ORIGINAL PAPER

Age-related changes in an insect mating signal have no effect on female choice

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Abstract The structure of male mating signals is often influenced by age. The causes and consequences of age-based signal variation have been much studied in visual, acoustic, and chemical signaling modalities, but are less explored in species that use vibrational signals for mate attraction. However, the complex structure of many vibrational signals makes them ideal for investigating the relationships between age, signal variation, and patterns of female choice. In the thornbug treehopper, *Umbonia crassicornis*, females mate more often with older males, but the mechanism underlying older male mating success is unknown. Our goals in this study were to determine whether male vibrational mate advertisement signals vary with age, and, if so, whether females prefer the signals of older males. We recorded male signals over four consecutive weeks beginning at the onset of signaling (a period spanning most of the male reproductive lifespan), and measured ten temporal, spectral, and energy-related parameters from each signal. Several features of male signals changed markedly with age. However, females responded similarly to signals representing males of different ages, indicating that they do not favor older males on the basis of long distance mate advertisement signals. Nonetheless, the results reveal that even in short-lived, determinate growth species age can have surprisingly large effects on mating signals, and we discuss some factors that may contribute to age-related signal variability in *U. crassicornis*.

Keywords *Umbonia crassicornis* · Membracidae · Vibrational communication · Age-based mate choice · Complex signals · Thornbug treehopper

Communicated by L. Simmons

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Introduction

Females in many animal species often mate with males of a particular age (Brooks and Kemp 2001). In some cases, this occurs as a consequence of male competition, as when older males are more successful at competing for mates or acquiring resources important for female reproduction (Howard 1978; Clutton-Brock and Albon 1979; Rohwer et al. 1980; Woodhead 1986; Wegge and Larsen 1987; Le Boeuf and Reiter 1988; Rasmussen et al. 2008). In other taxa, females may actively select males of a certain age independent of male competition, and much research has focused on identifying possible phenotypic cues involved in age-based female choice. Mating signals provide a source of potential cues, and in many species of vertebrates and some invertebrates, male mating signals vary with age. Some examples include odors (Lopez et al. 2003; Osada et al. 2003; Kaltenpoth and Strohm 2006), ornamentation or color patterns (Buchholz 1991; Johnstone 1995; Candolin 2000; Miller and Brooks 2005), courtship behavior (Höglund and Lundberg 1987; Felton et al. 2006), and acoustic mate attraction signals (McComb 1991; Simmons and Zuk 1992; Bertram 2000; Forstmeier et al. 2005; Poesel et al. 2006; Jacot et al. 2007; Hoikkala et al. 2008).

In many animals, males use substrate-borne vibrational signals for mate attraction (Hill 2008). Vibrational signals are often structurally complex, with distinctive components generated simultaneously by different mechanisms, i.e., they are multicomponent signals (Virant-Doberlet and Cökl 2004; Hebets and Papaj 2005; Cocroft et al. 2006; Elias et al. 2006). The expression of such complex signals is likely to be sensitive to developmental processes that affect the underlying neural and muscular systems controlling signal production, causing signals to change with age (Elias et al. 2006). However, even though vibrational communication is widespread—in the Arthropoda alone, it occurs in an estimated 195,000 species of insects (Cocroft and Rodrìguez 2005) and 35,000 species of spiders (Barth 1998)—only a handful of studies have investigated the influence of age on the expression of vibrational mating signals (Kumar and Saxena 1985; Zeigler and Stewart 1985; Moreira 1993; Sattman and Cocroft 2003). Nevertheless, the complex structure of many vibrational signals makes them ideal for investigating potential relationships between age, signal variation, and female choice.

Males of the thornbug treehopper, *Umbonia crassicornis* (Hemiptera: Membracidae), produce complex multicomponent vibrational signals during mate advertisement (Cocroft and McNett 2006). Females mate more with older (30 days) than younger (20 days) males under free-choice conditions, possibly in response to selection against inbreeding, since in this species older males cannot be siblings (De Luca and Cocroft 2008). However, the mechanism underlying older male mating success is unknown. One possibility is female choice based on age-related signal variation. In the study by De Luca and Cocroft (2008), mating occurred in a semi-natural environment in which vibrational advertisement signals were produced, but the signals of individual males were not recorded. However, in treehoppers and many other vibrationally communicating insects, females actively facilitate mate localization by duetting with signaling males (Henry 1994; Bailey 2003). Females in some treehopper species are highly selective at this stage of courtship, responding only to male signals with preferred traits (Rodríguez et al. 2006). There is an additional stage of courtship in which the male signals while in contact with the female in both *U. crassicornis* and the *Enchenopa* species studied by Rodríguez et al. (2006). However, in *Enchenopa*, female preferences measured on the basis of long-range advertisement signals (i.e., signals propagated over a distance of several centimeters, and prior to physical contact) predict the relationship between signal variation and mating success under semi-natural conditions (Sullivan-Beckers 2008). Female responses to male signals during the duetting phase of courtship thus appear to be a good proxy for female mating decisions.

Here, we test the hypothesis that female thornbugs choose males on the basis of age-related signal variation. This hypothesis predicts that (1) male signals contain reliable age cues, and (2) females prefer signal traits characteristic of older males. We used a longitudinal (within-male) approach to characterize age-based changes in male mate advertisement signals, beginning at the onset of signaling and extending throughout the relevant period of male sexual activity. We then conducted a playback experiment to test whether females prefer the signals of older males.

Materials and methods

Study species

Thornbug treehoppers range from Colombia to southern Florida, USA (Mead 1962). This species uses a variety of host plants, primarily in the Mimosaceae (McKamey and Deitz 1996). Females mate only once and lay a single clutch of eggs in their lifetime (Wood 1974). Females care for their eggs and nymphs by making feeding slits on the host plant and by protecting nymphs from predators (Cocroft 2002). Individuals begin dispersing from their natal site approximately 2–3 weeks after adult eclosion, with males usually leaving a few days before their sisters (Masters 1997). The maximum age of individuals in the field is unknown, but in a predation-free greenhouse environment adult males do not survive more than 50–60 days.

Insect maintenance

From 2001 to 2006, we made repeated collections from five field sites in Miami and Key Largo, FL, USA. We collected individuals as late-instar nymphs or teneral adults when they were still aggregated in their family group (~50–80 individuals per family). Approximately 1,000 individuals from 20 families were used to establish a greenhouse population at the University of Missouri (Columbia, MO, USA). Each family was enclosed within its own screen cage on a potted *Albizia julibrissin* (Mimosaceae) host plant. Approximately 1 week after individuals in a family eclosed to adulthood, we caged the sexes on different host plants to prevent sibling mating. We produced new families of insects by mating individuals from unrelated families every generation (about 3–4 months).

Recording of vibrational signals

In 2003 and 2005, we recorded signals from adult males from the greenhouse colony. We used males from eight families (four in 2003 and four in 2005). Males from 2005

were descended from insects collected in Florida in 2004, and thus, were not related to males used in 2003. We randomly selected eight to nine males from each family for a total of 66 males. We recorded each male once per week over four consecutive weeks, beginning at the start of signaling (~12 days after adult eclosion). During each recording session, we obtained three signals from each male for analysis.

We used a potted *A. julibrissin* plant to make recordings, placed on a vibration isolation table (Vibraplane 9100, Kinetic Systems, Inc., Boston, MA, USA). Males were recorded between 09:00 and 17:00 h and at temperatures of 25 ± 3°C. We placed one male at a time on the plant on a narrow stem and allowed it to settle for a few minutes. To stimulate male calling, we played a pre-recorded *U. crassicornis* male–female duet from a computer through a speaker located about 2 m from the plant. Air-borne vibrations imparted into the plant were sufficient to induce males to produce mate advertisement signals. Upon broadcast of the duet, males would immediately begin signaling. Males remained stationary and usually called in bouts of one to five signals. If a male gave fewer than three signals, the duet was immediately broadcast again to obtain more signals. If more than three signals were given, we randomly chose three from the bout for analysis.

Signals were transduced with a Polytec CLV1000 laser vibrometer with a CLV M030 decoder module (Polytec PI, Inc., Auburn, MA, USA) set at 5 mm/s/V sensitivity. A small piece (~1 mm^2) of reflective tape was affixed to the plant to increase the reflectance of the laser. To minimize substrate-related variation, the laser was focused on a point ≤10 cm from the signaling insect (Cocroft et al. 2006). We placed each male with his dorso-ventral axis parallel with the laser beam to ensure that the major axis of stem vibration was measured correctly (McNett et al. 2006). Recordings were low-pass-filtered at 5 kHz with the laser control module and high-pass-filtered at 60 Hz with a Krohn-Hite 3202 filter (Krohn-Hite Corporation, Brockton, MA, USA) before being recorded on a Macintosh computer using SoundEdit16 v.2 (Macromedia, Inc., San Francisco, CA, USA). We monitored signals during recording using an oscilloscope (Hameg HM 203-7) and a speaker (RCA Pro10W).

In 2005, we used the same recording protocol described above, but recorded males on a different *A. julibrissin* plant (stem diameter where males were recorded in 2003=6 mm; in 2005=4 mm). Most of the properties of *U. crassicornis* signals are highly repeatable when males signal on different individual host plants, and thus, use of two different plants should not influence our measurements of how traits change with age; however, we might expect minor differences in spectral components of the signals between the 2 years (Cocroft et al. 2006). Signals were acquired using a

National Instruments (NI) data acquisition board connected to a Dell computer. Custom software developed in LAB-VIEW v. 7 (National Instruments, Austin, TX, USA) and MATLAB v. 6.5 (The Mathworks, Inc., Natik, MA, USA) allowed us to quantify age-related variation in the peak amplitude (velocity) of vibrational signals.

Measuring signal parameters

The vibrational signals of *U. crassicornis* males contain two components produced simultaneously in different frequency ranges: an amplitude-modulated downward sweep from 200 to 130 Hz and a rapid series of broadband "clicks" in the 500–2,000 Hz range (Fig. 1). The low-frequency (LF) component is likely generated using direct muscle contractions that vibrate the abdomen, transmitting the signal into the substrate via the legs (B. Allison and C. Miles, personal communication). The high-frequency (HF) component is produced using a frequency multiplier system, likely a tymbal located on the abdomen (Ossiannilsson 1949; Gogala 1985; Čokl and Virant-Doberlet 2003).

We used Canary v. 1.2.4 in 2003 and Raven v. 1.2 in 2005 (both from the Cornell Laboratory of Ornithology, Ithaca, NY, USA), to quantify four temporal and five
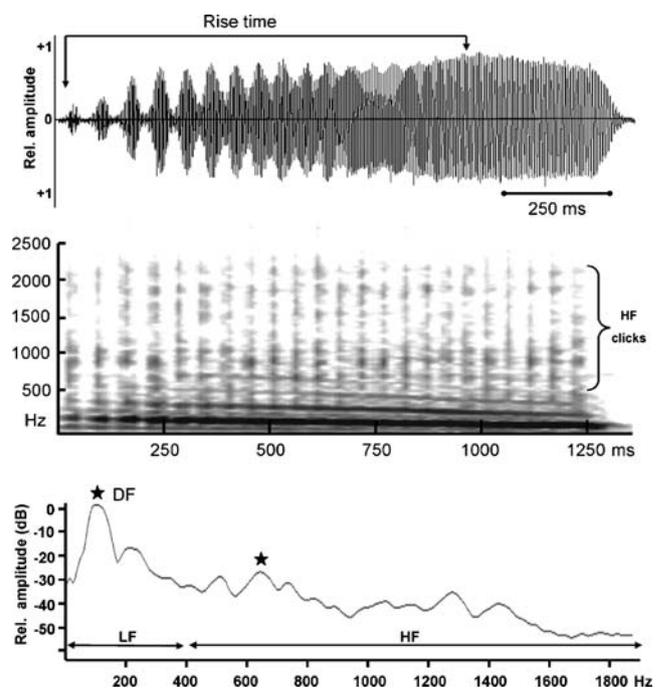
Fig. 1 Vibrational signal of a male *U. crassicornis*. *Top*, waveform. Amplitude modulation (AM) occurs over the first third to half of the signal. *Middle*, spectrogram. *Bottom*, amplitude spectrum. *Arrows* indicate the frequency range of the low- (LF) and high- (HF) frequency components, and *stars* mark the frequencies of peak amplitude in each. *DF* dominant frequency (for additional information, see Table 1)

spectral parameters for each signal we recorded. We also measured peak amplitude for signals in 2005. Table 1 provides a brief explanation of each parameter. Of the ten parameters measured, seven were significantly correlated with temperature, and thus, were corrected to a common temperature of 25°C. Three parameters (proportion of signals containing amplitude modulation (AM), HF click rate, and peak amplitude) did not vary significantly with temperature (all, $p \geq 0.19$).

Only males that signaled at each of the four age classes were included in the discriminant function analysis of age-related variation (see below). In 2003, mortality reduced the number of males from 32 to 16 by the end of the 4-week recording period. In 2005, all 34 males survived until the end of the recording period, but at 12 and 19 days, only 30 males signaled. For each male, we averaged parameter values from the three signals obtained at each age and used these mean values in subsequent analyses. In our reporting of age-based signal variation, we included data from all males regardless of whether they survived to the end of the experiment in order to show the full range of among-male variation.

Discriminant function analysis of age-related signal variation

We used discriminant function analysis (DFA) in JMP IN 5.1 (SAS Institute Inc. 2003) to classify signals according to age. From 2003, there were 64 cases (16 males × 4 age classes), and from 2005 there were 120 cases (30 males × 4 age classes). Because DFA requires a full data matrix (i.e., a measurement for each variable), peak amplitude was not included because we did not have measurements for all males across both years. The effect of age on this parameter was analyzed separately (see below). We first examined whether there were significant differences in signals obtained between the recording years and then examined whether signals could be reliably grouped according to male age.

Although DFA is an appropriate technique to investigate how well male signals can be discriminated by age (Blumstein and Munos 2005), the repeated measurements we took from each male over the 4 weeks violate the assumption of independence (Quinn and Keough 2002). Accordingly, we used the permuted DFA (pDFA) of Mundry and Sommer (2007) for significance testing. For full details, we refer readers to their paper. In brief, the pDFA uses a test statistic—the number of cases correctly assigned—and compares it to a distribution generated with random permutations of the original data set. The p value we report is the percentage of permuted data sets with a result (i.e., percent of correctly assigned cases) at least as large as our original data. We used a separate pDFA for signals recorded in the different years. Results of the pDFA were not qualitatively different from the DFA performed in JMP. For this reason, we report some additional statistics (i.e., eigenvalues and loadings) provided by the JMP program for descriptive purposes in order to identify the signal parameters that varied most with age.

We also report classification success from the DFA for all possible pair-wise comparisons between age groups, where the random expectation is a classification score of 50% (Blumstein and Munos 2005). In our case, there were four age groups and six possible pair-wise comparisons.

Table 1 Description of vibrational signal parameters measured in this study

Parameter	Description
Temporal	
Duration	Length of signal
Rise time	Time from start to maximum amplitude, expressed as a percentage of the total duration
Proportion of signals with amplitude modulation (AM)	Proportion of signals (out of 3) in one bout that contain AM
High-frequency (HF) click rate	Number of HF clicks per second in one signal
Spectral	
DF at beginning of signal	Frequency with the greatest relative energy during the first 200 ms
DF at middle of signal	Frequency with the greatest relative energy during the middle 200 ms
DF at end of signal	Frequency with the greatest relative energy during the final 200 ms
Dominant frequency (DF)	Frequency with the greatest relative energy in a signal
LF–HF amplitude difference	Difference in relative maximum amplitude between the low-frequency (LF) and high-frequency (HF) signal components, measured in decibels (dB)
Energy	
Peak amplitude	Peak intensity of a signal measured in velocity, expressed in decibels rel. 0.1 mm/s

For additional information, see Fig. 1

Male variation in signal traits

To compare age-related variation in signal traits within males to that occurring among males, we calculated coefficients of variation (CV=(mean/SD)×100) for each parameter. We calculated a CV for each male over his lifetime and then averaged the values of all males to obtain a measure of within-male variability in signal traits due to age. We also calculated among-male CVs, first at each age class, and then averaged the values across the four age classes. This provided a measure of among-male variability in signal traits. Analyses were conducted separately for signals recorded in different years.

Peak amplitude

To assess the effect of age on peak amplitude, we used a partially nested repeated-measures ANOVA in JMP IN 5.1. We log-transformed values to more closely approximate normality (Shapiro–Wilk W=0.982, p=0.08), and homogeneity of variance (Bartlett test, p=0.06), before proceeding (Zar 1984). Factors in the model included male family, male identity (nested within family), male age, and a family × age interaction term. Family and male were entered as random effects and age as a fixed effect. If a factor was significant at the α=0.05 level we used Student's t post hoc comparisons to evaluate which factor levels were significantly different from one another.

Design of stimuli for the playback experiment

To test female preferences, we conducted playback experiments using artificially generated male advertisement signals instead of pre-recorded natural signals, to ensure that stimuli differed only in the traits of interest, following the recommendation of McGregor et al. (1992) and McGregor (2000). Mean values for the parameters measured in 2005 (N=34 males) were used to construct stimuli using a custom-written program in Matlab v. 6.5. Four stimuli were made that each represented an "average" male signal from one of the four age classes described above. Our goal was to determine whether age-related variation was sufficient to influence female choice and not to assess the influence of individual signal traits on female preferences, which would require a different set of stimuli. Each stimulus was composed of a bout of three signals of equal amplitude, separated by an inter-signal interval of 2 s. A preliminary experiment testing the effectiveness of synthetic stimuli revealed no significant difference in female response compared to natural signals (proportion of females responding: natural=0.82, synthetic=0.91; χ^2=0.679, p=0.41, N=11 females). Prior to the playback experiment, we digitally filtered the stimuli at a distance of 5 cm from the transducer (see below) to compensate for the differential filtering of their frequency components from propagation along a plant substrate. For a detailed description of the compensation procedure, see Cocroft (1996).

Playback procedure

We performed the experiment during April–May 2007 in a climate-controlled room adjacent to the greenhouse where the *U. crassicornis* colony was kept. The temperature of the room was maintained at 25±1°C. Females used as test subjects were second-generation offspring of individuals collected from Florida in 2006, and thus, were not descended from insects whose vibrational signals were characterized in 2003 and 2005. Females were unmated and between 3 and 5 weeks old post adult eclosion. Each female was first tested at 21 days post-eclosion, at the onset of sexual maturity (but not necessarily of sexual receptivity). If a female was unresponsive, it was tested again every second day until it gave at least one response to playback of a male advertisement signal. Each female was thus tested within 2 days of the onset of sexual receptivity, ensuring that no female tested was hyper-receptive (which could lead to a lack of discrimination in responses). We placed females one at a time on a potted *A. julibrissin* plant (height=50 cm) during playbacks. We attached a magnet to the stem of the plant with wax (Endevco, San Juan Capistrano, CA, USA) and placed an electromagnet (Edmund Scientifics, Tonawanda, NY, USA) about 5 mm away (Rodríguez et al. 2006). The electromagnet received signals from a Dell PC notebook, amplified with a RadioShack MPA-250 amplifier. Stimulus presentation was controlled with a custom-written program in Matlab. To minimize noise generated by building vibrations, the plant rested on a flat piece of iron (50×50× 2 cm), resting on an inflated bicycle inner tube placed on the vibration isolation table. We further isolated the plant by placing it on a thin sheet of shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY, USA).

We recorded the playbacks using an accelerometer (Endevco 256-100 connected to an Endevco 133 signal conditioner) attached to the plant stem (diameter=3 mm) with wax 5 cm below the magnet. For testing, we placed one female at a time on the plant ≤5 cm from the magnet. Recordings were high-pass-filtered at 60 Hz with a Krohn-Hite 3202 filter and low-pass-filtered at 10 kHz with the Endevco signal conditioner. The filter output was acquired with a Marantz PMD-670 solid state recorder (Marantz America, Inc. Mahwah, NJ, USA), at a sampling rate of 44.1 kHz. We monitored the playbacks with headphones connected to the Marantz and visually with a Tektronix 2225 oscilloscope (Tektronix, Inc., Richardson, TX, USA).

We adjusted the amplitude of the stimuli at the point females were placed on the stem by using an oscilloscope

to calibrate the values. We used a peak amplitude of 0.18 mm/s, which corresponds to the average peak amplitude of a male signaling about 5 cm away, on the basis of measurements from 34 males recorded in 2005. We allowed females to settle for a few minutes after being placed on the plant, and they tended to remain in the same place during the playback. If a female moved, we terminated the trial and tested the female again later.

Female response analysis

Every female received four signal bouts in one single presentation, with each bout representing one of the four age classes. We randomized the order of the stimuli for each female, and stimuli were separated by an interval of 10 s. We tested a total of 50 females (ten females from each of the five families). We analyzed the recordings with Raven v. 1.2 and measured female signals given in response to the different stimuli (females respond immediately after the male signal). Our experimental unit is an individual female. We treated females as a repeated factor by including female identity in the model (see Rodríguez et al. 2006), nested within family. We used logistic regression in JMP v. 5.1 (SAS Institute Inc. 2003) to evaluate differences among females in their response to signals from the different age classes. We analyzed female responses in two ways: first, whether females replied to a particular stimulus, and second, the number of responses given to each stimulus. Because each stimulus was made up of three signals, and females give only one reply per signal, the total number of responses an individual female could give to a particular stimulus varied from 0 to 3.

Results

Age-based signal variation

Age is a significant source of variation in male signals (pDFA; $p=0.001$). There were differences between signals recorded in 2003 vs. 2005 (Fig. 2); almost 86% of the 184 cases could be correctly assigned to year. The first discriminant function (DF1) explained 100% of the variation (eigenvalue=1.261, canonical correlation=0.747). Dominant frequency had the highest loading, indicating that this parameter was the greatest source of signal discrimination between years. Based on the high percentage of cases correctly classified to year, we analyzed signals obtained in the different years separately to avoid confounding age-related variation with other potential sources of variation associated with the recording year.

For signals obtained in 2003, 73% were correctly assigned to their age class; the expected classification
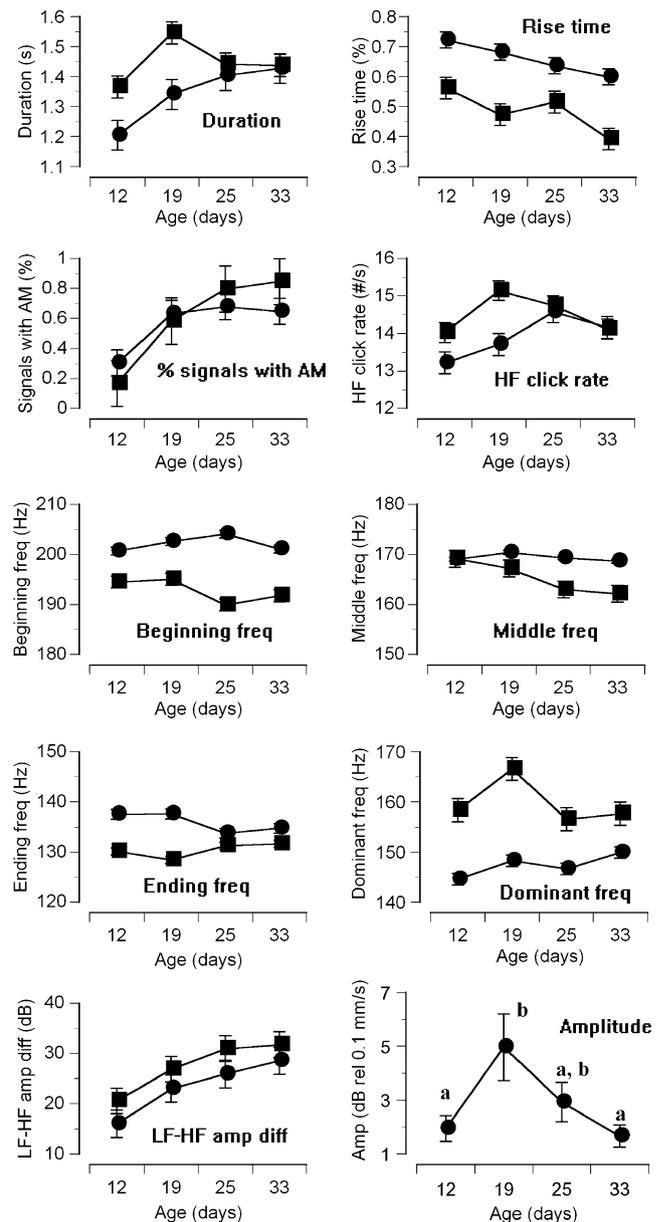
Fig. 2 Change with age in the values of signal parameters (mean ± SE). *Squares*, signals from 2003 ($N=32$ males); *circles*, signals from 2005 ($N=34$ males). For peak amplitude (*bottom right*), *letters* indicate age classes that are significantly different

success for four groups with signals randomly grouped by age class is 25%. DF1 explained 65% of the variation (eigenvalue=1.639, canonical correlation=0.788). Temporal parameters tended to have higher loadings than spectral ones (Table 2). The proportion of signals containing AM had the highest loading with DF1. For signals obtained in 2005, 59% were correctly assigned to their age class, again larger than the random expectation of 25%. DF1 explained 90% of the variation (eigenvalue=1.205, canonical correlation=0.739). As with signals from 2003, temporal parameters had higher loadings, although the rank order was slightly different

Table 2 Loadings from a discriminant function analysis assessing whether signals could be reliably grouped according to age

Parameter	2003	2005
Temporal		
Duration	0.164	**0.612**
Rise time	−0.482	−0.433
Proportion of signals with AM	**0.772**	0.445
HF click rate	0.175	0.502
Spectral		
Beginning frequency	−0.222	0.068
Middle frequency	−0.346	−0.046
Ending frequency	0.172	−0.299
Dominant frequency	−0.025	0.221
LF–HF amplitude difference	**0.591**	**0.824**

Signals recorded in different years were analyzed separately. Numbers in bold denote the two parameters with the highest loadings with the first canonical discriminant function

(Table 2). In 2005, the LF–HF amplitude difference had the highest loading with DF1.

Pair-wise classification scores were all greater than the random pair-wise expectation value of 50%, indicating that signals from different age classes were, for the most part, distinct from each other (Table 3). Signals from the youngest age class were most distinct from those of the three older age classes, with classification success ranging from 80% to 100%. Signals of 25- vs. 33-day-old males had the lowest scores and thus were most similar, but they were still grouped correctly over 64% of the time. Overall, classification scores for 2003 were similar to those for 2005, indicating that the pattern of age-related variation was comparable.

Male variation in signal traits

Within-male variability of signal traits was largest for most of the temporal parameters (except HF click rate), with coefficients of variation ranging from 10% to 95% (Table 4). In contrast, most spectral parameters (except the LF–HF amplitude difference) displayed much smaller coefficients of variation, ranging from 3% to 7% (Table 4). This pattern was not unexpected since the signal traits that varied most with age in the DFA were also traits with the largest within-male CVs. Among-male CVs were similar in magnitude to within-male values for nearly every signal trait (except peak amplitude; Table 4).

Signal amplitude

Peak amplitude varied significantly with male age ($F_{3, 87}=$ 4.54, $p=0.005$, Fig. 2 bottom right). Males aged 19 days

produced signals with the highest amplitude, while males aged 12 and 33 days produced signals with the lowest amplitude. The average difference in amplitude between 19- and 33-day-old males was about 3 dB (an intensity difference of 40%). There were also significant differences in peak amplitude among males within a family ($F_{30, 87}=$ 2.04, $p=0.006$) and between families ($F_{3, 30.3}=10.11$, $p=$ 0.001). There was no family × age interaction ($F_{9, 87}=1.16$, $p=0.329$), indicating that peak amplitude changed over time in a similar way across families.

Female response to age-related signal variation

Females did not respond differently to signals representing males of different ages (log-likelihood ratio (LLR) $\chi^2=$ 3.16, $df=3$, $p=0.37$). The proportion of females that responded to each stimulus ranged from 90% to 96%. Neither the family of origin, nor individual identity of a female, influenced the likelihood of response (family: LLR $\chi^2=-0.00013$, $df=4$; female: LLR $\chi^2=59.77$, $df=45$, $p=$ 0.07. Note that the negative χ^2 value for family was probably due to a rounding error, and no associated p value was provided in the statistical output).

As with the probability of response, there was no difference in the number of responses females gave to each stimulus (LLR $\chi^2=6.29$, $df=3$, $p=0.1$). The average number of responses per female for all stimuli ranged from 2 to 3 (Fig. 3). Family identity did not influence the number of responses given to a particular stimulus (family: LLR $\chi^2=0.00076$, $df=4$, $p=1.0$). However, there were individual level differences in the number of responses given to the different stimuli (LLR $\chi^2=171.79$, $df=45$, $p<0.0001$).

Discussion

This study is the first to show age-based variation in multiple features of a vibrational mating signal. The multicomponent

Table 3 Pair-wise comparisons of the percentage of cases correctly assigned to age class

Age comparison (days)	2003	2005
12 vs. 19	93	80
12 vs. 25	100	93
12 vs. 33	96	89
19 vs. 25	91	75
19 vs. 33	89	84
25 vs. 33	69	64

Signals recorded in 2003 and 2005 were analyzed separately but showed similar patterns. All scores were greater than the random pair-wise expectation of 50%, suggesting that signals from different age classes were largely distinct from one another

Table 4 Variation in vibrational signal traits expressed as coefficients of variation (CV)

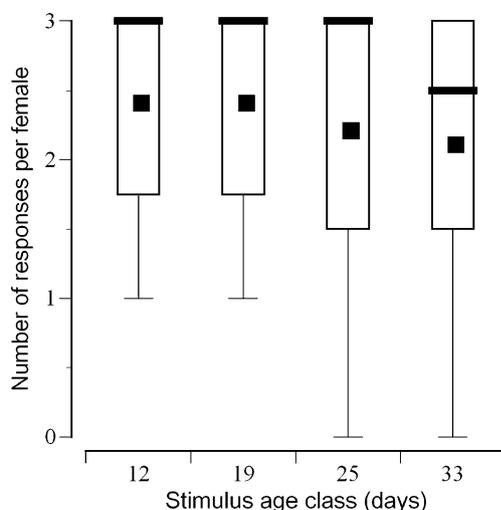| Parameter	2003		2005	
	Within male	Among male	Within male	Among male
Duration	10	10.6	11	12.9
Rise time	30	32	18.5	19
Proportion of signals with AM	94.9	95.9	82.7	78.8
HF click rate	5.8	5	6.7	8.3
Beginning frequency	4.9	6.4	2.9	4.2
Middle frequency	3.4	4.8	3.1	4.2
Ending frequency	4.5	5.5	3.8	5.0
Dominant frequency	6.9	7.6	5.3	6.4
LF–HF amplitude difference	28.2	27.5	31.5	28.8
Peak amplitude	–	–	37	47.3

Average within-male CVs are similar to among-male CVs, indicating that the magnitude of age-related variation within males is comparable to levels of variation among males

mate advertisement signals of *U. crassicornis* exhibit a level of plasticity not normally associated with insect mating signals and contain several age-dependent features that could potentially provide females with reliable age cues. Surprisingly, however, this variation does not influence female choice, at least in the context of long distance mate attraction. Nonetheless, our results indicate that even in short-lived, determinate growth species age can be a significant source of variation in mating signals.

Females in duetting species are expected to be highly selective when replying to long distance advertisement signals, in order to avoid the increased risks and energy costs associated with attracting a large number of males (Jennions and Petrie 1997; Greenfield 2002; Bailey 2003). However, such selectivity might not be expected in a

Fig. 3 *Boxplots* showing the number of responses per female to synthetic signals from different age classes. *Rectangles* indicate the 75th and 25th percentiles, *bottom whiskers* indicate the 10th percentile, and *dark horizontal bars* are the median. *Black squares* represent the mean. Each female received all four stimuli (*N*=50 females). There was no significant difference in the number of responses given to signals from different age classes

species with a mating ecology like that of *U. crassicornis*. Females often interact simultaneously with several males at close range (Wood 1974; Wood and Dowell 1985), and since female *U. crassicornis* mate only once (Wood 1974), responding to a wide range of signals may be adaptive by allowing females the opportunity to evaluate several competing males up close (Janetos 1980; Alexander et al. 1997; Bateson and Healy 2005). Also, by instigating agonistic interactions between rivals, females might be attempting to increase their chances of mating with a dominant high-quality male (Parri et al. 1998; Wong and Candolin 2005; McGhee et al. 2007). Females mate more often with older males, when both older and younger males are present (De Luca and Cocroft 2008), but whether this is a result of female choice or male–male interference competition is unclear.

Surprisingly, there have been few direct tests of the effect of age-related signal variability in species where females exert age-based mate choice. We know of only four such examples. One study in birds (O'Loghlen and Rothstein 2003) and three in insects (Ritchie et al. 1995; Hunt et al. 2004; Kemp 2008), all showed that when the parameter that varied most with age in the male's advertisement signals was experimentally manipulated, females chose the stimulus matching their age-based mating preference. This study provides a counter-example: although female thornbugs mate more often with older males, and although advertisement signals differ between older and younger males, females responded equally to signals representing all age classes. This was unexpected, given the high selectivity of females to male advertisement signals in a related species (Rodríguez et al. 2006). It is possible that female signal preferences in *U. crassicornis* are expressed only after males signal while in contact with the female. However, preference functions are expected to broaden as signal amplitude increases (Gerhardt and Huber 2002), so it is unlikely that uniform responses to signals representing males at 5 cm distance would be transformed into selective

responses to males in closer proximity. Males engage in aggressive interactions (kicking, wing buzzing) during competition for receptive females (P. De Luca and R. Cocroft, unpublished data), and the greater mating success of older males may thus be the result of male–male interference competition. Whether age-related signal variation is important in male–male competition has not been investigated.

What proximate factors might explain why some signal traits varied so strongly with age in *U. crassicornis*? One possibility is that trait expression is linked with the development of other processes occurring over the reproductive lifetime of males. For instance, the close synchrony between the onset of flight and signaling in *U. crassicornis* (both begin to be expressed at 10–15 days post-eclosion) suggests that these behaviors may share a common physiological basis. In addition, the flight motor pattern continues to develop through day 25 (Wood 1974), a period during which changes in signals are especially pronounced. Although the muscles responsible for producing the low-frequency signal component have not been identified in *U. crassicornis*, one likely source currently under investigation is the thoracic muscles that control flight (B. Allison and C. Miles, personal communication). The frequency range of the low-frequency component is within the range of wing beat frequencies reported for similarly sized insects (Casey 1989), and bi-functional flight muscles are commonly used by males in many insect groups in the production of acoustic or vibrational mating signals (Orthoptera: Hennig 1990; Hemiptera: Gogala 2006; Hymenoptera: Hrncir et al. 2006; Diptera: Kanmiya 2006).

Changes in signal features over time may also reflect age-related trade-offs between different aspects of mate acquisition behavior. Sexually mature males begin dispersing from their natal site when they are 15–20 days old (Wood and Dowell 1985). Recently dispersed, sexually active males might be at the peak of their physical condition, and therefore, able to produce higher amplitude signals when advertising for mates. As males get older, the decrease in the amplitude of their signals may be the result of a decline in condition, or changes in investment towards other aspects of mate acquisition, such as mate searching, courtship, or signaling effort. Age-based changes among different components of male mate acquisition behavior have been documented in many species (Marden and Waage 1990; Otronen 1995; Plaistow and Siva-Jothy 1996; Kemp 2002; Saino et al. 2002; Hunt et al. 2006; Fischer et al. 2008) and are currently being investigated in *U. crassicornis*.

In acoustic signaling systems, the amplitude of a signal can affect male mating success. Higher amplitude signals have greater broadcast distances, and females in many species prefer higher amplitude signals in playback tests

(Morris et al. 1978; Ryan and Keddy-Hector 1992; Forrest and Raspert 1994; Gerhardt and Huber 2002). For vibrationally communicating arthropods, this relationship is not as clear. The amplitude of a substrate-borne signal does not decrease monotonically with distance (Michelsen et al. 1982; Markl 1983), which makes amplitude an unreliable signal trait for female choice (Cocroft and Rodrìguez 2005; Hebets et al. 2008). However, the broadcast distance will still be greater, on average, for a higher amplitude vibrational signal, so the signals of younger *U. crassicornis* males will transmit farther. Female *U. crassicornis* are often sparsely distributed in their habitat (Wood and Dowell 1985; Masters 1997), and under these conditions, younger males producing higher amplitude signals may be more successful at stimulating a lone female to initiate a duet for pair formation (Bailey 2003).

The only spectral parameter that varied significantly with age was the relative amplitude of the low- and high-frequency spectral components. In some species of insects, females use the relative amplitude of low- and high-frequency song components to determine the distance to a signaling male (Morris et al. 1975; Keuper and Kuhne 1983; Latimer and Sippel 1987; Bailey et al. 1990; Römer and Lewald 1992). However, unlike these examples where females localize and approach singing males, female *U. crassicornis* remain stationary. Additional playback experiments have revealed that females respond equally well to synthetic signals lacking the high-frequency spectral component (P. De Luca and R. Cocroft, unpublished data), which suggests this signal trait is not necessary for female choice. Alternatively, the different components may be intended for different receivers (e.g., females and competing males), a phenomenon which occurs in the advertisement signals of many species (Andersson et al. 2002; Gerhardt and Huber 2002; Candolin 2003; Loyau et al. 2005).

Many studies have investigated age effects on mating signals in vertebrates, but studies in invertebrates are concentrated solely in arthropods, and most of those are limited to a single insect order and modality (i.e., acoustic signals in Orthoptera—see Judge et al. 2008). Additional taxonomic groups need to be examined, as well as other signaling modalities (e.g., chemical communication in Hymenoptera—Kaltenpoth and Strohm 2006). Our results add some much needed empirical data regarding the significance of age as a source of variation in invertebrate mating signals. While we found significant age-related changes in long distance mate advertisement signals in *U. crassicornis*, they do not underlie female choice of older males. Instead, the mating success of older males may be due to male competition during close range courtship encounters, and whether age-related changes in signals play a role here requires further study. In any case, results

of this study suggest that age effects on mate attraction signals may be pervasive, especially in species with complex multicomponent signals.

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