The Effect of Age on a Sexually Selected Acoustic Display

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Abstract

Models of sexual selection suggest that females should prefer to mate with older males because old age is evidence of heritable high viability. In a longitudinal analysis, we demonstrate that male field crickets (*Gryllus campestris*) alter their calling song with age. Carrier frequency, a calling song character related to growing condition and the main song component under female preference, changed towards higher sexual attractiveness with age. Body mass decreased slightly with age, while chirp rate, an indicator of current condition, remained stable. By choosing males singing at a low frequency, female field crickets would base their mate choice decision on a sexual trait that indicates superior growing conditions as juvenile and on viability, i.e. enhanced current condition as adult.

Introduction

Good genes sexual selection predicts that females choose males on the basis of traits that indicate high heritable viability resulting in offspring of higher fitness (Zahavi 1975). Recent good gene or indicator models have used age as indicator of genetic quality and suggest that females will benefit from choosing an old rather than a young male (Kokko 1998; Proulx et al. 2002). It has been argued that, simply by surviving, males provide information about their genetic quality in terms of viability. Selection should therefore favour mate choice decisions by females that incorporate traits indicating older age, because such traits would intrinsically reveal a male's overall ability to cope within a given environment.

There is a growing empirical evidence that females prefer to mate with old males compared with young males (Grant & Grant 1987; Zuk 1988; Cote & Hunte 1993; Enstrom 1993). Selection for old age, however, may come with costs in terms of genetically correlated responses that affect song effort (Hunt et al. 2006). Accordingly, theoretical models (Hansen & Price 1995; Kokko 2001) and some empirical studies

have found a female mating preference for young or intermediate-aged males (Ritchie et al. 1995; Jones et al. 2000). These differences in mating preference might be explained through the mating system and life history of the species. Where direct benefits prevail, females might benefit from mating with young males as has been suggested in nuptial feeding insects (Vahed 1998). In contrast, in species where indirect, genetic benefits are most important fitness determinants, females may choose a male on the basis of age as an indicator of viability genes. Despite the key role assigned to male age in female mate choice decisions, few studies investigated the variation in display with age within individual males.

Most studies investigating the effects of age on sexual display use a cross-sectional approach, where different individuals are compared between age classes (Simmons & Zuk 1992; Hasselquist et al. 1996; Walker & Cade 2003). This approach is problematic as it remains ambiguous whether the effect on the sexual trait is due to differential mortality, e.g. that attractive, good quality males have superior longevity, or due to a within-individual change in sexual attractiveness with age. Even longitudinal

approaches, where the same individuals are compared at different ages, hold the danger of non-random sampling, as the probability to re-capture an individual is often related to individual quality or condition (Gil et al. 2001; Garamszegi et al. 2005). What is needed are studies with a longitudinal approach where differential mortality, recapture rate or dispersal behaviour cannot confound the results.

In *Gryllus campestris*, males use their modified forewings to produce a long-range acoustic signal to attract receptive females. Song characteristics (e.g. chirp rate, carrier frequency and loudness) are determined by wing properties and the rate and pattern of wing movement. Females are attracted to males calling at a low frequency (Simmons 1995; Simmons & Ritchie 1996; Scheuber et al. 2004), which is an indicator of superior nymphal condition (Scheuber et al. 2003b). Other calling song characteristics have been shown to be less important, and are only incorporated when calling frequencies between displaying males are equal (Scheuber et al. 2004). Based on a cross-sectional field sample, Simmons (1995) described that old males in good body condition generally call at lower carrier frequencies and enjoyed the highest mating success. However, the between-male comparison does not reveal whether high-quality males with low carrier frequencies lived longer (survivor bias) or that the carrier frequency of the calling song changes with age in individual males. To understand the functional significance of age-specific variation in sexual signals and the associated female preference, discriminating between survivor-bias and individual improvement with age is essential. Based on the longevity data from the field (Holzer et al. 2003; Jacot et al. 2004), we studied the calling song of individual male field crickets at a young (day 8) and an old (day 30) adult stage and discussed the results in relation to male attractiveness.

Methods

Male field crickets used in the present study were offspring originating from our laboratory stock population. Groups of 30 newly-hatched nymphs were kept in plastic containers ($l*w*h = 38*20*25$ cm^3). Rearing containers contained sand substrate, egg cardboards for shelter, a water vial, and a plastic tray with ad libitum food (i.e. fish chow, a 1:1 mixture of JBL Novovert and JBL Novogrand, JBL GmbH/Neuhausen, Germany). Temperature in the climate chamber was maintained at 26°C and relative humidity at 65%. The light–dark cycle was 14:10 h with lights-on at 10 PM. Rearing containers were checked daily for newly eclosed adult males. Forty-one adult males were housed individually in plastic jars ($l*w*h = 13*9*18$ cm^3), containing a single cardboard shelter, ad libitum food and a water vial. No mortality was observed during the experimental period, thereby excluding the possibility that presented findings are due to non-random sampling, i.e. differential mortality.

Adult body mass was measured on the first day, the eighth day and the 30th day following the adult eclosion. On day 8 (young) and day 30 (old), we audio recorded the male calling song between 13:00 and 17:00 h (i.e. within the first 5 h of the dark phase) to obtain a qualitative measure of the calling behaviour. All recordings were performed at a standard temperature of 26°C and 65% relative humidity. Recordings were done with a microphone (SONY ECM-16; Sony, Tokyo, Japan), which was located in the lid of the housing container and connected to a digital tape recorder (SONY TCD-D100; Sony, Tokyo, Japan). Prior to audio recording, we removed the cardboard shelter and positioned the microphone at a distance of 16 cm ($\pm$1) above the base of the housing container. For data analysis, we digitized a 30-s sequence taken at least 2 min after the start of calling activity. Using CANARY software (Charif et al. 1995), we evaluated chirp rate (no. of chirps/s), syllable number (no. of syllables/chirp), chirp duration (ms), interchirp duration (ms), chirp intensity (fW/m^2), and carrier frequency (kHz) according to standard criteria (Scheuber et al. 2003a). The carrier frequency of the calling song is the frequency of the basal tone that contains the most energy.

Data analyses were performed by the SPSS 14.0 statistical package. All data were presented as means $\pm$ one SE. To analyse the variation in body mass and calling song characteristics between age classes, we used restricted maximum likelihood mixed models with age classes as fixed factor and individual as random term. To account for multiple testing in the analysis of the calling song, we used Bonferroni correction and adjusted the level of significance α for the six tests to 0.0083.

Results

Forty-one male crickets were recorded on day 8 and on day 30 post-adult eclosion. One male was excluded from all analyses because of an abnormal calling song on day 30. The calling song of this individual had approximately nine syllables per chirp and resembled the aggressive call used in agonistic displays (Alexander 1961).

Effect of Age on Body Mass

Mean (±SE) body weight of males has been measured for a sample of 28 males and varied between 0.97 g (±0.02) on adult eclosion, and 1.10 g (±0.02) and 1.06 g (±0.02) on the occasion of the first and second recording the calling song (comparing days 0, 8 and 30: $F_{2,54} = 83.14$, $p < 0.001$). A post hoc Tukey–Kramer comparison between the age classes revealed a significant increase during the first eight days post-eclosion ($p < 0.001$) and a decrease in weight from day 8 to day 30 ($p = 0.004$).

Effect of Age on Calling Song Characteristics

Male field crickets altered their calling song with age. Carrier frequency varied significantly with age (Table 1). Old males called at a lower carrier frequency compared with young males. The mean within-individual change in carrier frequency was 80 Hz, resulting from a decline in frequency from 5078 ± 22.24 Hz on day nine to 4998 ± 28.33 Hz on day 30. Syllable number and chirp length were highly correlated ($r = 0.813$, $p < 0.0001$) and both affected by age (Table 1). Chirps of old males were slightly shorter with a lower average number of syllables compared with chirps of young males. We did not detect an effect of age on other calling song characteristics, although males tended to sing at lower amplitude with older age (Table 1; chirp energy). The change in carrier frequency or any other calling song characteristic was not related to initial carrier frequency (Fig. 1), initial body mass or subsequent change in body mass.

Discussion

This study revealed systematic changes in the calling song of male field crickets with age. Most pronounced was the decline in carrier frequency with age. As females show a preference for calling songs
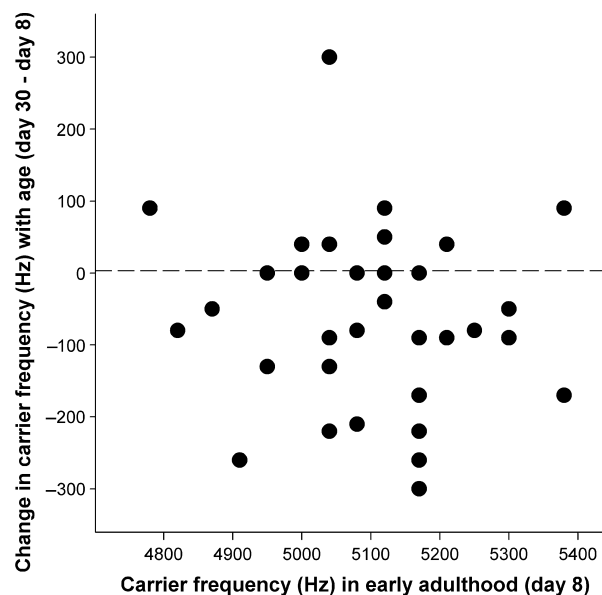
Fig. 1: The change in carrier frequency (CF_{30}–CF_8) in relation to the carrier frequency on day 8. Negative values on the y-axes indicate a decrease in carrier frequency with age

of lower carrier frequency (Scheuber et al. 2004), our results suggest that sexual attractiveness of individual males increases with age.

One explanation for changes in male sexual display with age is related to seasonal variation in optimal investment in signalling (Velez & Brockmann 2006). Variation in environmental conditions, i.e. denser vegetation late in the season, might affect optimal signalling towards lower frequencies. Alternatively, males could make a final or terminal investment with old age. As the residual reproductive value decreases with age, individuals are expected to increase their current investment in reproduction and signalling (Polak & Starmer 1998; Candolin 2000; Bonneaud et al. 2004). However, variation in dynamic signalling traits, such as the time spent calling with age (Bertram 2000; Hunt

Table 1: Means (±SE) and test statistics for calling song characters in relation to cricket age

Variable	Age class		dF	F	P
	Young	Old			
Carrier frequency (kHz)	5.08 ± 0.023	4.99 ± 0.029	1,39	15.88	<0.001*
Chirp rate (chirps/s)	3.86 ± 0.83	3.91 ± 0.83	1,39	0.28	0.60
Syllable number (syllables/chirp)	3.67 ± 0.078	3.36 ± 0.088	1,39	11.08	0.002*
Chirp length (ms)	99.99 ± 2.46	91.46 ± 2.46	1,39	9.93	0.003*
Interchirp duration (ms)	163.57 ± 6.32	169.05 ± 4.61	1,39	1.13	0.295
Chirp energy (fW/m^2)	27.73 ± 3.57	17.36 ± 2.15	1,39	6.10	0.018

*Significant at the $\alpha = 0.0083$ level (Bonferroni corrected).

et al. 2006), does not indicate terminal investment in other cricket species. In addition, it is unclear by what costly process a male cricket could strategically achieve a decline in carrier frequency with age, as this calling song component is closely related to structural components of the modified forewings.

The decline in carrier frequency with age is more likely a result of aging, reflecting a progressive change in structural properties of the forewing through wear and tear over time. Physical properties of the sound-producing stridulatory file are known to deteriorate with age in bushcrickets. Pegs on the file wear down and even break off through use and are known to alter the calling song and thereby also female preference (Hartley & Stephen 1989; Ritchie et al. 1995). In addition, the colour of the sound-producing harp gets darker with age (Jacot A., pers. obs.), probably because of a change in melanization or sclerotization (Hooper et al. 1999). The 'clock-work cricket' model (Elliott & Koch 1985) proposes that the carrier frequency is determined principally by the mechanical resonance of the wing cells that radiate sound. A possible, potentially condition-dependent mechanism that needs further investigations is that enhanced wing melanization within a lifetime lastingly affects the resonance properties of the harp and thereby the carrier frequency of the calling song.

Syllable number and chirp length, two highly inter-correlated song parameters, were additionally affected by age. Interestingly, a study in the house cricket (*Acheta domesticus*) revealed high heritability on immune parameters that were correlated to syllable number (Ryder & Siva-Jothy 2001). However, syllable number and chirp length have never been shown to convey information about current adult condition (food availability in *G. campestris*: (Scheuber et al. 2003a), past nymphal condition (food availability in *G. campestris*: (Scheuber et al. 2003b), immune status in *G. campestris* (Jacot et al. 2005), and immunocompetence in *A. domesticus* (Ryder & Siva-Jothy 2000). In contrast to these findings, syllable number was used in mate choice decisions in other cricket species (in *G. integer*: (Wagner et al. 1995), in *G. lineaticeps*: (Wagner 1996), and it is likely that such temporal properties of long-range calls are more important in species recognition than as quality indicators. Future studies are urgently needed to demonstrate the importance of each calling song component on female sexual preference (Scheuber et al. 2004). Such experiments might also reveal the relative importance of amplitude or loudness of the calling song, which tended to decline

with age in male field crickets *G. campestris*. The lesser amplitude may reduce the detectability of males by females and counteract the beneficial effect of declining carrier frequency on male mating success with age.

In a previous study, we demonstrated that superior nymphal nutritional condition reduced carrier frequency by around 130 Hz (Scheuber et al. 2003b) while this study demonstrates that old crickets sang 80 Hz lower than young crickets. In both studies, the change in frequency was smaller than what has been used to elicit a female response (Scheuber et al. 2004). As discriminative abilities, i.e. the stimulation of the tympanal membrane of female field crickets, depend on signal amplitude (Kleindienst et al. 1983), one predicts that small changes in frequency ($\sim$ 100 Hz) will mainly be perceived in songs of low amplitude. It thereby remains unclear if and under what conditions, the observed change will affect female mate choice. If females would mainly base their mate choice decision on carrier frequency, they will select males on the basis of superior nymphal condition but also in relation to viability, which is related to adult nutritional condition and adult immune status (Jacot et al. 2004). In conclusion, these data suggest that directional female preference for low carrier frequency will favour old aged males with good overall condition that had superior past condition in addition to prime current condition.

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