Long-distance signals influence assessment of close range mating displays in the field cricket, *Gryllus integer*

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Male sexual displays often include components detected across long distances, and those perceived only at close range. Understanding what information females gain from each component of a complex display and how they use these signals to make decisions are questions of major interest in sexual selection research. We evaluated content-based hypotheses ('redundant signals' and 'multiple messages') for the courtship displays of field crickets (*Gryllus integer*) by measuring female responses to males' long-distance calling song (calls) and close-range chemical cues. Females' responses to a male's calls and chemical cues were uncorrelated, supporting the 'multiple messages' hypothesis. We also tested the 'inter-signal interaction' hypothesis by investigating how long-distance calls influence evaluation of close-range courtship. The relationship between long- and close-range signals was complex and conditional: females accepted close-range courtship more quickly after exposure to attractive calling song than they did after exposure to either unattractive calling song or silence, and unattractive calls were no more or less effective than silence. This inter-signal interaction could affect our understanding of mate choice in species with multiple mating signals because it implies that females may save time and energy by not assessing the close-range signals of attractive long-distance signalers. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 856–865.

ADDITIONAL KEYWORDS: acoustic signals – chemical cues – courtship – mate choice – multimodal signals – multiple cues – multiple messages – redundant signals.

INTRODUCTION

Female mating decisions are a complex force acting on the evolution of male phenotypes (Andersson, 1994). For example, females may perceive signals at different spatiotemporal scales (Candolin & Reynolds, 2001; Robson, Goldizen & Green, 2005), evaluate multiple components of the same signal (Gerhardt, 1991), and integrate signals that span several sensory modalities (Partan & Marler, 2005). Studying mate choice as a process rather than an outcome can provide insight on questions as diverse as the true costs of mating selectivity (Candolin, 2003), the origin and evolution of correlated traits (Johnstone, 1996),

and the role of sexual selection in speciation (Schluter & Price, 1993; Uy & Borgia, 2000).

Several reviews highlight hypotheses for the function of complex, multicomponent courtship displays (Candolin, 2003; Hebets & Papaj, 2005; Partan & Marler, 2005). For example, the redundant signals (or backup signals) hypothesis (Møller & Pomiankowski, 1993; Johnstone, 1996) suggests that each signal transmits similar information about male quality. Females might benefit from using redundant indicators of quality to check male honesty or to increase their assessment accuracy (Hebets & Papaj, 2005). By contrast, the 'multiple messages' (Møller & Pomiankowski, 1993; Johnstone, 1996) hypothesis holds that signals reflect different aspects of male quality and therefore vary independently of each other. Considering how females integrate and prioritize

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these signals is also crucial (Partan & Marler, 2005). For example, the 'inter-signal interaction' hypothesis (Hebets & Papaj, 2005) proposes that one signal affects the probability that females detect or respond to other signals.

Male field crickets provide females with acoustic signals and chemical cues, presenting an opportunity to test the redundant signals, multiple messages, and inter-signal interaction hypotheses. Most previous research on mate choice in crickets measures female responses to male calling song, a long-distance signal. Calling song is species-specific but females also discriminate among conspecific calls based upon their duration, rate, and frequency (Gerhardt & Huber, 2002). At close range, females use their antennae to detect male cuticular hydrocarbons (Adamo & Hoy, 1994; Tregenza & Wedell, 1997). Chemical cues can indicate sex (Otte & Cade, 1976; Tregenza & Wedell, 1997; Leonard & Hedrick, 2009a), species (Mullen *et al*., 2007), and relatedness (Simmons, 1990). Male cuticular hydrocarbons are heritable and particular subcomponents are associated with reproductive success (Thomas & Simmons, 2008, 2009). Even more importantly for the present study, female discrimination among chemical cues suggests that these cues function in mate choice. For example, female *Gryllus bimaculatus* use pheromones to discriminate against related males (Simmons, 1989, 1990), and female *Gryllus integer* preferentially associate with the chemical cues of males that later win intrasexual contests (Kortet & Hedrick, 2005). Despite this evidence that chemical cues function in mate choice, no previous study has explored how their attractiveness relates to that of other mating signals. We use the term 'chemical cues' rather than 'signals' or 'pheromones', reserving the term 'signal' for a trait that has shown clear evidence of selection for its effect on receivers (*sensu* Greenfield, 2002).

Males also produce a close-range courtship song, which may be condition-dependent (Hack, 1998; Rantala & Kortet, 2003; but see also Wagner & Reiser, 2000; Gray & Eckhardt, 2001) and influences mate choice in some species (Wagner & Reiser, 2000 Gray, 2004; but see also Nelson & Nolen, 1997; Tregenza *et al*., 2006). Although the role of courtship song in the intraspecific mate choice of *G. integer* is unclear, female *G. integer* require courtship song before mating. They cannot be coerced to mate because they must mount the male to receive a spermatophore.

Given the natural history of field crickets, both the redundant signals and multiple messages hypotheses provide plausible explanations for the presence of both long-distance and close-range communication. Because males usually do not produce courtship song until they have antennated the female (Tregenza & Wedell, 1997), a simple model of female crickets'

decision-making involves sequential assessment of three display components (calling song, chemical cues, and, finally, courtship song; Adamo & Hoy, 1994). Although females generally do not approach males with unattractive calls, they probably can be successfully courted by males who produce only close-range courtship, such as satellite males situated near callers or mobile males encountered in areas of high density (Hissmann, 1990; Cade & Cade, 1992; Zuk, Rotenberry & Tinghitella, 2006). Thus, female crickets might use redundant long-distance and close-range signals to verify that a courting male is the caller they approached; alternatively, nonredundant long- versus close-range signalling could allow females to efficiently assess which males merit further evaluation using additional criteria (Candolin, 2003). Because most studies measure female responses to either calling song or close-range signals, interactions between these stages of assessment are virtually unexplored (but see Wagner & Reiser, 2000). A male's calling song affects the probability that females will approach him (Gerhardt & Huber, 2002), although it is unknown whether calling song also influences assessment of close range signals or whether evaluation at close range operates independently of call assessment.

We tested the redundant signals and multiple messages hypotheses in *G. integer* by conducting two experiments. Experiment 1 examined whether the attractiveness of a male's calling song, courtship song, and chemical cues are related. If each signal or cue conveys information about the same aspect of male quality (redundant signals hypothesis), then the attractiveness of different signals or cues from the same male should be positively correlated (Hebets & Papaj, 2005; Partan & Marler, 2005). If signals or cues provide females with information about different aspects of male quality (multiple messages hypothesis), then the attractiveness of different signals or cues should not be positively correlated.

Experiment 2 examined how successive stages of signalling contribute to female mating decisions. We maintained signals in their usual sequence (calling song, then close-range courtship) but manipulated the attractiveness of a male's calling song. We compared the responses of different females to the same male, after exposure to either attractive calling song, unattractive calling song or silence. If long-distance signals influence females' close-range assessment of the male, then females pre-exposed to an attractive calling song should mount the male sooner than females exposed to unattractive songs or silence. Pre-exposure to unattractive calling song could also potentially bias females against the male, resulting in a longer latency to mount than that of females pre-exposed to silence. Alternatively, if females assess close-range signals independently from long-distance

signals, then their responses to the male should not depend upon the attractiveness of previous call exposures.

MATERIAL AND METHODS
GENERAL

We conducted our study on field crickets, *G. integer*, collected from fields surrounding Davis, CA, in 2005–2007. Prior mating histories of our subjects were unknown. The calling song of male *G. integer* consists of chirps with two or three sound pulses each (carrier frequency of approximately 4.2 kHz). Males call in trains of chirps, defined as bouts, comprising a period of calling with no pause greater than 100 ms (Fig. 1A) (Hedrick, 1988). A male's calling bout length is heritable, and females preferentially approach sources of calls with long bouts versus those with short bouts (Hedrick, 1986, 1988). By contrast to calling song, courtship song in *G. integer* consists of 4.2 kHz sound pulses interspersed with higher amplitude, higher frequency (13 kHz) single sound pulses (Fig. 1B). Previous research on *G. integer* showed that male chemical cues may also function in mate choice because females preferentially associated with the chemical cues of males that later won intrasexual contests with size-matched rivals (Kortet & Hedrick, 2005).

We housed crickets individually (males: waxed-paper containers, diameter 7.6 cm, height 5.7 cm; females, plastic boxes, length × width × height, 29.2 × 15.2 × 8.9 cm) with chick starter available *ad libitum* and water vials stuffed with cotton. We weighed all crickets at capture, and measured their pronotum widths. Behavioral trials were conducted in an acoustically-isolated chamber (length × width × height, 3.0 × 3.0 × 3.0 m) at 27 ± 1.5 °C, illuminated by a 25-W red incandescent light (14 lux at 60 cm from the experimental arena, described below). Call playbacks were presented via Harmon-Kardon speakers (#19.5), at 70 dbA SPL (measured 10 cm from the speaker, Simpson Sound Level Meter, Model #886, fast RMS). We used AV BIO-STATISTICS software (A. Vainikka, http://personal.inet.fi/koti/ansvain/avbs/) to document female responses to male songs and chemical cues, and JWATCHER, version 1.0 (http://www.jwatcher.ucla.edu) to score videos of courtship behaviors (recorded using a Canon ZR-300 MiniDV camera). Trials occurred in sand-lined arenas of variable dimensions (details given below). For chemical cues trials, we used fresh sand for each trial; for all other trials, we overturned sand between trials.

When necessary, we transformed data using $\log_{10}(x)$ when data were nonzero or $\log_{10}(x+1)$ to meet the assumptions of normality and equal variances; when this was not possible, we used nonparametric analyses (Zar, 1984; Siegel & Castellan, 1988). For multiple comparisons on the same data set, we used Bonferroni-adjusted values of α (Day & Quinn, 1989).

EXPERIMENT 1: ARE FEMALE RESPONSES TO MALE ACOUSTIC SIGNALS, CHEMICAL CUES, AND COURTSHIP CORRELATED?

We randomly assigned crickets to 70 experimental male-female pairs, and then measured the response of the female to the same male's calling song (day 1),
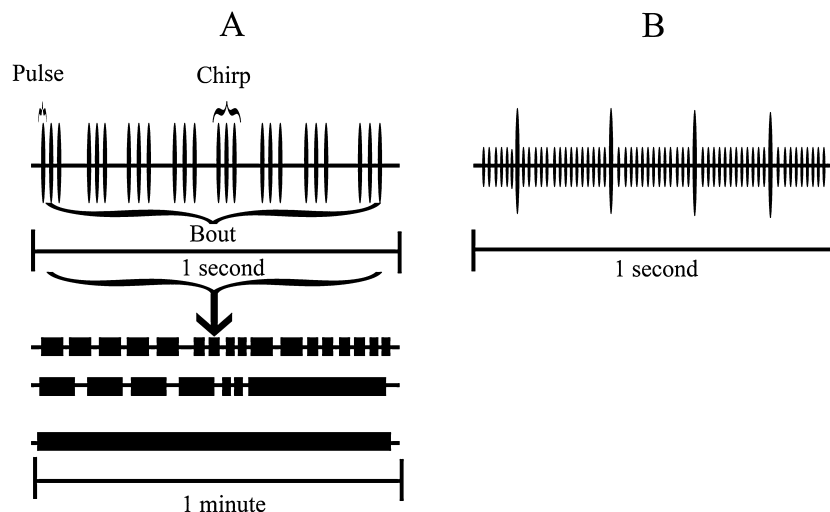
Figure 1. (A) Male *Gryllus integer* calling song. Top: males call in chirps consisting of two or three sound pulses; chirps are grouped into calling bouts (a period of calling with no pause greater than 100 ms). Bottom: males vary in the proportion of time spent calling in short bouts less than 5 s in duration (Hedrick, 1988). (B) Male *G. integer* courtship song. Males produce 4.2 kHz pulses, punctuated by relatively higher amplitude 13-kHz single sound pulses.

courtship song (day 1), chemical cues (day 2), and full courtship (day 3). We ran all trials between 14:00 and 20:00 h (Pacific Standard Time). We did not randomize the order of isolated signal or cue trials to control for carry-over effects, although females had 24 h between each trial from which data were analyzed (see Results), so we expected that any such effects would be minimal. A standard order ensured that signal/cue collection would have similar effects on male behavior and that experience during courtship trials would not alter signal production.

Recording calling song and measuring female response

We used the time that individual females spent near a playback of the call to assess call attractiveness. Time spent in association is widely used as a measure of female preferences for male phenotypes (Wagner, 1998; Walling *et al*., 2010), and phonotaxis (orienting toward or approaching calls) is commonly used to assess the attractiveness of male calls (Gerhardt & Huber, 2002). We recorded male calling songs by holding a male for 48 h in a small arena (length × width × height, $20.3 \times 20.3 \times 10.2$ cm) inside an acoustically isolated chamber (length × width × height, $60.0 \times 35.0 \times 30.0$ cm) at 27 ± 1.5 °C, and recording digital WAV files of his song using a Sony 959a microphone positioned 5.0 cm above the arena center, sound software (Adobe Audition 1.0, Adobe Systems Incorporated; sample rate: 44 kHz, resolution: 16-bit) and a laptop computer. From calls recorded during this period, we selected an approximately 10-min sample of steady calling (mean ± SD, 578.9 ± 67.1 s).

We ran calling song trials in a $53.3 \times 36.8 \times 30.5$ cm (length × width × height) arena. Speakers were on opposite sides of the arena, one broadcasting the 10-min sample of the male's calling song, and the other silent. The position of the broadcasting speaker was switched between trials. At the start of the trial, we released the female from a plastic vial midway between the two speakers after a 1-min acclimation period; for the next 10 min, we recorded the time spent within one body length (approximately 4 cm) of each speaker.

Recording courtship song and measuring female response

To elicit courtship song, we paired the male with a stimulus female in a small arena (length × width × height, $20.3 \times 20.3 \times 10.2$ cm). A different female was used for each male. Using the recording equipment described above, we held the microphone approximately 1 cm behind the male's wings to record a variable-length sample of courtship song (mean ± SD, 66.2 ± 52.3 s).

We measured female responses to courtship song in trials 40–60 min after calling song trials, using the same procedures and arena. We were unable to record courtship song from 11 males. To ensure that each of these 11 males' assigned female underwent the same number of trials as other females, we presented a courtship song randomly selected from our pool of males (we did not analyze data from these 11 trials, reducing sample size for courtship song comparisons to 59 pairs).

Collecting male chemical cues and measuring female response

We collected chemical cues by placing a male on a filter paper disc (diameter 3.4 cm) inside a Petri dish (diameter 3.5 cm) for 9 h. Immediately before the trial, we removed the male and transferred the paper to the experimental arena (length × width × height, $20.3 \times 20.3 \times 10.2$ cm). This technique allowed us to collect chemical cues from live males and permitted subsequent courtship trials using these same males. Previous studies showed that female crickets discriminate between filter papers previously occupied by crickets varying in sex (Leonard & Hedrick, 2009a), relatedness (Simmons, 1990), and fighting ability (Kortet & Hedrick, 2005). We positioned the male's paper as well as a same-sized blank control piece of filter paper on opposite sides of the arena, 1.5 cm apart. At the start of the trial, we released the female from a plastic vial in the center of the arena; for the next 10 min, we recorded the time females spent touching each paper disc with their antennae. Because crickets detect chemicals through antennal contact (Rence & Loher, 1977), we only analyzed data for females that touched the male's paper for longer than 1 s ($N = 61$). Two males died before the chemical cues trials, reducing the sample size to 59 male-female pairs.

Courtship trials

We measured the female's response to the male's complete courtship display in courtship trials during which calling song, chemical cues, courtship song, and tactile stimuli (plus any perceptible visual stimuli) were available. Before trials began, we held the female in a screened cylinder (diameter 10.8 cm, height 6.4 cm) in the center of the arena (length × width × height, $20.3 \times 20.3 \times 10.2$ cm) for 10 min during which we played back the male's calling song (previously heard by the female in her calling song trial) from a speaker located 5.0 cm behind the arena (males did not hear the call). After stopping the playback, we introduced the male to the arena, removed the screened chamber, and videotaped courtship until the female mounted the male. Trials lasted a maximum of 30 min. We excluded trials in which the

male did not produce courtship song ($N = 15$). Five males died before the courtship trial, resulting in a sample size of 50 pairs. When scoring videotapes, we measured the latency to mount, defined as the time between the onset of male courtship song and female mounting. This measure is often used to assess male crickets' attractiveness in 'no-choice' trials (Shackleton, Jennions & Hunt, 2005; Sakaluk & Ivy, 2007); we assumed that latency reflects females' reluctance to mate, rather than males'. We assigned females that did not mount a maximum latency of 30 min.

EXPERIMENT 2: DOES CALLING SONG AFFECT FEMALE RESPONSE TO CLOSE-RANGE SIGNALLING?

In 36 replicate courtship trials, we compared responses to the same male of females randomly assigned to three treatment groups ($N = 108$ females, $N = 36$ males). At the start of a trial, we transferred a female to a screened pre-exposure chamber (height 20.3 cm; diameter 7.6 cm) in the center of the arena (length × width × height, $18.0 \times 18.0 \times 10.0$ cm). Within each replicate, the three females experienced one of three 15-min acoustic pre-exposures: silence, a call with short bouts (mean ± SD, 0.95 ± 0.27 s, all < 5 s) or a call with long bouts (mean ± SD, 296.0 ± 21.5 s, all ≥ 5 s). We presented playbacks from a speaker 10 cm from the arena center (SPL 70 dbA, fast RMS, 10.0 cm from the speaker). In previous isolated presentation trials using these stimuli (Leonard & Hedrick, 2009b), female *G. integer* spent more time in association with the long bout playback than the short bout playback. Playbacks were constructed from the same digital chirp (pulse period = 0.13 s, chirp pause = 0.39 s, three pulses per chirp, average values for the Davis, CA, population of *G. integer* at 25 °C, Hedrick & Weber, 1998) and thus were matched for all acoustic parameters besides calling bout length, unlike in Experiment 1.

At the end of the acoustic pre-exposure period, we introduced the male into the mating arena (males did not hear the playback), removed the female's chamber and videotaped interactions for 15 min. We stopped trials when the female mounted the male, gently separating the pair to prevent spermatophore transfer. Between trials, we returned the male to his container for a 15-min rest period during which we pre-exposed the next female. We excluded some trials ($N = 3$) from the analysis because the male failed to court each female. We rotated the order of pre-exposures across replicates.

When reviewing videos of courtship, we measured both the female's latency to mount (with non-mounters assigned a maximum latency of 900 s) and the proportion of females within each treatment group that mounted the male.

RESULTS

EXPERIMENT 1: ARE FEMALE RESPONSES TO MALE ACOUSTIC SIGNALS, CHEMICAL CUES, AND COURTSHIP CORRELATED?

First, we verified that females responded to our presentations of isolated signals. As a group, females spent more time near the active speaker versus silent speaker in call trials (Wilcoxon signed rank test: $Z_{70} = 3.972$, $P < 0.001$), and spent more time with male papers versus control papers in chemical cues trials (Wilcoxon signed rank test: $Z_{58} = -4.672$, $P < 0.001$). However, females did not spend more time near the speaker playing back courtship song versus the silent speaker (Wilcoxon signed rank test: $Z_{58} = 0.492$, $P = 0.626$). Hence, female *G. integer* did not demonstrably respond to the isolated presentation of courtship song. Although the reasons for this are unclear, we speculate that females may require priming by a combination of other stimuli before courtship song becomes relevant. Because an investigation of this possibility was beyond the scope of the present study, we restricted our analysis to the results of the calling song, chemical cues, and courtship trials.

Did the attractiveness of a male's calling song predict the attractiveness of his chemical cues? With a sample size of 58, our power to detect a correlation of at least $r = 0.36$ between female responses to male calling song and responses to chemical cues was 0.80 (SIGMAPLOT, version 11.0, Systat Software). Nonetheless, we found no association between the time females spent near a male's calling song playback and time spent with that male's chemical cues ($\log_{10}(x + 1)$ transformed data: $r = -0.009$, $N = 58$, $P = 0.944$; Fig. 2).

We next investigated whether a female's response to any one signal predicted her response to the male's full courtship display. Across all females, including those that did not mount, a female's latency to mount males in courtship trials was neither related to her response to his calling song, nor chemical cues, nor to a simple interaction between these factors (multiple linear regression on $\log_{10}(x)$-transformed latency data: $r^2 = 0.006$, $F_{3,45} = 0.0851$, $P = 0.968$). Restricting analysis to only those females that mounted the male did not change these results (multiple linear regression on $\log_{10}(x)$-transformed latency data: $r^2 = 0.0603$, $F_{3,41} = 0.856$, $P = 0.472$).

COURTSHIP FACTORS ASSOCIATED WITH LATENCY TO MOUNT

We also considered male body size (pronotum width) and body condition (residual of mass on pronotum width, to the third power; Jakob *et al*., 1996)
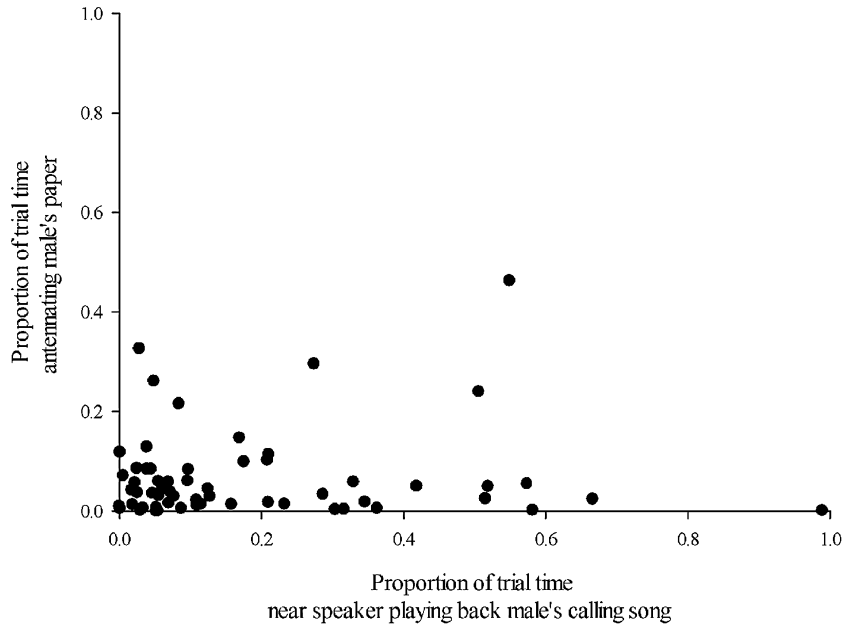
Figure 2. Experiment 1 showed no correlation between the time females spent near the speaker playing back the male's calling song and the male's chemical cues. For statistical analysis, see text.

because these traits influence mating success in other cricket species (Simmons, 1988; Bateman, Gilson & Ferguson, 2001). Neither male body size, nor condition explained how quickly females mounted (multiple linear regression on $\log_{10}(x)$ transformed latency data: $r^2 = 0.003$, $F_{2,49} = 0.223$, $P = 0.639$). However, we noted a trend for females to discriminate against males in low condition ($r = -0.191$, $N = 50$, one-tailed $P = 0.091$; three of four males not mounted at the end of the trial were in the lowest quartile).

EXPERIMENT 2: DOES CALLING SONG AFFECT FEMALE RESPONSE TO CLOSE-RANGE SIGNALLING?

To determine whether females evaluate close-range courtship signals independently from long-distance signals, we compared the responses of females to the same male's close-range courtship after different acoustic pre-exposures. Females pre-exposed to the call with long bouts mounted the male more quickly than females pre-exposed to either the call with short bouts or silence (Friedman repeated measures analysis of variance: $\chi^2 = 25.528$, d.f. = 2, $P < 0.001$; Tukey's test $P < 0.05$: silence versus long, short versus long; not significant: silence versus short; Fig. 3). Also, a larger percentage of females pre-exposed to the long playback mounted the male than those females pre-exposed to the short or silence treatments (long: 96%; short: 76%; silence: 82%; Cochran's Q-test: $Q_2 = 7.09$, $P < 0.05$).

DISCUSSION

Mate choice is often a sequential process based upon signals received at different distances or time points (Gibson, 1996; Candolin, 2003; Suk & Choe, 2008). Whether females use each signal to make an independent assessment of male quality, or whether earlier signals shape responses to later signals, is an important question for researchers studying complex mating displays (Rowe, 1999; Hebets & Papaj, 2005; Partan & Marler, 2005). The results obtained in the present study demonstrate that mating signals encountered early in a temporal sequence may bias females' assessment of close-range displays. We found that the attractiveness of long-distance and close-range signals are not necessarily correlated (Experiment 1); moreover, when we exposed females to male signals in a sequence similar to that often encountered in the field (Experiment 2), females pre-exposed to highly-attractive calling song mounted the same male faster than females pre-exposed to less attractive calling song or silence.

Although numerous species use acoustic signals (Gerhardt & Huber, 2002) and chemical cues (Johansson & Jones, 2007; Coleman, 2009) during courtship, the present study is the first, to our knowledge, to consider content-based hypotheses for both of these modalities within one species. The results obtained are inconsistent with the 'redundant signals' hypothesis for *G. integer*'s acoustic signals and chemical cues: male crickets with attractive calls do not
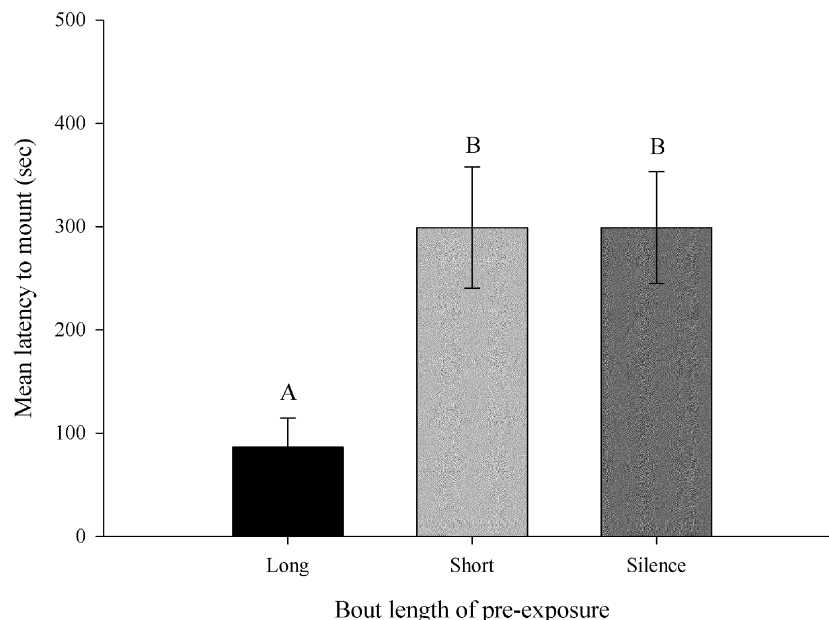
Figure 3. Responses of groups of three females exposed to different calls before being paired with the same male (Experiment 2). Females pre-exposed to a long-bout call mounted the male more quickly than females pre-exposed to a short-bout call or silence. Different letters indicate statistically significant differences.

necessarily have attractive chemical cues, and we could influence a females' response to a particular male by altering her perception of his calling song.

COMPLEX MATING SIGNALS IN CRICKETS

Many studies have measured female crickets' responses to acoustic (Gerhardt & Huber, 2002) or chemical (Simmons, 1990) stimuli, although few have considered how multiple components of the male display influence mate choice. Among these, studies within the acoustic modality do not support the 'redundant signals' hypothesis. In *Gryllus campestris*, some aspects of call structure reflect adult diet quality, whereas others reflect juvenile diet quality (Scheuber, Jacot & Brinkhof, 2003a, b, 2004). Similarly, in *Gryllus lineaticeps*, diet quality affects males' calling song, but not courtship song; furthermore, chirp rates in calling song (a target of female preference) versus courtship song are uncorrelated (Wagner & Hoback, 1999; Wagner & Reiser, 2000).

Our experiments on acoustic and chemical modalities also do not support a redundancy hypothesis. Nonredundant indicators of quality could influence sexual selection on male advertisement signals in this and similar systems. For example, if long- versus close-range components signal different aspects of male quality, then females risk mating with males other than those whose calls attracted them from a distance, and if females encounter males at close range in high-density populations, they may make decisions based upon aspects of quality other than those encoded in calling song. In *G. integer*, calling song can inform a female about a male's heritable call attractiveness (Hedrick, 1988), body condition (Hedrick, 2005), predator-avoidance behavior (Hedrick, 2000), and the presence of a spermatophore (Loher, 1989), whereas chemical cues transmit information about a male's fighting ability (Kortet & Hedrick, 2005). The available evidence to date suggests that calling song and fighting ability are not necessarily correlated (A.V. Hedrick, unpubl. data).

The findings obtained in the present study are also consistent with additional hypotheses besides the 'multiple messages' hypothesis. For example, certain display components could be directed towards females and others towards male competitors, or components might reflect selection for dual roles in mate attraction and intrasexual competition (Berglund, Bisazza & Pilastro, 1996). Male *G. integer* respond to both the calling song and chemical cues of competitors (Leonard & Hedrick, 2009a, b); although calling song clearly functions in mate attraction (e.g. Experiment 2; Hedrick, 1986), a long-bout call also attracts males who might displace the caller (Leonard & Hedrick, 2009b). Moreover, although female *G. integer* discriminate among male chemical cues (Kortet & Hedrick, 2005), perhaps these cues function primarily for signalling fighting ability to other males.

Even if all courtship signals are directed exclusively at females, they might transmit messages unrelated to quality, such as species identity or location (Hebets & Papaj, 2005). Indeed, we have found that the close-range courtship song of *G. integer* likely functions in species recognition, and that one function of chemical cues is sex recognition (Leonard & Hedrick, 2009a). Finally, our results might simply reflect differences among *G. integer* females in their prioritization of calling song versus chemical cues (Candolin, 2003). However, we think that this is unlikely because almost all females mounted males significantly more quickly after exposure to attractive versus unattractive calling song (Experiment 2).

Interaction between stages of mate assessment

The results obtained in the present study establish that long-distance and close-range signals can interact to influence female mating decisions. Although assessment of multiple signals takes time and energy (Fawcett & Johnstone, 2003), females might reduce assessment costs if they use signals early in the encounter sequence to determine which males merit further evaluation (Candolin, 2003). However, if an early signal dominates female assessment, then it may not matter how attractive close-range signals are: female *G. integer* may reduce costs by not assessing the downstream signals of males with highly-attractive calls (but see Wagner & Reiser, 2000). Although Experiment 2 demonstrated that exposure to attractive calling song speeds up close-range courtship, information provided by males at close range is clearly relevant to females in many cases (Shackleton *et al.*, 2005). For example, we could not predict females' latency to mount the male from the females' (previously measured) responses to his calling song (Experiment 1), and female *G. integer* obviously respond to male chemical cues (Experiment 1; Kortet & Hedrick, 2005; Leonard & Hedrick, 2009a). This suggests a possible conditional interaction (Patricelli, Uy & Borgia, 2003; Coleman, Patricelli & Borgia, 2004) between long-distance and close-range signalling. For example, highly-attractive calling songs might lead females to accept a wide array of males' close-range displays, whereas exposure to average or mediocre calling songs might lead them to accept a narrower set of close-range courtship displays.

In conclusion, the interface between females' long-distance and close-range decision-making during mate assessment reveals an under-appreciated complexity. Not only do female field crickets likely receive different information at each stage, but also a female's response to the complete display cannot be predicted from signals in isolation. Sequential interactions may shape mate assessment because an attractive signal early in the sequence not only increases the probability that a female will continue to evaluate the male, but also may affect females' assessment of downstream signals. Thus, when mating displays consist of a series of signals, untangling complex inter-signal interactions may be required to understand the form and force of selection exerted by female choice on male phenotypes.

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