Single versus multiple cues in mate discrimination by males and females

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Courtship communication can function in both species recognition and mate choice. Little is known about how animals prioritize signals or cues for species identification versus intraspecific mate choice when several information sources are available, such as when communication spans several sensory modalities or spatiotemporal scales. Cricket courtship, for example, involves transmission of acoustic signals as well as chemosensory contact. We explored how chemical cues function in sex and species recognition for both male and female crickets, and then evaluated their use in a mating context where additional stimuli were available. First, we observed the response of female and male *Gryllus integer* to the chemical cues of conspecifics and sympatric *G. lineaticeps*. Males' strongest response was to conspecific female chemical cues. Although females responded most strongly to male chemical cues, they did not show species discrimination. Next, we compared the responses of male and female *G. integer* to conspecifics and heterospecifics in mating trials. Females directed more aggressive behaviour and less chemosensory behaviour towards heterospecific males, but males courted females of both species with equal intensities. These results suggest a sex-based difference in cue usage: for males, the additional stimuli present during mating trials appeared to override species-specific chemical cues, whereas for females, additional stimuli apparently bias responses towards conspecifics.
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Courtship commonly involves the exchange of signals spanning several sensory modalities and spatiotemporal scales. One content-based explanation for complex signalling is that beyond transmitting information about quality, components of a mating display indicate species identity, an approach recently formalized as the species-recognition hypothesis for complex displays (Candolin 2003; Hebets & Papaj 2005). This use of multiple signals or cues is thought to be especially likely when the values of traits that females use to assess conspecific male quality overlap with heterospecific male trait values, creating a conflict between species recognition and mate choice (Pfennig 1998). For example, female swordtail fish (*Xiphophorus pygmaeus*) prefer larger males, and accordingly prefer males of a larger sympatric species in visual choice tests (Ryan & Wagner 1987; Hankison & Morris 2002). It is only when females have access to both species-specific chemical and visual cues that they prefer conspecific males (Hankison & Morris 2003). These results suggest that by integrating information from several sensory modalities, females can balance preferences for high-quality males with preferences for conspecific males: they shift their responses depending on the information available. While

several recent models address the cognitive processes underlying the integration of multiple signals in species recognition versus mate choice (Castellano & Cermelli 2006; Phelps et al. 2006), relatively few empirical studies have explored how individuals use multiple signals or cues to select mates that are not only conspecifics, but also of high quality (McClintock & Uetz 1996; Hankison & Morris 2003; Gumm et al. 2006; Phelps et al. 2006). Many courtship displays involve a particular species-specific component, but little is known about how individuals prioritize this information when faced with additional signals and cues. Moreover, since courtship communication is often studied from the perspective of female receivers (e.g. Candolin 2003), few studies have directly compared the kinds of information that both sexes gain from signals exchanged in courtship. In this paper, we examine how male and female field crickets, *Gryllus integer*, use chemical cues exchanged during courtship in intraspecific mate choice and species recognition, and then ask how these cues are used in a mating context where additional stimuli are available.

In crickets, sexually receptive females respond to male acoustic signals (calls) by moving towards the calling male. Male calls are species specific and ordinarily serve to attract conspecific females from a distance (e.g. Alexander 1962). Besides having species-specific preferences for the temporal properties of their own species' calling song (but see Doherty & Howard 1996), female crickets also show intraspecific preferences for characteristics of the song such as amplitude, frequency, duration and rate of signalling (reviewed in Gerhardt & Huber 2002). Once a female has

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reached a calling male, however, courtship becomes multimodal: males switch to a softer 'courtship song' and both sexes contact each other with their antennae and palps, a tactile and chemosensory interaction that characterizes much of courtship (Alexander 1961; Rence & Loher 1977; Adamo & Hoy 1994).

In areas of high density, male and female field crickets may encounter conspecifics and heterospecifics at close range; under these circumstances, males do not need to attract females from a distance, and often do not call (personal observation; see also Alexander 1961; Cade & Wyatt 1984; Hissmann 1990; Gray 2004). Instead, they proceed directly to multimodal close-range courtship, in which acoustic, chemical and tactile signals or cues could provide information either similar to that of the male's call or information unique to the interaction (reviewed in: Rowe 1999; Candolin 2003; Partan & Marler 2004; Hebets & Papaj 2005).

Courtship song is thought to function in intraspecific mate choice (Wagner & Reiser 2000; but see Nelson & Nolen 1997), but its role in species recognition is less well understood: in the single field cricket species pair studied (the sister species *G. texensis* and *G. rubens*: Fitzpatrick & Gray 2001; Gray 2004), courtship song biased female responses towards conspecifics, but not to the degree necessary to prevent interspecific matings (Gray 2004).

More information is available on the function of chemical cues exchanged in courtship. Herein we conservatively refer to chemical 'cues' rather than chemical 'signals' or pheromones, reserving the term 'signal' for stimuli that both mutually benefit both senders and receivers and that have been selected for their effects on receivers (sensu Greenfield 2002). Chemical cues are used in sex recognition by male crickets (e.g. Rence & Loher 1977; Hardy & Shaw 1983; Adamo & Hoy 1994; Tregenza & Wedell 1997), and contact chemical cues can provide female crickets with information on a male's relatedness (Simmons 1990; Thomas & Simmons 2008), prior mating history (Ivy et al. 2005) and fighting ability (Kortet & Hedrick 2005). Within a genus, cricket species can differ in their cuticular hydrocarbons (*Laupala* spp.: Mullen et al. 2007, 2008) and some studies have suggested that males might use chemical cues to distinguish conspecifics from heterospecifics (Paul 1976; Otte & Cade 1976; but see Hardy & Shaw 1983). Thus, chemical cues could function in both mate choice and species recognition (e.g. Alexander 1961; Gray 2004; Kortet & Hedrick 2005). To determine whether chemical cues function in species recognition by *G. integer* and how individuals prioritize information obtained from these cues during courtship, we examined how both male and female crickets use close-range chemical cues in mating decisions. Specifically, we investigated whether decisions based on chemical cues alone match those made when additional sensory cues (e.g. courtship song, tactile and visual stimuli) are available during close-range courtship.

METHODS

We conducted four interrelated experiments designed to identify what kind of information close-range chemical cues provide to male and female crickets, and asked how that information is used by each sex in a mating context.

First, we measured the response of both field-caught males (experiment 1) and females (experiment 2) to conspecific male and female contact chemical cues, as well as to chemical cues collected from heterospecifics of the opposite sex and to blank controls. We measured males' production of courtship song after antennal contact with females, an assay that rendered our results comparable to previous research using similar protocols (e.g. Rence & Loher 1977; Adamo & Hoy 1994; Tregenza & Wedell 1997). Female crickets do not produce courtship song, so we measured their responses using a different assay (similar to: Simmons 1990; Kortet & Hedrick 2005): we observed their response to chemical cues on

paper. Since these protocols differed, we also ran experiment 3 on laboratory-reared crickets (chemical cues on paper for both sexes) to permit direct sex-based comparisons. We tested three non-mutually exclusive hypotheses about the role of chemical cues in mating. The first hypothesis is that chemical cues simply provide information about the presence of another cricket; this hypothesis predicts that males and females will react more strongly to presentation of cricket chemical cues than to presentations of control stimuli. The second hypothesis is that chemical cues contain information about the sex of their producer, predicting a stronger response to chemical cues from the opposite sex than to chemical cues of the same sex. The third hypothesis is that cricket chemical cues contain information about the species of their producer, predicting that males and females should respond more strongly to conspecific chemical cues than to those of heterospecifics.

Finally, in experiment 4 we observed inter- and intraspecific pairings to determine how information from chemical cues is integrated with other courtship signals in mating decisions. We made the following predictions.

(1) If response to a single (contact chemical) cue accurately predicts the outcome of multimodal courtship interactions, then selectivity for conspecific chemical cues should predict the degree of species discrimination in mating trials. For example, species discrimination in response to chemicals should be matched by courtship behaviour directed exclusively towards conspecifics, and a lack of discrimination between conspecific and heterospecific chemicals should correspond with indiscriminate courtship behaviour in mating trials.

(2) If response to a single cue (contact chemical) does not predict the outcome of multimodal courtship, then selectivity for conspecific chemical cues will not predict species discrimination in mating trials. For example, if chemical species-recognition cues are overridden by the additional acoustic, visual and tactile stimuli exchanged during courtship, then crickets should show species recognition in response to chemical cues but mate indiscriminately in mating trials.

If signals other than chemical cues provide the mechanism of species recognition, crickets should react similarly to conspecific and heterospecific chemical cues but direct courtship only towards conspecifics in mating trials.

Subjects

All four experiments were conducted on *G. integer* and *G. lineaticeps*, sympatric species whose mate choice and signalling behaviour are characterized by work spanning two decades (*G. integer*: Hedrick 1986, 1988; Hedrick & Weber 1998; *G. lineaticeps*: Hoback & Wagner 1997; Wagner & Hoback 1999; Wagner & Reiser 2000). Although they are not closely related within the genus (Huang et al. 2000), these species' distributions overlap throughout California (Singing Insects of North America Web site, T. Walker, Webmaster; http://buzz.ifas.ufl.edu), and in Davis, CA, U.S.A., both species are commonly observed occupying the same cracks in the ground (personal observation). *Gryllus lineaticeps* tend to be larger, but the body sizes of the species overlap (masses of field-caught crickets used in mating study: $\overline{X} \pm$ SE: male *G. integer*: 0.554 ± 0.093 g; male *G. lineaticeps*: 0.694 ± 0.180 g; female *G. integer*: 0.580 ± 0.120 g; female *G. lineaticeps*: 0.758 ± 0.190 g). The two species' calling songs differ markedly (Fig. 1a), despite an overlap in carrier frequency (*G. integer*: 4.2–4.9 kHz; *G. lineaticeps*: 4.5–5.17 kHz; Hedrick & Weber 1998; Wagner & Basolo 2007; A. S. Leonard, unpublished data, Singing Insects of North America Web site, T. Walker, Webmaster; http://buzz.ifas.ufl.edu). The species' courtship songs are more similar than their calling songs: in addition to low-amplitude pulses in the 4–5 kHz range, they each are characterized by higher-amplitude single sound pulses with
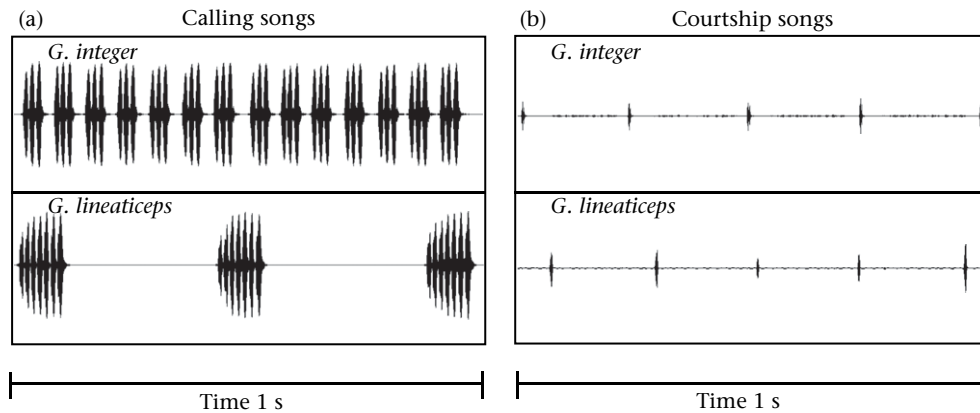
Figure 1. (a) Calling songs of *G. integer* and *G. lineaticeps*. (b) Courtship songs of *G. integer* and *G. lineaticeps* (high-amplitude component).

frequency peaks at 4–5 and at 14–15 kHz (Wagner & Reiser 2000; A. S. Leonard, unpublished data; Fig. 1b). There is overlap in the duration (*G. integer*: 4.6–10.1 ms; *G. lineaticeps*: 2.3–95.7 ms) and in the rate (*G. integer*: 3–6 pulses/s; *G. lineaticeps*: 2–8 pulses/s) of louder pulses as well as in the rate of softer pulses (Wagner & Reiser 2000; A. S. Leonard, unpublished data). Playback trials of courtship songs revealed that females of both species do not preferentially associate with conspecific or heterospecific courtship song (A. S. Leonard, unpublished data). Thus, females may not be able to use courtship song alone to discriminate between species at close range. Postinsemination reproductive isolation mechanisms are a possibility in this species pair (as in ground crickets; Gregory & Howard 1994); however, for other *Gryllus* species, postzygotic isolation mechanisms appear to be absent (e.g. the sister taxa *G. rubens* and *G. texensis*; reviewed in Gray & Cade 2000).

We collected male and female crickets from fields surrounding Davis, CA, U.S.A. (2006–2007). We housed crickets in the laboratory on a 12:12 h light:dark cycle in individual containers (males: 7.6 diameter × 5.7 high; females: 29.2 × 15.2 × 8.9 cm, L × W × H), and provided them with cotton-plugged water vials and ad libitum access to Purina chick starter. Since crickets for experiments 1 and 2 were collected in the field, their previous mating history and their exposure to conspecifics and heterospecifics were unknown. In experiments 3 and 4, we used virgin laboratory-reared offspring of field-caught mothers as subjects, and housed them individually (as described above) after the penultimate instar. We attempted to transform all data to meet the assumptions of normality and equal variances; when this was not possible, we used nonparametric tests. We used two-tailed significance tests (except for directional hypotheses, which were one-tailed tests), unless otherwise noted.

EXPERIMENT 1: RESPONSE OF FIELD-CAUGHT MALE *G. INTEGER* TO CONSPECIFIC AND HETEROSPECIFIC CHEMICAL CUES

Methods

Presentation cylinder

The presentation cylinder we used in experiment 1 permitted subjects to contact only the antennae of the stimulus cricket (Fig. 2). This degree of contact approximates antennal contact during natural encounters. The presentation cylinder consisted of an opaque plastic vial (54 mm × 17 mm diameter) with a small window at one end (15 × 4 mm) through which the stimulus cricket's antennae (or control bristles) protruded and generally remained motionless (A. S. Leonard, personal observation); any withdrawn antennae were gently extracted with a bent insect pin.

A soft cotton plug in the open end of the presentation cylinder oriented the stimulus cricket forward. A clean, separate presentation cylinder was used for each treatment group, preventing cross-contamination of chemical cues.

Presentation of stimulus antennae

We used 120 male *G. integer* as subjects in this experiment. The antennae of 27 *G. integer* and 23 *G. lineaticeps* females as well as 27 *G. integer* males were used as stimuli in trials. Those crickets whose antennae were used multiply as stimuli were given at least 24 h between trials to minimize the accumulation of male chemical cues on their antennae. Although studies on other field cricket species have established that the male courtship song is triggered by chemical, not tactile, stimulation (Rence & Loher 1977; Hardy & Shaw 1983; Adamo & Hoy 1994), we included a control treatment to establish baseline rates of response to a stimulus that visually and mechanically resembled cricket antennae but did not contain cricket chemical cues. These control stimuli were fashioned from natural bristles cut from a 1-inch (2.54 cm) paintbrush (Ace Hardware One Coat no. 11023). Since bristles resemble cricket antennae in thickness ($\overline{X} \pm$ SD: bristles: 140.0 ± 32.0 μm; antennae: 150.0 ± 22.0 μm, five of each measured at midlength) and colour, previous studies have also successfully used this technique to mimic antennae (e.g. Hack 1998). For each control presentation ($N = 30$), fresh bristles were cut and mounted within the presentation cylinder using duct tape.
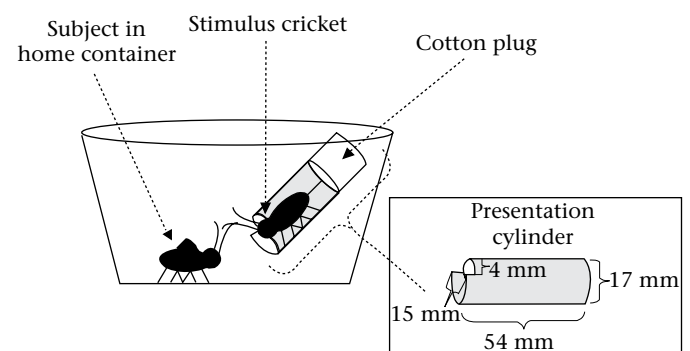
Figure 2. Detail of antennal presentations to field-caught male *G. integer*. Subjects remained in their home containers, while a stimulus cricket restrained in the presentation cylinder was gently lowered down. Antennal contact occurred between the subject and stimulus cricket through a small window in the presentation cylinder.

Trials

We conducted trials between 0800 and 1600 hours (Pacific Standard Time, PST), and used males only if they produced calling song during the 30 min preceding the trial. Just before the trial, we moved subjects within their home containers into an acoustically isolated room. The room was illuminated by a 25-W red incandescent light (14 lx at 60 cm) to mimic nocturnal conditions. The lids of the subjects' containers were then removed, and they were given a 2 min adjustment period.

At the start of the trial, we brought the presentation cylinder (with the stimulus cricket or control bristles inside) into the chamber and lowered it towards the male's home container. An observer (A.S.L.) gently touched the male's antennae with the antennae of the cricket (or control bristles) inside the presentation cylinder. Antennal contact with the subject was maintained continually in a uniform manner; note that previous studies report that varying the tactile component of antennal stimulation has little effect on the production of male courtship song (Rence & Loher 1977; Loher & Rence 1978). Trials lasted for a maximum of 10 min and stopped when the male produced courtship song. We recorded whether the male produced courtship song and the latency to produce courtship song among responding males. When conducting our three comparisons of the proportion of males in each treatment group that produced courtship song, we used z tests with the Yates correction for continuity (Glantz 2005) and a Bonferroni-adjusted α of 0.016. For our two latency comparisons (see below), we used t tests with an α of 0.025 (Day & Quinn 1989).

Results

Production of courtship song

Figure 3a shows the percentage of males that produced courtship song in response to antennae or bristles. Control bristles were only minimally effective in eliciting courtship song (from 6.7% of males), whereas 57.7% of males exposed to cricket antennae (male, conspecific female and heterospecific female) produced courtship song (control = 2/30, crickets = 52/90; z test with Yates correction: $z = 4.67$, one-tailed $P < 0.001$).

Males also biased their production of courtship song towards female antennae. Whereas only 33% of males exposed to conspecific male antennae produced song, 70% of males exposed to either conspecific or heterospecific female antennae did so (male = 10/30, female = 42/60; z test with Yates correction: $z = 3.094$, one-tailed $P = 0.001$).

Finally, males were more likely to produce courtship song in response to females of their own species than in response to *G. lineaticeps* females (90% versus 50%), showing clear evidence of chemical-based species recognition (*G. integer* females = 27/30, *G. lineaticeps* females = 15/30; z test with Yates correction: $z = 3.099$, one-tailed $P = 0.001$).

Latency to produce courtship song

Since only two males responded to the control antennae, we restricted our analysis of latencies to males that responded to cricket antennae. We found no significant differences in latency to produce courtship song in response to male versus female antennae (t test on square-root-transformed data: $t_{50} = 1.814$, one-tailed $P = 0.038$) or in response to conspecific female versus heterospecific female antennae (t test on square-root-transformed data: $t_{40} = -0.573$, one-tailed $P = 0.715$).

## EXPERIMENT 2: RESPONSE OF FIELD-CAUGHT FEMALE *G. INTEGER* TO CONSPECIFIC AND HETEROSPECIFIC CHEMICAL CUES

Methods

Subjects and chemical cue sources

We used 100 female *G. integer* as subjects in this experiment. Three groups of crickets served as sources of chemical cues: female *G. integer* ($N = 24$), male *G. integer* ($N = 20$) and male *G. lineaticeps* ($N = 19$). In each case, we collected chemical cues from the stimulus cricket by placing the cricket on a piece of filter paper (1.27×5.08 cm) within an opaque black film canister (5.08 cm $\times 3.18$ cm diameter) for 24 h before the trial. We used a fresh canister for each trial to prevent contamination between treatments.

Trials

In all trials, we presented females with a canister containing a piece of filter paper. Canisters had previously housed one of four treatments: No cricket (a control measure of response to a canister containing unscented paper), *G. integer* male, *G. integer* female or *G. lineaticeps* male. Trials took place between 1200 and 1700 hours (PST) in an acoustically isolated room. Before the trial, we removed the stimulus cricket from the canister and placed the canister (with paper inside) in the centre of an opaque plastic arena ($14.0 \times 26.7 \times 40.0$ cm, H $\times$ L $\times$ W) filled to a depth of approximately 1 cm with sand. Canisters were partially buried in the sand
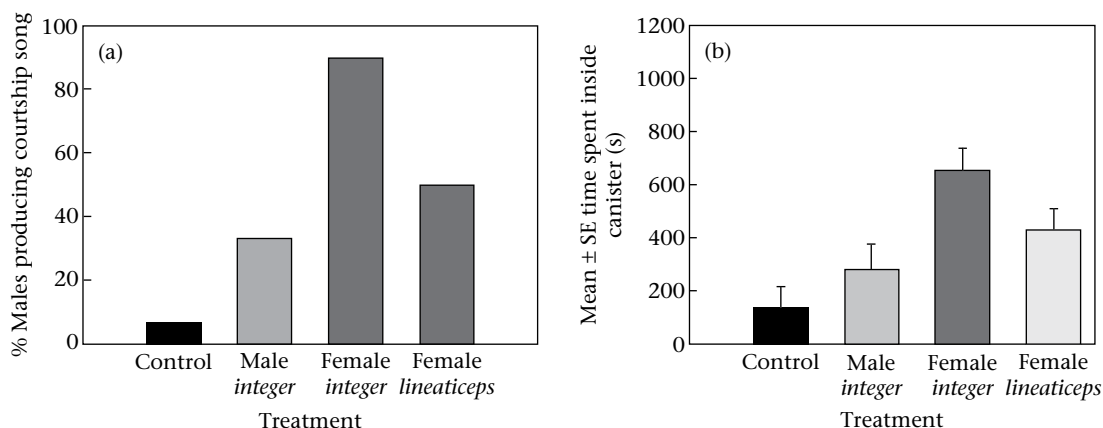
Figure 3. (a) Percentage of field-caught male *G. integer* that produced courtship song in response to cricket antennae and control bristles. (b) Mean ± SE time that laboratory-reared virgin male *G. integer* spent in unscented control vials and in canisters previously occupied by conspecific and heterospecific females and conspecific males.

and oriented towards the observer so that the female's approach could be observed. Trials were conducted under red light (14 lx at 60 cm) to mimic nocturnal conditions. The trial began when a subject was brought into the chamber, transferred into a plastic vial and placed in the experimental arena adjacent to the wall behind the film canister. We released the female from the plastic vial and observed her for 30 min. An event recorder program (AV Bio-Statistics: http://web.telia.com/~u25601709/avbs) was used to record when the cricket antennated the canister opening and when she entered the canister. Between trials, the sand was thoroughly mixed and the walls of the arena were wiped with a damp towel.

Previous studies (Rence & Loher 1977; Hardy & Shaw 1983; Adamo & Hoy 1994) suggest that antennal contact is necessary for crickets to respond to foreign chemical cues. Thus, all our subjects were females that contacted the opening of the canister with their antennae. We analysed data from trials using two measures: (1) time spent within the canister and (2) the proportion of the treatment group that entered the canister. For time spent within the canister, we used Mann–Whitney U tests to make our three planned comparisons across treatment groups (Ruxton & Beauchamp 2008). For the proportional data, we used z tests with the Yates correction for continuity (Glantz 2005). In both cases, we used a Bonferroni-adjusted α of 0.016 (Day & Quinn 1989).

Results

Females in treatment groups differed in terms of total time spent in canisters (Fig. 4a): females spent less time in control canisters than they did in canisters previously occupied by crickets (Mann–Whitney U test: $U = 1537.0$, $N_1 = 25$, $N_2 = 75$, one-tailed $P < 0.001$). Females also spent more time in canisters with male chemical cues than they did in canisters with female chemical cues ($U = 959.0$, $N_1 = 25$, $N_2 = 50$, one-tailed $P < 0.001$). However, females did not differ in the time spent with male cues from each of the two species ($U = 288.5$, $N_1 = N_2 = 25$, one-tailed $P = 0.324$). Females entered cricket-scented canisters at a higher rate than they did control canisters (cricket-scented $= 65/75$, control $= 16/25$; z test with Yates correction: $z = 2.208$, one-tailed $P = 0.013$). Females also entered male-scented canisters at higher rates than they did female-scented canisters (males $= 48/50$, females $= 17/25$; z test with Yates correction: $z = 3.002$, one-tailed $P = 0.0015$). However, females did not differ in the rate at which they entered conspecific and heterospecific male canisters (conspecific $= 24/25$, heterospecific $= 24/25$; z test with Yates correction: $z = -0.722$, one-tailed $P = 0.235$).

EXPERIMENT 3: RESPONSES OF LABORATORY-REARED FEMALES AND MALES TO CONSPECIFIC AND HETEROSPECIFIC CHEMICAL CUES

Methods

Subjects

We used 120 male and 120 female laboratory-reared crickets that were the first laboratory generation from field-caught mothers. All crickets were virgins. As in experiment 2, we exposed these subjects to chemical cues collected from conspecifics of the same and opposite sexes, as well as heterospecifics of the opposite sex. For female subjects, we collected chemical cues from 28 male and 28 female *G. integer* and from 14 male *G. lineaticeps*. For presentation to male subjects, we collected chemical cues from 29 *G. integer* males and 29 *G. integer* females and from 16 *G. lineaticeps* females.

Trials

Trials proceeded as described in experiment 2. We observed the responses of four treatment groups of females to canisters containing filter paper previously occupied by a *G. integer* female ($N = 30$), *G. integer* male ($N = 30$), *G. lineaticeps* male ($N = 30$) or control (no scent; $N = 30$). Similarly, we observed the responses of four groups of males to canisters previously occupied by a *G. integer* female ($N = 30$), *G. integer* male ($N = 30$), *G. lineaticeps* female ($N = 30$) or control ($N = 30$). Our statistical analysis conformed to that used in experiment 2, with a Bonferroni-adjusted α of 0.016 for each comparison (Day & Quinn 1989).

Results

Laboratory-reared females spent more time inside cricket-scented canisters than they did inside the control canisters (Mann–Whitney U test: $U = 1966.0$, $N_1 = 30$, $N_2 = 90$, one-tailed $P < 0.001$; Fig. 4b). They also spent more time in male-scented canisters than they did in female-scented canisters ($U = 1320.5$, $N_1 = 30$, $N_2 = 60$, one-tailed $P < 0.001$), but they did not differ in the time spent in conspecific versus heterospecific male canisters ($U = 464.0$, $N_1 = 30$, $N_2 = 30$, one-tailed $P = 0.364$). In terms of propensity to enter canisters, female *G. integer* entered canisters previously occupied by crickets at a higher rate (75%) than they did unscented control canisters (30%) (cricket-scented $= 68/90$, control $= 9/30$; z test with Yates correction: $z = 4.221$, one-tailed $P < 0.001$). They also entered male canisters at a higher rate than they did female canisters (male $= 53/60$, female $= 15/30$; z test with Yates correction: $z = 3.729$, one-tailed $P < 0.001$). However, they did not show
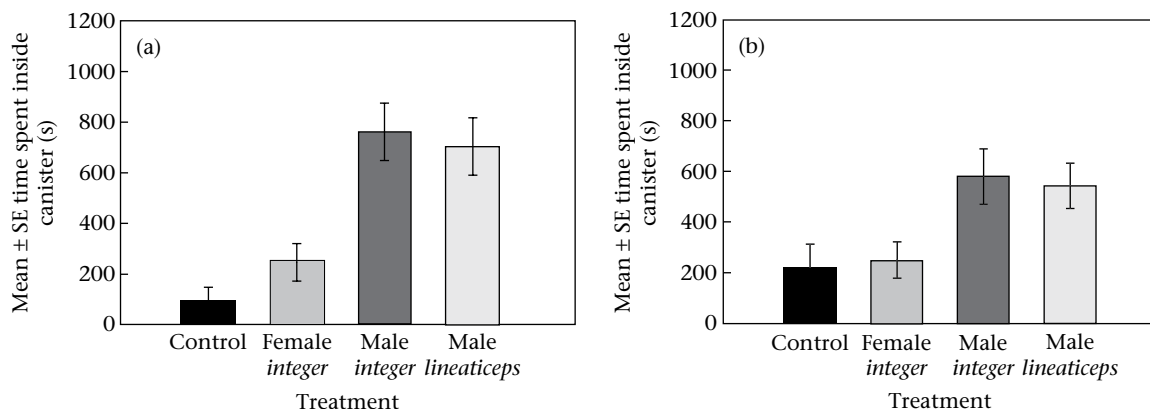
Figure 4. Mean ± SE time that (a) field-caught female *G. integer* and (b) laboratory-reared (virgin) female *G. integer* spent in unscented control vials and in canisters previously occupied by female and male *G. integer* and male *G. lineaticeps*.

a propensity to enter conspecific male canisters at a higher rate than they did heterospecific canisters (*G. integer* = 23/30, *G. lineaticeps* = 30/30; *z* test with Yates correction: $z = 2.413$, one-tailed $P = 0.992$).

Males spent more time in cricket-scented canisters than they did in control canisters (Mann–Whitney *U* test: $U = 2024.0$, $N_1 = 30$, $N_2 = 90$, one-tailed $P < 0.001$; Fig. 3b) and they spent more time in female- versus male-scented canisters ($U = 1283.0$, $N_1 = 30$, $N_2 = 60$, one-tailed $P < 0.001$). Males also spent more time in canisters of conspecific females than they did in canisters of heterospecific females ($U = 304.0$, $N_1 = 30$, $N_2 = 30$, one-tailed $P = 0.015$). Male *G. integer* entered canisters containing cricket chemical cues at a higher rate than they did control canisters (cricket-scented = 64/90, control = 9/30; *z* test with Yates correction: $z = 3.779$, one-tailed $P < 0.001$); similarly, they entered female canisters at higher rates than they did male canisters (female = 50/60, male = 14/30; *z* test with Yates correction: $z = 2.781$, one-tailed $P < 0.010$). They showed a trend to enter conspecific female canisters at a higher rate than they did heterospecific female canisters (*G. integer* = 28/30, *G. lineaticeps* = 22/30; *z* test with Yates correction: $z = 1.732$, one-tailed $P = 0.041$).

EXPERIMENT 4: MATING DISCRIMINATION OF MALE AND FEMALE *G. INTEGER*

Methods

Subjects

We conducted four types of mating trials on field-caught crickets: (1) *G. integer* males with *G. lineaticeps* females ($N = 20$), (2) *G. integer* females with *G. lineaticeps* males ($N = 20$), (3) *G. integer* males with *G. integer* females ($N = 20$) and (4) *G. lineaticeps* males with *G. lineaticeps* females ($N = 20$). On laboratory-reared crickets, we ran similar trials ($N = 20$ per group), but we did not run the intraspecific *G. lineaticeps* trials (4) because of low availability of laboratory-reared *G. lineaticeps*. These pairings allowed comparison of male *G. integer* behaviours directed towards female *G. integer* and *G. lineaticeps* as well as a comparison of female *G. integer* behaviours directed towards male *G. integer* and *G. lineaticeps*. We used males only if they produced calling song during the 30 min before the start of the trial.

Trials

We conducted trials in a large (3 × 3 m) acoustic chamber illuminated by a 25-W incandescent red light (14 lx at 60 cm). The mating arena consisted of a plastic box (20.3 × 20.3 × 10.2 cm, L × W × H) filled with sand. After a 2 min adjustment period, we introduced the female into the arena. Between trials, the sand was mixed thoroughly and the walls of the arena were wiped with a damp cloth. To prevent cross-contamination of species-specific chemical cues, we conducted each of the four trial types in separate arenas. Videotaping of the trial began when the male was transferred into the arena and lasted until the female mounted the male or for a maximum of 15 min.

Analysis

Videotapes were analysed using JWatcher v.1.0. (http://www.jwatcher.ucla.edu). We scored cricket behaviours using an ethogram similar to that developed by Adamo & Hoy (1994). Several components of mating interactions were analysed and are listed below.

Propensity to mount: the proportion of females that mounted the male. A mount was considered an attempt to mate if the female mounted the male's back with at least half of her body contacting his for at least 2 s (even if spermatophore transfer was unsuccessful).

Aggression: the total number of bites and kicks directed at the opposite sex.

Chemosensory investigation: the percentage of total trial time spent contacting the other cricket with antennae and palps.

Initiation and duration of courtship song: the latency for males to produce courtship song following antennal contact, as well as the percentage of total trial time that the male spent producing courtship song.

Results

Propensity to mount

Female *G. integer* mounted male *G. integer* at higher rates than they did male *G. lineaticeps*: among field-caught crickets, 11/20 conspecific pairings resulted in mounting, while only 2/20 heterospecific pairings did so (*z* test with Yates correction: $z = 2.701$, $P = 0.007$). Laboratory-reared female *G. integer* also discriminated: 8/20 mounted *G. integer* males, while only 1/20 mounted male *G. lineaticeps* (*z* test with Yates correction: $z = 2.272$, $P = 0.023$). Similarly, while 6/20 field-caught *G. lineaticeps* females mounted conspecific males, only 3/20 mounted *G. integer* males, although this difference was not statistically significant, possibly because of small sample sizes (*z* test with Yates correction: $z = 0.757$, $P = 0.449$).

Aggression

Comparing the average total number of bites and kicks per trial directed by females towards males, both field-caught and laboratory-reared female *G. integer* (Fig. 5a) tended to be more aggressive towards heterospecific males than towards males of their own species (laboratory-reared: Mann–Whitney *U* test: $U = 288.5$, $N_1 = N_2 = 20$, $P = 0.009$; field caught: $U = 300.0$, $N_1 = N_2 = 20$, $P = 0.0105$). Field-caught female *G. lineaticeps* were similarly more aggressive towards heterospecific males than towards conspecific males ($U = 355.5$, $N_1 = N_2 = 20$, $P < 0.001$).

Comparing both types of mating trials involving field-caught *G. integer* males (Fig. 5a), males directed more bites and kicks at heterospecific females than they did at conspecific females (Mann–Whitney *U* test: $U = 250.00$, $N_1 = N_2 = 20$, $P = 0.020$). However, males may have been defensively responding to elevated aggression of female *G. lineaticeps*: across all trials where biting and kicking occurred, female crickets (both species) were the initial aggressor 80% of the time. When we analysed the behavioural sequences of aggressive interactions, we found that male *G. integer* initiated aggressive interactions with heterospecific females at the same rate as they did with conspecific females (in 3/20 of each kind of trial). However, female *G. lineaticeps* initiated aggression in 15/20 trials with male *G. integer*, whereas female *G. integer* did so in only 6/20 similar trials. Finally, laboratory-reared *G. integer* males were as aggressive towards female *G. integer* as they were towards female *G. lineaticeps* (Mann–Whitney *U* test: $U = 233.5$, $N_1 = N_2 = 20$, $P = 0.221$).

Chemosensory behaviour

Besides directing less aggressive behaviour towards conspecific males, field-caught female *G. integer* also showed more chemosensory behaviour with conspecifics, spending a greater proportion of total time close to conspecific males, and touching males with their antennae and palps (Mann–Whitney *U* test: $U = 55.0$, $N_1 = N_2 = 20$, $P < 0.001$; Fig. 5b). Laboratory-reared female *G. integer* showed a similar difference in their behaviour towards conspecifics and heterospecifics (Mann–Whitney *U* test: $U = 34.0$, $N_1 = N_2 = 20$, $P < 0.001$). Male *G. integer*, however, did not show
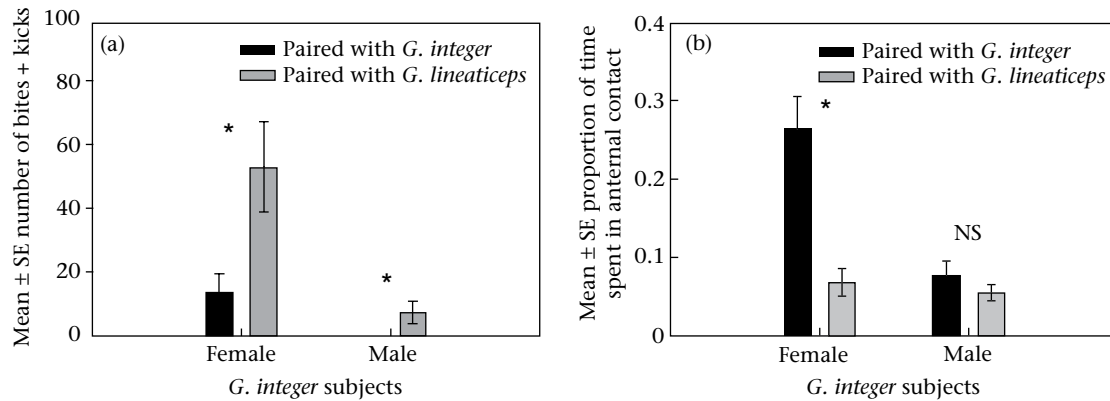
Figure 5. (a) Mean ± SE number of bites and kicks (combined) by field-caught female *G. integer* towards conspecific males and heterospecific (*G. lineaticeps*) males and females. (b) Mean ± SE proportion of time that field-caught female and male *G. integer* spent in antennal contact with conspecific and heterospecific males and females.

a corresponding difference in their chemosensory response to the two species of females. Comparing the proportion of time that field-caught males spent contacting females with antennae and palps, we did not detect any significant species differences (t test on arcsine square-root-transformed data: $t_{38} = 0.816$, $P = 0.419$; Fig. 5b), nor did we find such differences between laboratory-reared males ($U = 173.0$, $N_1 = N_2 = 20$, $P = 0.473$).

Female *G. lineaticeps* spent most of their time in close association with conspecifics (t test: $t_{38} = -9.210$, $P < 0.001$) as did male *G. lineaticeps* (Mann–Whitney U test: $U = 118.0$, $N_1 = N_2 = 20$, $P = 0.027$).

Initiation and duration of courtship song

Latency to produce courtship song did not differ significantly between field-caught males in the presence of *G. integer* and *G. lineaticeps* females (t test on log-transformed data: $t_{38} = -0.390$, $P = 0.699$). Laboratory-reared *G. integer* males actually produced courtship song slightly faster when paired with female *G. lineaticeps* (t test on log-transformed data: $t_{38} = 2.292$, $P = 0.028$). There was also no difference in the percentages of total trial time that *G. integer* males spent producing courtship song when paired with the two species of females (field-caught males: t test on arcsine square-root-transformed data: $t_{38} = 0.456$, $P = 0.651$; laboratory-reared males: t test: $t_{38} = -0.312$, $P = 0.757$).

We observed a similar lack of species discrimination among *G. lineaticeps* males: they did not produce courtship song sooner in the presence of conspecific versus heterospecific females (Mann–Whitney U test: $U = 249.0$, $N_1 = N_2 = 20$, $P = 0.156$), nor did they spend a greater proportion of total trial time producing courtship song with conspecific versus heterospecific females ($U = 144.5$, $N_1 = N_2 = 20$, $P = 0.112$).

DISCUSSION

Mating decisions are often studied in the context of female preferences for a single male signal, yet multiple signals or cues are often exchanged during courtship (Candolin 2003). This exchange of additional information involves assessment costs (e.g. time) for females as well as production costs for males (Partan & Marler 2004). What benefits does it confer? In field crickets, a male's long-distance calling song often predicts his mating success (e.g. Hedrick 1988), so why do field crickets engage in close-range courtship? Although calling song is the principal mechanism for species isolation (Alexander 1962), female field crickets may encounter sympatric species at close range in areas of high density (e.g. Gray 2004). Therefore, we investigated the possibility that, in addition to playing a role in intraspecific mate choice (e.g. Wagner & Reiser

2000; Gray 2004; Kortet & Hedrick 2005), close-range courtship signals might function in sex and species recognition by both male and female *G. integer*.

We found clear evidence of sex recognition using chemical cues among both males and females but species recognition only among males. However, the results of our chemical cue trials did not predict the outcomes of realistic, multimodal courtship interactions. Although male *G. integer* were less likely to produce courtship song in response to heterospecific female chemical cues and spent more time in association with conspecific female chemical cues, in the mating trial they were equally likely to produce courtship song in response to heterospecific females. Both field-caught and laboratory-reared female *G. integer*, in contrast, did not differentiate between conspecific and heterospecific chemical cues; however, when presented with male heterospecifics, they were more aggressive and spent less time in antennal contact with these males than with conspecifics. This suggests that to predict the outcomes of natural mating interactions, researchers must not only identify signals or cues of potential importance, but also ask how they are prioritized and integrated with other sources of information (Rowe 1999; Candolin 2003; Partan & Marler 2004; Hebets & Papaj 2005).

Males Preferred Conspecific Chemical Cues But Courted Females Indiscriminately

Our work used a novel technique to confirm previous reports that male antennal contact with conspecific female pheromones triggers the production of courtship song (Tregenza & Wedell 1997). Males preferentially responded to female chemical cues regardless of whether these cues were on female antennae or on substrate previously occupied by females. Our experiments also provide new evidence that male crickets gain information about species identity via chemical cues. A previous study on this topic reported mixed results, and focused on volatile rather than contact chemical signals (Otte & Cade 1976).

Males tended to produce courtship song more often in response to female antennae than in response to male antennae, regardless of species. This result suggests that some components of female-specific cuticular compounds may be shared across species. To date, analysis of field cricket cuticular hydrocarbons has largely focused on intraspecific sex differences (Warthen & Uebel 1980; Tregenza & Wedell 1997; Thomas & Simmons 2008). For example, in *Gryllus bimaculatus*, male courtship song is triggered by recognition of sexually dimorphic concentrations of several cuticular hydrocarbons (Tregenza & Wedell 1997; Nagamoto et al. 2005). A comparative study (e.g. Mullen et al. 2007 on *Laupala* spp.) using

phylogeny, gas chromatography and behavioural assays could shed light on the evolution of the compounds that trigger this response in field crickets.

Although chemical cues may provide males with information about species identity, additional cues available in mating trials appear to mask or outweigh this information. Fitness costs of interspecific matings are expected to be lower for males than for females (Andersson 1994; but see Howard et al. 1998), yet a male cricket's investment in close-range courtship may comprise time and opportunity costs as well as the energy required to produce courtship song and a spermatophore (Hack 1998; Wagner 2005). We therefore expected to find some level of species discrimination among males (e.g. Gray 2004). However, both *G. integer* and *G. lineaticeps* males presented with heterospecific females showed no evidence of discrimination, even though experiments 1 and 3 established that *G. integer* males can distinguish conspecific from heterospecific chemical cues.

While the causes of this 'overshadowing' of the chemical cues alone by the presentation of a female are unknown, it may occur because courtship provides more chemical stimulation than just antennal/paper exposure, or because courtship provides visual and tactile information unavailable in the antennal/paper exposure. In the first case, males might simply be less motivated to mate when they have access to less chemical information. Alternately, species differences in female chemical signals might be overshadowed by sensory inputs from other modalities during mating interactions. Such a conflict could occur if cues that males use to modulate their courtship of conspecific females overlap between species. For example, if male *G. integer* prefer larger females, this could contribute to male *G. integer*'s vigorous courtship of generally larger *G. lineaticeps* females.

Females Preferred Conspecific Males But Not Their Chemical Cues

Removing or inactivating antennal female chemoreceptors reduces or abolishes mounting of males (Loher & Rence 1978; Adamo & Hoy 1994; Balakrishnan & Pollack 1997), but it is unclear whether female crickets deprived of chemosensory information do not mate because they cannot determine the male's sex and/or because they cannot recognize him as conspecific. Our results suggest that deantennated females may lose information about a male's sex rather than a male's species. Other studies have presented females with a choice between conspecific and hetero-specific male chemical cues (e.g. Bell 1980). However, without also presenting conspecific female chemical cues, this test does not distinguish between conspecific attraction and sex recognition. Here, we show that among both field and laboratory-reared crickets, females preferentially responded to male chemical cues, but unlike males, females did not preferentially respond to their own species' cues. Sex differences in chemical species discrimina-tion exist in a variety of taxa (e.g. Verrell 2003; Barbosa et al. 2006), but a higher level of discrimination is usually associated with the sex that actively searches for mates. Female field crickets, however, are the searching sex. Thus, the clear species discrimination shown by females in response to long-distance acoustic signals is not matched by their response to close-range chemical cues, perhaps because in most circumstances, response to calling song precedes their exposure to chemical cues, and this response leads them to conspecific males.

In further contrast to males, species discrimination by females increased with access to more information: in courtship trials, females directed more aggression and less chemosensory behav-iour towards heterospecific males. Most studies of cricket hybrid-ization provide few behavioural details regarding why particular crosses succeed or fail (e.g. Alexander & Bigelow 1960; Smith & Cade 1987), but our results suggest that female behaviour (in both

G. integer and *G. lineaticeps*) at close range can function as a pre-zygotic mating barrier. Previous research on species discrimination by females has focused on response to male calling song (e.g. Alexander 1962; Gray & Cade 2000; but see Doherty & Howard 1996), and both *G. integer* and *G. lineaticeps* females preferentially approach conspecific calling song (although they do not avoid heterospecific male calling song; A. S. Leonard, unpublished data). However, calling song functions largely to attract females from a distance (Gerhardt & Huber 2002). Our study focused on close-range interactions between *G. integer* and *G. lineaticeps* because these two species often co-occur in the same microhabitat. How do females distinguish conspecifics from heterospecifics, given that males of different species may display equally vigorous courtship? Our results imply that chemical cues alone may not be sufficient for species discrimination. Since female *G. integer* do not discriminate between conspecific and heterospecific courtship songs in phono-taxis trials (A. S. Leonard, unpublished data), our work suggests that, for females, chemical cues and courtship songs may interact to bias response towards conspecific males, a possibility also sug-gested by Gray's (2004) study of *G. texensis* and *G. rubens*.

These results indicate that researchers applying a complex sig-nalling perspective should ask how information from a single signal is integrated with other kinds of information in realistic behav-ioural interactions. Recently, Phelps et al. (2006) and Castellano & Cermelli (2006) questioned the traditional view of species recog-nition and mate choice as hierarchically distinct perceptual processes. Their models suggest that mating decisions reflect a cognitive process geared towards selecting the most attractive mate, where attractiveness reflects multiple preference strengths functioning in different contexts (Phelps et al. 2006). Our experi-ments were not designed to address a particular perceptual hypothesis, but a nonhierarchical interpretation of our results suggests that the strength of female preference for male chemical cues is lower than that expressed when multiple male traits are available for evaluation.

Most studies of variation in response to multiple mating signals make comparisons across species or within a single sex (reviewed in: Candolin 2003; Hebets & Papaj 2005). To our knowledge, our study is the first to report variation between males and females of the same species: the sexes differ in their prioritization of signals when making mating decisions. This intraspecific comparison illustrates how selection has shaped the way animals integrate signals in multiple sensory modalities when they are making decisions critical to reproductive success.

Acknowledgments

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