodness of fit test. Data transformation was not needed when data followed normal distribution and where variances were homogenous. However, when the assumption of normality and homoscedasticity was violated, data were analyzed using Kruskal–Wallis test (Zar, 1996). Tukey test and Nemenyi test were used to compare the means whenever there was significant difference among the levels of a factor. F test for variance was applied prior to t tests to determine the difference between two means.

Playback Experiments

Playback experiments allowed us to examine the role of male and female acoustic signals on the behavior of female *B. incisa*. We created playback signals by digitizing the analog signal. The selected signal was then frequency-filtered (high-pass with a corner frequency of 150 Hz) and played through a D-A SoundFX® programmable board via the same type of stylus as used for recording. The number of females responding to the calls and the degree of their response were recorded for each experiment. In addition any acoustic response was recorded on tape by a second recording stylus, which was placed close to the focal female and from this stylus we were able to detect both stimulus and response on the same recording track.

Females (10 for each treatment) aged 4–5 days old were randomly assigned to each treatment. Each female was placed onto a crab-grass leaf blade where the stimulus and the pick up styli were already set up. Once they were put onto a plant, most females went through a series of characteristic cleaning movements (78%) before they produced any call. We allowed females to clean and beat their wings for about 3 min before presenting a stimulus call.

Stimulus signals were produced continuously for 30 s. The female's response, as a duet in was quantified during this period and then during a period of 5 min from when the stimulus was stopped. The control treatment was to allow females to remain on the same grass stem, close to the stimulating and recording styli, for the same time as that used in the experiment, but with no acoustic stimulus. Females that failed to respond to any one of the six stimulus types presented in random order (see results below) after 5.5 min were first presented with a male calling signal and then

male–female duetting calls. Females that failed to respond after this process were discarded.

As indicated above, females may emit very faint and infrequent secondary signals of low amplitude in addition to the “true” calling signal and such calls are easily identified. However, the responses of the female used during the response experiments were always the duetting signals.

RESULTS

Male *B. incisor* produce six types of signals, marking different behavioral activities including cleaning, calling, precopulatory behavior, copulation, postcopulatory behavior, and aggression.

Male Wing Fluttering

Signals produced while the male is apparently cleaning are undoubtedly a by-product of leg, wing, and abdominal movements (Fig. 1a). In this context, wing fluttering was the most common behavior. We noted that this signal was produced in three contexts: (1) when males were first put on the plant, (2) after a burst of calling where there was no female response, and (3) after the completion of mating. Despite these signals being essentially a by-product of cleaning there was a surprisingly high degree of stereotypy in temporal acoustic structure with differences only in the amplitude of the pulse train.

Male Calling Signal

Calling signals are repeated group of signals consisting of four parts (Fig. 1b and c) were parts “a” and “b” form the main call component and parts “c” and “d” more rarely introduced within the call. Within each of the four signal types there is a complex of pulses produced by unique body movements and groups of the signal, especially components “a + b” may last several seconds. The repetition on these two call elements ranges from 1.4 to 1.7 calls per second. However, towards the end of a bout of calling the interval between calls lengthens and the call slows down. The fine structure of the “a” component of the call shows a group of fully amplitude-modulated pulses of increasing length (10–15 ms), while the “b” component has a longer signal envelope with a shallower modulation (<40% modulation) lasting some 80–100 ms. The calls, and especially parts “a + b” are

frequency modulated with the pitch falling from 1.5–2 kHz to 600–700 Hz (Fig. 1c). The first part of the signal has high transients, which are reflected in their broader frequency band and the inclusion of harmonics.

Analysis of the calls of 26 males of unknown age and mating history showed at least two combinations of the basic signal structure. First, calls that included elements “a,” “b,” and “c” were the most common (81%) and second, males that produced only elements “a” and “b” formed some 11% of the total number of calls analyzed.

The “a + b” calls were often followed by a series of additional fully modulated pulses lasting some 150–400 ms. The amplitude of these pulses varied from 20 to 30% of the main signal to the same level or louder. Rarely (2 from 26 males) a fourth type of signal was produced (Fig. 1b; component “d”), after the fully modulated “c” section and prior to any following “a + b” calls. This component consists of a series of fully modulated pulses of extremely short duration. Males may add up to 15 pulses to the main call. This signal may be analogous to the searching signal of brown planthoppers, which change their signal type as they approach the female (Ichikawa, 1976).

Variation in Male Calling Signal

Figure 2 shows rank coefficient of variation of between-male calls, where the most variable character was the interval between components “b” and “c” (character 10 in Fig. 2), and the least variable character was the interval between calls (character 1). There was also high variation in interval between bouts of calling (character 9), which is usually a result of males changing the number of pulses and adding secondary pulses between calls. These data are derived from the 36 males used to establish variation in call structure due to age and mating history.

Signal Variation, Age, and Mating History

Age of the males had no effect on the form of the signal for each of the six parameters measured (Table I). However, within each age class, mating history appeared to have a significant influence on all signal characters, except number of pulses and the length of the main signal (Table II). Mated males sang more vigorously with higher signal rate, shorter signal interval, more additional pulses, and hence a longer signal. Calls showed less variability in the number of pulses in the “a + b” component between males for all treatments (C.V. 4 day unmated 18.8%, 4 day mated 16.7%, 10 day

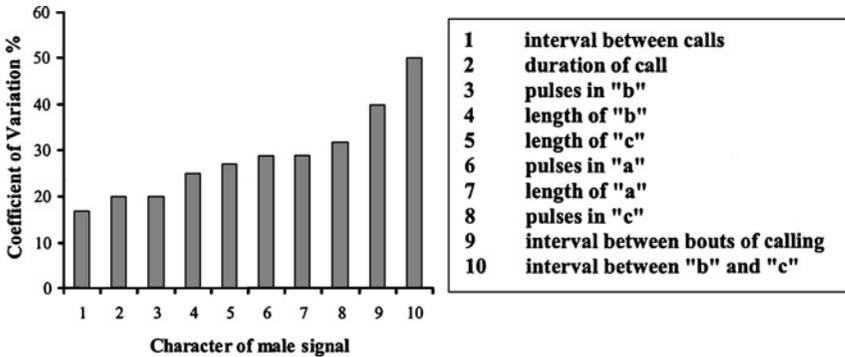


Fig. 2. Ranked variability (coefficient of variation as %) of three of the components of the male signal of *Balclutha incisica*; static characters are those with less variation than more dynamic characters.

unmated 20.8%, 16 day unmated 16.2%), compared with the number of pulses in the second component “c” (C.V. 4 day unmated 88.9%, 4 day mated 56.9%, 10 day unmated 114.3%, 16 day unmated 60.2%). This result suggests that parts “a + b” are critical components in species’ identity while part “c” expresses a qualitative aspect of the calling male. There was no difference between signal variation in unmated and mated males.

There was a positive correlation within unmated individuals between number of pulses in the main component “a + b” and the number of pulses in the additional component “c” (Spearman rank correlation, $r_s = 0.622$, $n = 26$, $P < 0.001$). Surprisingly, in mated males the correlation was negative (Spearman rank correlation, $r_s = -0.833$, $n = 10$, $P = 0.0048$). There was no correlation between variability of signal length and the signal interval within individual unmated males (Spearman rank correlation, $r_s = -0.0677$, $n = 26$, $P > 0.50$), although a positive correlation occurred

Table I. Measures of the Signal Characters of Unmated Male *Balclutha incisica* (Mean \pm SE) According to Age

Signal characters	4 days	10 days	16 days	P value (ANOVA)
<i>n</i>	10	8	8	
Signal interval (s)	0.680 \pm 0.031	0.721 \pm 0.060	0.626 \pm 0.035	0.3921
Length of signal (s)	0.390 \pm 0.046	0.352 \pm 0.048	0.449 \pm 0.051	0.5691
Number pulses (a + b)	7.800 \pm 0.366	7.194 \pm 0.048	7.972 \pm 0.237	0.4177
Length of “a + b” (s)	0.157 \pm 0.007	0.147 \pm 0.008	0.151 \pm 0.009	0.6436
Number pulses in “c”	1.679 \pm 0.396	2.403 \pm 0.872	3.278 \pm 0.600	0.1445
Call rate (per s)	1.460 \pm 0.066	1.444 \pm 0.056	1.596 \pm 0.063	0.2099

Table II. Measures of Signal Characters (Mean \pm SE) of Male *Balclutha incisa* With Mating: Comparison Between 4-Day-Old Unmated and 4-Day-Old Mated Males

Signal characters	Unmated	Mated	Significance <i>t</i> test (one-tailed)
<i>n</i>	10	10	
Signal interval (s)	0.68 \pm 0.03	0.59 \pm 0.03	<i>P</i> < 0.05
Length of call (s)	0.39 \pm 0.05	0.49 \pm 0.03	<i>P</i> < 0.05
Number pulses (a + b)	7.80 \pm 0.37	7.82 \pm 0.29	NS
Length of "a + b" (s)	0.16 \pm 0.01	0.16 \pm 0.01	NS
Number pulses in "c"	1.68 \pm 0.40	4.47 \pm 0.59	<i>P</i> < 0.001
Signal rate (per s)	1.46 \pm 0.07	1.70 \pm 0.08	<i>P</i> < 0.05

when the males were mated (Spearman rank correlation, $r_s = 0.8273$, $n = 10$, $P = 0.0054$).

Precopulatory Signal

Precopulatory signals of *B. incisa* are produced when a receptive female is nearby. The signal consists of repeated signal elements including single- or double-grouped pulses (Fig. 3i and ii). Precopulatory signals invariably end when genital contact is achieved.

Occasionally, signals, structurally similar to the precopulatory signal, were produced after mating. Males producing these signals appeared more inclined to attempt remating with very short copulation intervals.

Copulation Signals

Copulatory signals (Fig. 4i) are produced 2–3 min following initial genital contact ($n = 10$ pairs). They are similar to the "a" component of the calling signal except that they are emitted irregularly and at very low intensity extending over 200 ms. There is no partially modulated pulse equivalent to the "b" component of the calling signal associated with this signal. A possible function of this signal is to maintain contact with the female; we observed that when females produced movements signifying the end of copulation the male would increase the number of copulatory signals.

Postcopulatory Signals

Males, once they have withdrawn their genitalia, and usually when the female is still close by, emit postcopulatory signals. The signals are distinct

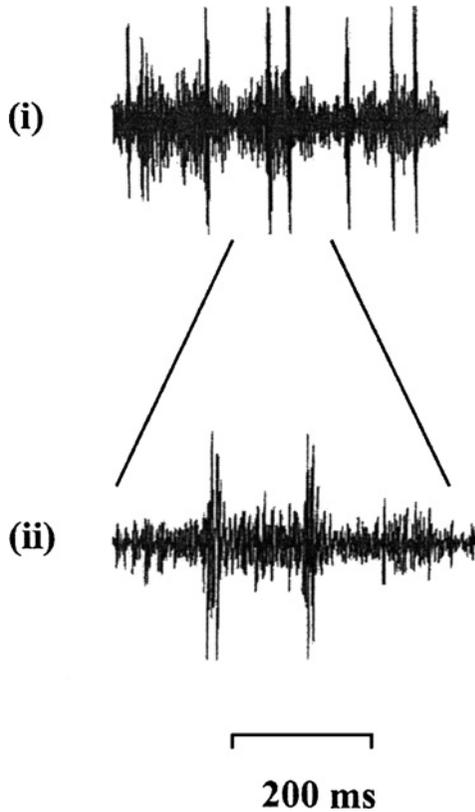


Fig. 3. Precopulatory signals of male *Balclutha incisa* showing the detail of the doublet call.

from cleaning or calling signals and consist of long heterogeneous groups of pulses repeated with an interval of about 200 ms (Fig. 4ii). The structure of the initial part of the signal is similar to the “a” component of the calling signal, but at much lower amplitude, while the main element appears to have little structure, except for repeated doublets.

Aggressive Male Signals

Aggressive signals are produced by males in the presence of other calling males close by. The signal consisted of an unstructured train of pulses (Fig. 4iii) and is, in many ways, similar to the postcopulatory signal, except there is no evidence of discrete chirps.

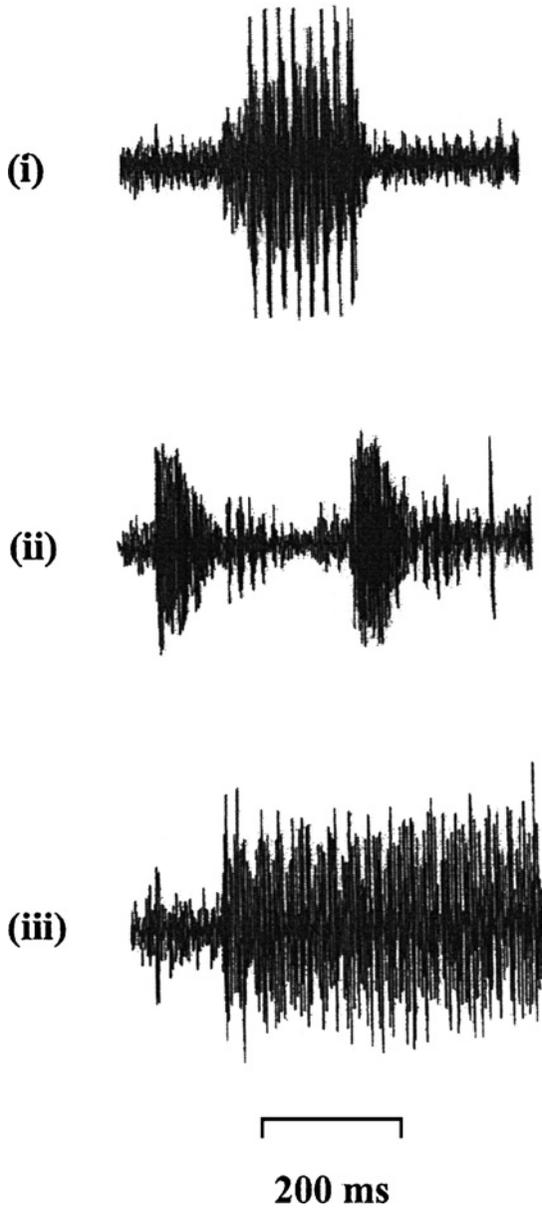


Fig. 4. Three signal types of *Balclutha incisa*: (i) copulatory signal, (ii) postcopulatory signal, and (iii) aggressive signal. All figures are drawn to the same time scale.

Female Signals

As noted, female Auchenorrhyncha make their calls by abdominal vibrations (Ichikawa, 1976) and *B. incisa* follows this general pattern of signal production (Fig. 5a). The female produces two kinds of signals, an introductory signal, in response to the male signal where the abdomen vibrates as if in preparation for the main signal. This signal is very short, irregular, and of very low frequency and energy. Interestingly, low amplitude signals are also produced after bursts of cleaning activity.

Females may also produce a call either spontaneously or as part of a duet (Fig. 5b). The pulse structure of the female's spontaneous calls is irregular and often produced in short bursts of 0.205 ± 0.007 s ($n = 20$). By contrast, signals produced when the female is duetting are distinctly periodic

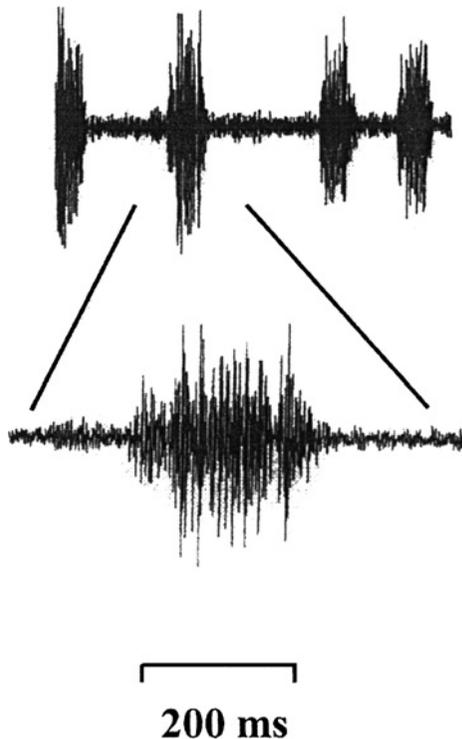


Fig. 5. (a) Female reply signal of *Balclutha incisa*.
 (b) A duet with male and female calls are marked by symbols.



Fig. 5. Continued.

and repeated in a series of chirps lasting some 100–150 ms. Pulses within the signal appear unstructured and are difficult to differentiate from the male's calling signal.

Variability of Female Signal

Females call both spontaneously and in response to the male calling signal as a duet. We measured signal length and number of calls per 10 s

Table III. Signal Characters (Mean \pm SE) of Young (4 Days) and Old (16 Days) Virgin Female *Baldutha incisa*

Signal characters	4-day old	16-day old	Significance <i>t</i> test (two-tailed)
<i>n</i>	10	10	
Number of calls/10s	8.43 \pm 0.76	3.76 \pm 0.69	P < 0.001
Signal length (s)	0.20 \pm 0.01	0.21 \pm 0.01	NS

from when the prerecorded signal stopped (Table III). Signal length was less variable, compared with the number of signals within 10 s of the cessation of the stimulus in both young and old females. The C.V.s of signal length for young and old females were 23.8 and 20.6%, respectively, compared to 34.1 and 67.7% for the number of signals per 10 s in young and old females. Age was found to influence the number of calls in the 10 s following the cessation of the stimulus, while the signal length remained similar with age.

Male/Female Duetting

When responding to males, the female places her signal immediately after the “a + b” component, with a mean (\pm SE) interval of 136 ms \pm 7 ms (*n* = 8 duets) (Fig. 5b). This structured duet is produced when the female first detects the male signal and when the male is stationary. The female continues to reply once the male begins its searching pattern, but the reply appears less structured.

Playback Response of Females to Male Signals

There was a significant treatment effect on the female’s responsiveness to playback calls during the 30 s of signal ($\chi^2 = 34.5396$, *P* < 0.001). The type of male signal also influenced the proportion of females that continued to call during 300 s after the stimulating signal ceased ($\chi^2 = 17.0796$, $0.005 < P < 0.01$). The mean number of female responses among the male signals tested during the playback was significantly different (Kruskal–Wallis analysis of variance with tied ranks (Zar, 1996): *H* = 42.7726, *P* < 0.001). Similarly, the mean number of calls following the stimulus also showed high heterogeneity (*H* = 21.5462, $0.001 < P < 0.005$).

Three of the male’s signals, the calling, precopulatory, and postcopulatory signals produced the strongest female response. Among these three signals the calling signal generated the highest response rate (Fig. 6); 70% of females continued to call after this stimulus had ceased.

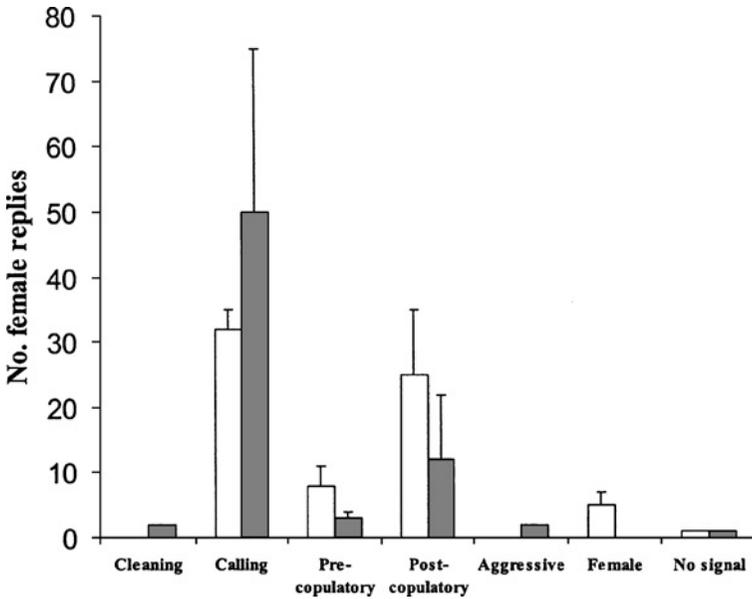


Fig. 6. Mean number (\pm SE) of female responses to the different signal types of male and female *Balclutha incisa* during 30 s of playback (white) and 300 s following cessation of the signal (dark) ($n = 10$). The control is with no sound for 330 s. Females reply most readily to male calling signals and also postcopulatory signals.

The second most preferred signal was the postcopulatory call. There was no difference between the female's response to this signal compared with the precopulatory signal (Table IV). However, after the male's signals ceased, only 20% of the females extended their calling behavior, which was no different from the control situation. The other two male signals, cleaning and aggressive signals were clearly not important in male-female pairing; female responsiveness to these signals was not significantly different from the control. Similarly, females did not react to their own calls.

DISCUSSION

Male Calling Signal

As with many plant and leafhoppers, the calling signals of *B. incisa* are complex. Usually, they have at least three parts; a short series of fully modulated pulses (component "a") followed by a section with partial modulation (component "b") and then a series of short pulses ("c") before the next

Table IV. The Vibrational Responses of Females to Different Types of Prerecorded Signal During and After Playback

Type of signal	% female responded		Number of responses (mean ± SE)	
	During playback	After playback	During playback	After playback
Male cleaning	0	20	0.0 ± 0.0a	0.5 ± 0.34ad
Male calling	100	70	31.1 ± 2.78b	49.9 ± 26.19b
Male precopulatory	70	20	8.2 ± 3.35d	1.4 ± 1.11ac
Male postcopulatory	70	50	22.3 ± 6.27d	12.7 ± 5.20b
Male aggressive	0	10	0.0 ± 0.0a	0.4 ± 0.40ac
Female calling	50	0	3.1 ± 2.55c	0.0 ± 0.000a
Control	20	30	0.7 ± 0.51 7ac	0.8 ± 0.42cde

Notes. Same letter the measurement in the same column indicates no significant difference at 5% level.

signal cycle. The frequency modulation of the signal appears as a highly repeatable phenomenon showing consistency between males and also between substrates. The presence of high frequencies in the “a” component is most easily explained by the transient nature of the signal, and also by the side banding as a consequence of rapidly produced pulses (Bennet-Clark and Young, 1992). Once the modulation envelope is reduced there is an expected reduction in the upper harmonic component and thus, there are fewer transients. If the harmonic structure of the bending waves can be detected by conspecifics the presence of these harmonics could have a role in detecting the proximity of the signaller.

The calling signal can be divided into three functional parts. First, the signal interval and signal duration combine to give the overall temporal structure of the song. This signature appears to provide species’ identity, and equates to the temporal structure of calls described for other leafhoppers and planthoppers (Claridge, 1985a). A second group of signal characteristics, measured as the number of pulse components in both “a” and “b” parts of the signal indicates the amount of energy each male is putting into the call. Such fine level structures contribute to the total vibrational power of the signal, and when, as expected, females respond preferentially to signals with more information, these components may well reflect male mating status or quality. We predict that sexual selection should act on these signal elements. Finally, the pause between two calls may also reflect metabolic energy available for calling but also, because males are moving between bouts of calling, such periods of silence may become vulnerable allowing competing males to take over the duet with responding females; the female has no awareness of which male is initiating the duet.

The calling signal produces the most consistent response from the female. However, variation in the call's temporal characteristics, as measured by coefficient of variation can vary from as low as 16% to as high as 50%. Although we have no comparative information for other plant or leafhopper species, figures for variation in frog calling make a useful comparison. In frogs, so-called static characters, which are those most concerned with species' identity, may fall beneath 5% while dynamic characters, usually concerned with male quality, exceed 15% (Gerhardt, 1991). Hence, signal characters with the lowest C.V.s in leafhoppers can be considered as static species-fixed elements; they include the interval between syllables, the duration of a single call, the number of pulses in, and the length of the call.

The Influence of Age and Mating History on Signal Variance

Critical to any signal under selection through female choice will be the degree to which the signal varies with both age and mating status. We measured six components of the male signal and none appeared to be affected by male age. However, there was a significant difference in female response to four of the six components when comparing unmated with mated males. While there was no influence of mating status on the "species" static components of the signal (components "a + b"), factors that indicated energy, such as interval, rate, length, and additional pulses were significantly different.

Where males only provide sperm in polygamous mating systems there should be strong selection on males to hide the effects of previous matings from subsequent females. Male refractory periods are not uncommon and are usually attributable to the need of males to replenish accessory gland products rather than sperm (Simmons, 2002). As with other planthoppers and leafhoppers males mate frequently (Ott, 1994) and in this case *B. incisa* appear to produce potentially "dishonest" signals once they have mated. Do females detect these postcopulatory displays and reject such courting males as these males increase the energy they put into almost every aspect of postcopulatory signalling?

There are a number of adaptive explanations. First, females may be attracted to the intensity of male courtship, and once mated, males may take advantage of this generally adaptive preference by increasing their calling rate; they are in a target-rich environment with sexually active females. Such dishonesty can only remain as an evolutionary stable strategy if the incidence of post-male calling and courtship is relatively low within a population, for if the event was common, females would use this as a signal marking spent males, enabling them to avoid these males. Second, males with reduced quantities of sperm would have to increase competition for

access to females; increasing signal rates may be part of this process. We have shown elsewhere that unmated males provide accessory gland products that potentially reduce female receptivity (Bailey and Nuhardiyati, in press), and we assume that this induced-female refractory period forms part of a postmating guarding behavior. Males that only have sperm to offer females may be under strong selection to overcome this female refractory period by producing an above average signal. Finally, if the presence of accessory gland fluid prevents females from mating again, perhaps in her lifetime, there is conceivably an adaptive advantage for females to mate with males that are low in accessory gland fluids. Simply put, the mating system suggests that there is an increase in net benefit to females responding positively to high intensity male signals; these are generally associated with higher quality males.

Duetting and Female Responsiveness

Females appear to duet with calling males in a highly structured way. They answer the “a + b” component of the signal with a latency close to 135 ms. The adaptive function of such timed calling patterns has been subject to considerable experiment within the duetting Orthoptera, where females reply within species distinct latencies following the male call (Zimmermann *et al.*, 1989; Robinson, 1990; Heller and Helversen, 1993, and see review Bailey, 2003).

Female duetting allows us the opportunity to test female preferences, and the simplest metric is the number of responses she makes both during the male signal and in a period following the call. Hence, one way of detecting a female's response to male calls is to measure the way she responds within a duet. There were two measures of female responsiveness: calls made during the male signal and those continuing calls once the male calls have stopped. We show significant differences in responsiveness between call types, with, not unexpectedly, the calling signal creating the most effective female response both within the male calling period and in the continuing period when the male was silent. Interestingly, females were responsive to the postcopulatory signal, and in this situation 20% of females responded by continuing to call when the stimulus stopped.

Precopulatory, Copulatory, and Aggressive Signalling

Most male leafhoppers emit faint short songs during copulation (Heady and Denno, 1991), but Claridge (1985b) points out that for many acoustic Auchenorrhyncha, precopulatory signals form a continuum with the calling signal. Signals produced by males during copulation have been

reported in the planthopper *Prokelisia marginata* (Heady and Denno, 1991) as well as in three species of *Nephotettix* (Nuhardiyati, 1998). Also signals produced by the cicadellid *Amrasca devastans* were considered as distinct precopulatory or copulatory signals, but it was unclear as to which sex was producing the call (Saxena and Kumar, 1984). In *B. incisa*, precopulatory signals are clearly produced by the male and the signal, although containing elements of the calling signal, appears markedly different.

For most insects the role of the precopulatory signal is to maintain contact between the pairs (Alexander, 1967), whereas the function of the copulatory signal may be to ensure that other males do not interrupt copulation (Wilcox and Stefano, 1991). Once the pair is coupled the signal is unlikely to have a role in maintaining copulation (but see Eberhard, 1996, for discussions on "copulatory courtship"). Interestingly, copulatory signals by male *B. incisa* were produced more often just before copulation concluded, when the female started to kick the male with her hind legs or drag the male away, eventually forcing the male to break genitalic contact (Nuhardiyati and Bailey, in press). A similar role has been suggested for copulatory signals in *Prokelisia marginata*, where the signal was associated with uncoupling (Heady and Denno, 1991).

Postcopulatory signals are usually emitted after separation and while the female is still nearby. Such postcopulatory behavior may either be a form of mate guarding, both reducing the chance of female remating and also lessening the chance of immediate male takeovers, or a signal to attract a new female. The chances of male takeovers might be high in dense populations as the male will seek courtship with another female immediately after copulation, and often females will be attracted to this postcopulatory signal. Further, as is common in the delphacid relatives, males are capable of copulating frequently and within a short time of the last copulation (see review by Ott, 1994).

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