

Signal components, acoustic preference functions and sexual selection in a cricket

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In many sexually reproducing organisms, females choose mates based on multiple male traits. This study examined how two temporal components of the male mating call – chirp rate and chirp duration – affect female mating preference in five populations of a widely distributed North American cricket, *Allonemobius socius* (Orthoptera, Gryllidae). Chirp rate and chirp duration of the *A. socius* mating call were varied independently, and the responses of virgin females to these experimentally manipulated calls were repeatedly measured using a sequential single-stimulus design. Significant among- and within-population variation in chirp-duration preferences of females were found. Contrary to many previous studies, call chirp rate had no effect on female phonotaxis. Also there was no evidence of an interaction between chirp rate and chirp duration on female response to male mating calls. Moreover, female responsiveness to average and above-average chirp duration appeared to decline with female (adult) age. Overall, these results suggest evolved differences among populations in chirp-duration preferences, and that selection can act within populations on female chirp-duration preference. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 83, 461–472.

ADDITIONAL KEYWORDS: age effect – *Allonemobius* – calling song – ‘no choice’ paradigm – phonotaxis – signal component.

INTRODUCTION

Sexual selection results from variation among males in their ability to compete for mates or in their attractiveness to females (Darwin, 1871). Once controversial (Andersson, 1994), sexual selection through female mate choice now remains a vigorous area of theoretical and empirical study (Heisler, 1984, 1994; Eberhard, 1996; Gavrillets, Arnqvist & Friberg, 2000). Today, many behavioural biologists focus attention on why female mating preferences evolve (Andersson, 1994; Jennions & Petrie, 1997).

Despite broad interest in the evolution of female mating preferences, most studies examine group preferences rather than preferences of individuals (Jennions & Petrie, 1997; Wagner, 1998). In group-preference studies, the experimenter typically exposes

each female only once to a given stimulus or pair of stimuli, establishing preference when females of a given population respond more strongly or more often to one trait value than they do to alternative values. Such tests allow rapid assessment of population-level preferences. In contrast, documenting individual preferences requires many preference trials with each female. As a result, few studies, for example, Godin & Dugatkin (1995), Wagner, Murray & Cade, 1995), and Brooks & Endler (2001a, b), have examined variation in mating preferences within and among females.

Measuring individual preferences allows examination of the fitness consequences of preference variation. Females might benefit from different preferences in two general ways. First, different preferences based on a given male trait may confer different fitness benefits (Zeh & Zeh, 1997a, b; Tregenza & Wedell, 1998, 2000), or else arise when the benefits of mating with preferred males outweigh the costs of searching for or associating with them. In such cases, variation among females in physiological condition, age, and other factors can influence these trade-offs. Second, females

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may receive fitness benefits by using different male traits in mate choice. Different male traits can vary in relative importance to females (Jang & Greenfield, 2000), perhaps providing information about different mating benefits (e.g. Moller & Petrie, 2002; Wagner & Harper, 2003). Should females vary in how these different mating benefits affect net fitness, females may then differ in how they rank male traits when choosing mates. Understanding how selection shapes preference, therefore, requires careful analysis of individual preferences.

This study examines sources of variation in female mating preference of *Allonemobius socius* (Orthoptera, Gryllidae), a ground-dwelling cricket inhabiting grassy fields throughout the North American temperate zone (Vickery & Johnstone, 1973; Tanaka & Brookes, 1983; Howard & Furth, 1986; Mousseau & Roff, 1989). The male-limited mating call of *A. socius* consists of repeating short chirps, and varies in chirp rate, chirp duration and other components (Howard & Furth, 1986; Mousseau & Howard, 1998). To date, only one study has examined female call preference in this species: in a test of whether reinforcement accounts for reproductive character displacement between *A. socius* and the closely related *A. fasciatus*, Doherty & Howard (1996) failed to find a preference in *A. socius* females for songs typical of conspecifics over songs typical of *A. fasciatus*.

Here, after confirming species specificity of female phonotaxis, we measured the responses of individual females from five geographically distinct *A. socius* populations to male mating calls that varied in both chirp rate and chirp duration. We presented a random sequence of population-specific mating calls to each female (see Discussion of sequentially vs. simultaneously presented stimuli in Wagner (1998) and in Bush, Gerhardt & Schul, 2002), and then applied repeated-measures analysis of variance to female responses to assess population- and individual-level variation in chirp-rate and chirp-duration preferences.

MATERIAL AND METHODS

FIELD COLLECTIONS AND STOCK MAINTENANCE

Crickets (total $N \sim 1500$) were caught with a collecting net at several sites around south-eastern USA (Fig. 1). They were later identified from publicly available taxonomic keys as *A. socius* (Walker & Moore, 2001). Adults were placed individually in separate polystyrene cages ($H \times W \times D = 4 \times 11 \times 9$ cm) and kept at room temperature ($22\text{--}24$ °C). Each cage was provisioned ad libitum with dry cat food, cardboard cuttings, and a water vial. Field-caught juveniles, in contrast, were housed in plastic bins ($H \times W \times D =$

$21 \times 30 \times 21$ cm) and reared to adulthood under ecologically plausible conditions (14 h daily light and 30 ± 1 °C). All juvenile rearing bins were stocked with cat food, unbleached paper, and several water vials.

Gravid field-caught females were allowed to oviposit in dampened cheesecloth rolled into 7-dram vials, and these were kept at 12 h daily light and 25.5 ± 3 °C. Females not ovipositing after 7 days were presumed virgin, and each was arbitrarily and exclusively paired with same-population males. The large collection of males and females minimized incestuous (e.g. parent-offspring) matings.

Eggs were collected 7 d after initial male–female pairing. Newly hatched juveniles were transferred to polystyrene cages ($H \times W \times D = 4 \times 11 \times 9$ cm) with < 10 nymphs per cage, and reared to adulthood at 14 h daily light and 30 ± 1 °C. Females were isolated from males as early in nymphal development as possible, or else immediately after the final moult.

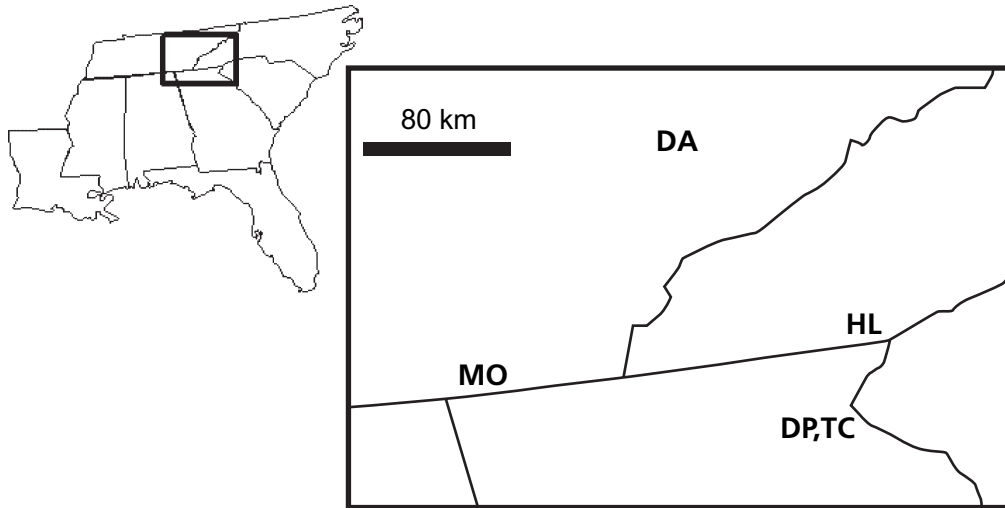
CONSTRUCTION OF SYNTHETIC CALLS

Mating calls of field-collected males were recorded at 23 ± 1 °C in anechoic boxes fitted with microphones (Sennheiser model ME-64). Natural calls were sampled at 22 kHz and saved as 12-bit digitized files using a multichannel event recorder and 'Spike2' software (Cambridge Electronic Design). Customized scripts (Andrew Hill & Cambridge Electronic Design) facilitated analysis of digitized calls.

We then used an *A. socius* sound file from publicly available taxonomic keys (Walker & Moore, 2001) to construct synthetic calls, allowing independent variation of chirp rate and chirp duration without altering the basic pulse structure from which other call traits are derived (Bennet Clark, 1989; Moore, 1989). For example, decreasing chirp rate in a 3-s digitized sample required increasing only the intervals between adjacent chirps (i.e. the interchirp intervals). Similarly, we increased chirp duration by increasing only the intervals between pulses (i.e. the interpulse intervals), maintaining identical interpulse intervals throughout. Thus, other properties of the *A. socius* call, such as pulse duration, pulses per chirp, and dominant frequency, remained identical across synthetic calls. We modelled synthetic calls after the natural call characteristics of each study population (Fig. 1).

MEASURING CALL PREFERENCE

Test subjects were offspring of field-matured *A. socius* females, and had <24 h exposure to calling males before commencement of behavioural trials. Hence, except for slight differences in population density among replicate rearing containers, all test subjects experienced similar juvenile and adult environments.



	MO	DA	DP	TC	HL
Site Elevation, m	203	281	314	314	1237
No. of Males, <i>N</i>	29	159	93	104	13
CR (\pm SD), #/s	3.02 \pm 0.54	3.11 \pm 0.64	3.40 \pm 0.71	3.36 \pm 0.69	3.06 \pm 0.37
CD (\pm s.d.), ms	92.7 \pm 9.4	91.5 \pm 9.8	81.0 \pm 10.0	83.8 \pm 8.7	95.6 \pm 10.3
Correlation, $r_{CR,CD}$	-0.030	-0.055	-0.158	-0.143	-0.237

Figure 1. Sampling of *Allonemobius socius* in the south-eastern USA, along with mean call chirp rate (CR) and chirp duration (CD) of field-caught males. Site elevation is given in metres above sea level (Wood, 1996). All calls were recorded at room temperature (22–24 °C). No phenotypic correlation between CR and CD differed from zero (5% Type I error).

Female behaviours were recorded under infra-red light (wavelength >700 nm) with a camcorder mounted above an arena ($L \times W \times H = 80 \times 30 \times 30$ cm) housed in an echo-dampened room. Near one end of the arena, we placed one speaker (Radioshack catalogue no. 40–1400) encircled by a metallic wire (diameter ~ 17.5 cm) defining an area <10% of that of the entire arena floor, with which we scored phonotaxis. Near the opposite end of the arena, we fixed a holding cell from which one female would be released into the arena.

We allowed each female 5 min to acclimate to testing conditions while we calibrated a continuously looped call stimulus to 66 ± 2 dB sound pressure level (SPL) [fast root mean squared (RMS), Brüel & Kjær Type 2236 sound level meter] at ~ 5 cm from the base of the speaker's front grill. Test subjects were allowed to exit the holding cell voluntarily. Females remaining motionless in the holding cell for > 10 min were gently prodded with a pen to exit through an opening at the base of the holding cell. Each videotaped trial began

when the exiting female lifted one hind leg up off the floor of the holding cell, and continued for a targeted duration of 20 min. Except for one trial lasting only 10 min (due to video equipment malfunction), variation in trial duration reflected extremely precise noting of elapsed time by the experimenter (i.e. entering spreadsheet data and, after a trial, reaching for a light switch).

Females each underwent 25 trials involving unique combinations of population-standardized chirp rates and chirp durations, plus one trial using a heterospecific (*A. tinnulus* Fulton) call obtained unmodified from publicly available taxonomic keys (Walker & Moore, 2001). Females also underwent separate, silent trials under otherwise identical test conditions to discount putative olfactory cues.

Females that completed all 25 trials involving *A. socius* stimuli were analysed for positive phonotaxis, quantified initially as the proportion of the total trial duration spent inside the speaker area (delimited

by the circular wire). Responses of females completing silent and heterospecific-stimulus trials were measured likewise but analysed separately.

We minimized 'carry-over effects' in repeated phonotaxis measurements (Wagner, Smeds & Weigmann, 2001) by allowing 12–96 h between adjacent trials per female. Each call stimulus (or null stimulus, as in the silent trials) was presented in arbitrary order and only once per female.

STATISTICAL ANALYSES

To minimize heteroscedasticity (Sokal & Rohlf, 1981), we transformed time-dependent observations (i.e. adult age, trial duration and phonotaxis) to normalized ranks, which allow for statistical tests that attain asymptotic relative efficiencies (\sim statistical power) equal to or greater than those of any parametric or non-parametric test (Conover, 1999: 396). Unlike ranks per se, normalized ranks facilitate unbiased tests of interactions (Conover, 1999: 419).

To analyse female phonotaxis, we constructed the following ANOVA model:

$$Y_{ijkl} = \mu \dots + POP_i + FEM_{j(i)} + CRAT_k + CDUR_l + (POP \times CRAT)_{ik} + (POP \times CDUR)_{il} + (FEM \times CRAT)_{jk(i)} + (FEM \times CDUR)_{jl(i)} + (CRAT \times CDUR)_{kl} + (POP \times CRAT \times CDUR)_{ikl} + \epsilon_{(ijkl)},$$

where Y_{ijkl} is normalized-rank stimulus response, $\mu \dots$ is a common mean, POP_i is a random-effect term denoting population origin, $FEM_{j(i)}$ is a random-effect term denoting female nested within population, $CRAT_k$ is a fixed-effect term denoting population-standardized chirp rate (repeated factor), $CDUR_l$ is a fixed-effect term denoting population-standardized chirp duration (repeated factor), and $\epsilon_{(ijkl)}$ is the random-effect error term. We declared interaction effects random when involving a random-effect main factor.

Because test subjects were measured once per stimulus (given the repeated-measures design), the error term in the above model is not only unreplicated, but also includes a term denoting three-way interaction among test subject, chirp rate and chirp duration, i.e. $(FEM \times CRAT \times CDUR)_{jkl(i)}$, as well as terms denoting interactions with female age (analysed separately; see below). However, the lack of within-subject, within-treatment replication only produces conservative tests of interactions (Neter, Wasserman & Kutner, 1990: 1050).

We obtained descriptive statistics, correlation estimates, and mean squares (under the GLM procedure) with SAS for Windows 6.12 (SAS Institute, 1999). Only 15 of the original 23 females completed the minimum required 25 trials. Hence, the total sample size was $15 \times 25 = 375$ repeated observations on 3 ± 1 (mean \pm SD) females per population: three females

from the DA population, four from DP, two from HL, two from MO, and four from TC (see Fig. 1). Finally, we used the VARCOMP procedure in SAS 6.12 to assess the effect of female adult age on call preference. We quantified relative contribution of each term in the following ANOVA model to total variance in normalized-rank stimulus response:

$$Y_{ijklmn} = \mu \dots + POP_i + FEM_{j(i)} + AGE_{k(ij)} + CRAT_l + CDUR_m + (POP \times CRAT)_{il} + (POP \times CDUR)_{lm} + (FEM \times CRAT)_{jl(i)} + (FEM \times CDUR)_{jm(i)} + (AGE \times CRAT)_{kl(ij)} + (AGE \times CDUR)_{km(ij)} + (CRAT \times CDUR)_{lm} + \epsilon_{n(ijklm)}.$$

This is similar to our repeated-measures ANOVA model, except for the incorporation of a term denoting female age (in weeks posteclosion) nested within test subject and population, $AGE_{k(ij)}$, and the absence of three-way interactions. Non-independence of error terms renders the above model inappropriate for formal significance testing, but provides quantitative description of the relative importance of different factors to female phonotaxis. We chose the method of restricted-maximum likelihood (REML) to estimate variances (e.g. Shaw, 1987), and omitted three-way interactions to enable convergence of the REML algorithm.

RESULTS

VALIDITY OF BEHAVIOURAL MEASUREMENTS

Despite differences in trial duration among test subjects (one-way ANOVA, $F_{21,512} = 16.02$, $P < 0.0001$), stimulus response remained uncorrelated with trial duration ($r = -0.47$, two-tailed $t = 2.14$, $P = 0.0691$, $N = 16$). Thus, it appears that our phonotaxis measurements were not confounded with female habituation to stimuli, or other similar experimental artefacts.

RESPONSES TO NON-SPECIFIC STIMULI

There was no difference among populations in their tendency to associate with the silent speaker (one-way ANOVA, $F_{4,10} = 1.04$, $P = 0.4337$). In silent trials, there was no significant variation among populations in female age (one-way ANOVA, $F_{4,10} = 2.77$, $P = 0.0615$).

Also, populations did not differ from each other in response to heterospecific calls (one-way ANOVA, $F_{4,12} = 0.55$, $P = 0.7048$). There was no significant variation among populations in the ages of females tested with heterospecific calls (one-way ANOVA, $F_{4,12} = 1.74$, $P = 0.2066$).

EXPERIMENTAL FACTORS AFFECTING CALL PREFERENCE

The CRAT and CDUR main effects examined variation in response to across-population variation in

A. socius call chirp rate and chirp duration, respectively (i.e. species-level preferences). We found no significant species-level preference based on chirp rate, but significant species-level preference based on chirp duration (Table 1).

The POP main effect examined variation among populations in general responsiveness (i.e. the mean, or else summed, female response to all stimuli, regardless of preference for any stimulus). There was no significant variation among populations in female responsiveness (Table 1). The POP \times CRAT and POP \times CDUR interactions examined variation among populations in female preference based on chirp rate and chirp duration, respectively. There was no significant variation among populations in female chirp-rate preferences, but there was significant variation among populations in female preference for chirp duration (Table 1). Unlike with call chirp rate, there appeared to be subtle and systematic increases in phonotaxis with increasing chirp duration in three *A. socius* populations, while the remaining populations showed maximum responses near the mean value of chirp duration (Fig. 2).

The FEM(POP) main effect examined variation among females within a population in overall responsiveness to call stimuli, and appeared statistically significant (Table 1). The FEM(POP) \times CRAT and FEM(POP) \times CDUR interaction effects examined vari-

ation among females within populations in response to chirp rate and chirp duration, respectively. Females within populations appeared not to vary in their chirp-rate responses, but varied in their chirp-duration responses (Table 1). Females appeared to differ in location of peak preference and in shape of preference functions (Fig. 3), suggesting within-population variation in strength of individual response to preferred values of chirp duration.

The CRAT \times CDUR effect examined the interaction between chirp rate and chirp duration on female response across populations. At the species level, female response based on chirp rate showed no dependence on chirp-duration values, and vice versa (Table 1). The POP \times CRAT \times CDUR effect examined variation among populations in the intensity of interaction between chirp rate and chirp duration. There was no significant variation among populations in the nature of interaction between these two male traits (Table 1).

VARIANCE CONTRIBUTION OF ADULT AGE

The AGE(POP, FEM) term quantified the effect of adult age on female response to call stimuli. Despite lacking formal tests of REML variances (in SAS 6.12), we could infer statistical significance of the AGE(POP, FEM) term because its variance contribution exceeded the smallest known non-zero variance, i.e. FEM(POP) \times CDUR (Tables 1 and 2). Similarly, we could infer a significant contribution from the interaction between female age and chirp duration, AGE(POP, FEM) \times CDUR, because its variance contribution exceeded the FEM(POP) \times CDUR variance

Table 1. Repeated-measures analysis of variance on conspecific call preference of female *Allonemobius socius*

Source of variation	d.f.	Type III MS	F
(1) Population	4	6.3235	0.65 ^{NS}
(2) Female within population	9	9.7937	27.20 ^{***}
(3) Call chirp rate	4	0.5505	0.94 ^{NS}
(4) Call chirp duration	4	14.2736	4.38 [*]
Interaction of (1) & (3)	16	0.5879	1.65 ^{NS}
Interaction of (1) & (4)	16	3.2600	4.75 ^{***}
Interaction of (2) & (3)	36	0.3557	0.99 ^{NS}
Interaction of (2) & (4)	36	0.6861	1.91 ^{**}
Interaction of (3) & (4)	16	0.4318	1.33 ^{NS}
Interaction of (1), (3) & (4)	64	0.3250	0.90 ^{NS}
Error†	169	0.3600	

^{NS} $P > 0.05$, ^{*} $0.05 > P > 0.01$, ^{**} $0.01 > P > 0.001$, ^{***} $P < 0.001$.

†Technically, the error mean-squares (MSE) contains the interaction among test subject, chirp-rate preference, and chirp-duration preference, i.e. FEM(POP) \times CRAT \times CDUR, as well as those arising from age of test subject, which is not incorporated in this analysis. See Methods for details.

Table 2. Restricted-maximum likelihood estimation of variance components for female response to male mating call in *Allonemobius socius*

Source of variation	Relative contribution (%)
POP	0.0
FEM(POP)	19.4
AGE(POP, FEM)	8.8
CRAT	0.6
CDUR	27.6
POP \times CRAT	0.0
POP \times CDUR	11.1
FEM(POP) \times CRAT	0.7
FEM(POP) \times CDUR	4.7
AGE(POP, FEM) \times CRAT	0.0
AGE(POP, FEM) \times CDUR	5.5
CRAT \times CDUR	0.0
Error	21.6
Total	100.0

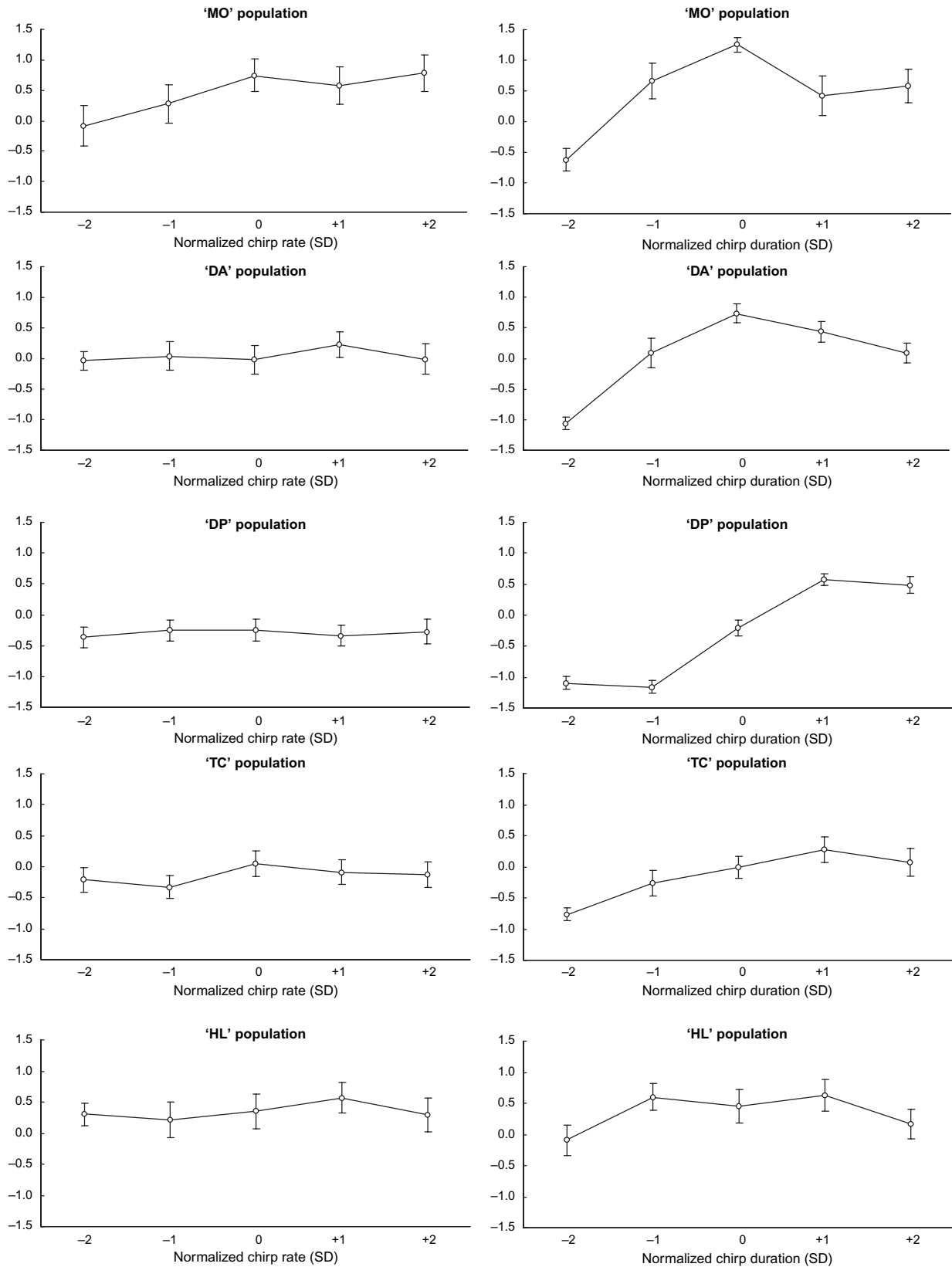


Figure 2. Preference functions of *Allonemobius socius* females for chirp rate and chirp duration of male mating calls. The vertical axis in each panel is normalized-rank stimulus response (mean \pm SE).

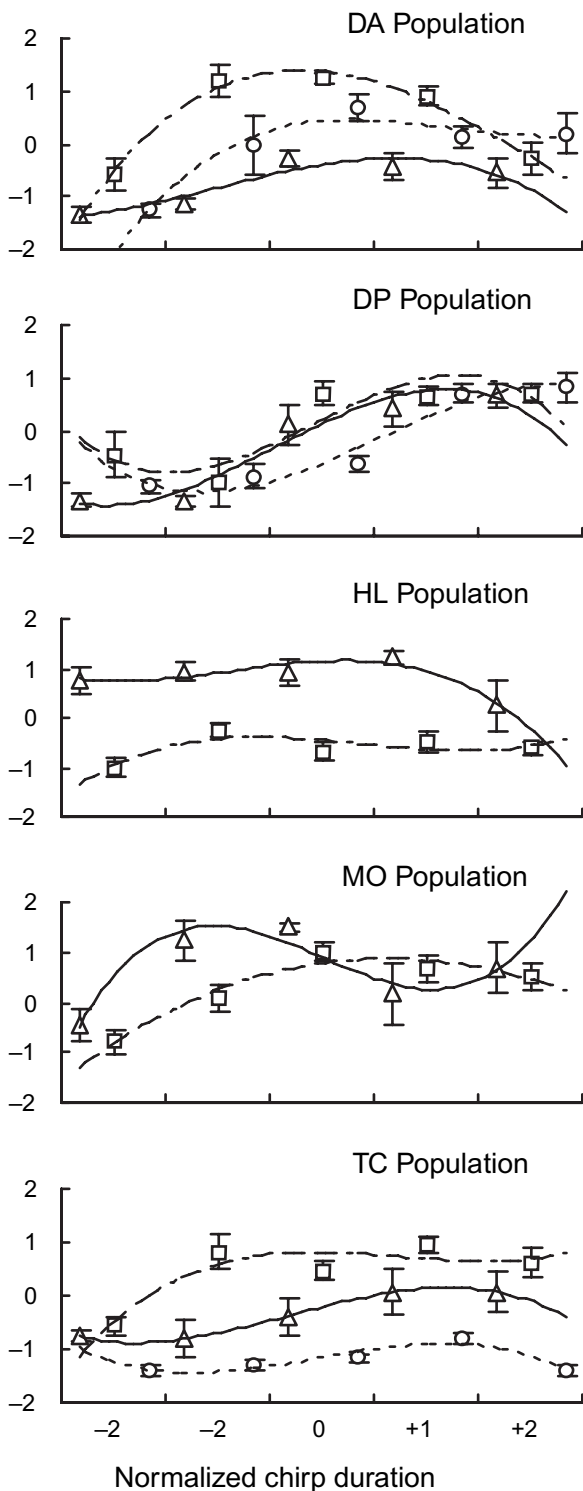


Figure 3. Variation in shape of individual preference functions for *Allonemobius socius* mating call. Cubic regressions (range of $r^2 = 0.65\text{--}1.00$) are fitted to mean response averaged across chrip-rate responses of select females (female 1 = Δ , female 2 = \square , female 3 = \circ) to population-specific chrip duration (in SD). The vertical axis in each panel is normalized-rank stimulus response (mean \pm SE).

term. Phonotactic response to preferred values of chrip duration appeared to decline with age of test subjects (Fig. 4). In contrast, the interaction between female age and chrip rate contributed nothing to female phonotaxis (AGE(POP, FEM) \times CRAT in Table 2), which remained consistent with the repeated-measures ANOVA results: chrip rate and interactions involving chrip rate showed no effect on female phonotaxis (Table 1).

DISCUSSION

These findings illustrate the complex nature of variation in female *A. socius* mating preferences. Our use of sequential, single-stimulus presentations and crossed-nested ANOVA with repeated factors allowed examination of among- and within-population preference variation, as well as the potential interaction of preferences based on two male traits.

POPULATION VARIATION IN RESPONSIVENESS AND PREFERENCE

There was no significant variation among the study populations in overall responsiveness of females to call stimuli (Fig. 2). Females from all populations, thus, appeared to respond equally well to preferred mating calls, and equally well to less preferred mating calls.

There was neither a significant main effect of chrip rate on female responses nor significant variation among populations in female chrip-rate preferences, suggesting no chrip-rate preference at the species level and no variation among populations in chrip-rate preferences (Fig. 2). In contrast, variation in chrip duration had a strong effect on female responses. First, there was a main effect of chrip duration. At the species level, females showed preferences based on chrip duration. Second, there was significant variation among populations in chrip-duration preferences. Across populations, females were least responsive to chrip-duration values at -2 SD from the population mean (Fig. 2). Above -2 SD, female preferences either remained consistently high (e.g. in the HL and MO populations), or else peaked at or slightly above the mean value for chrip duration (Fig. 2). Hence, chrip-duration preference appears to have stabilizing and directional components, the stabilizing component consisting of preferences at or above the mean chrip duration of the population, and the directional component consisting of preferences for chrip-duration values above the lowest extreme. Together, these call preferences suggest selection favouring *A. socius* mating calls with chirps near or slightly above the mean chrip duration. While female mating preferences are often described

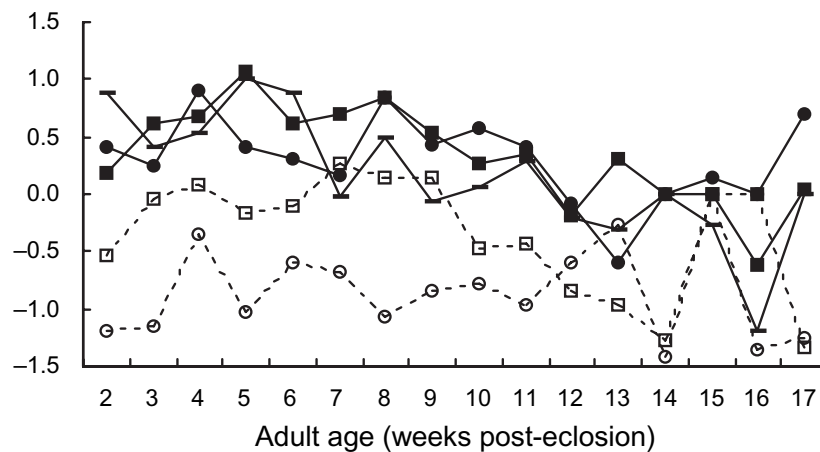


Figure 4. Decline in *Allonemobius socius* female responsiveness to male mating call with increasing adult age. Weeks 3–13 post-eclosion define the middle 90th percentile for stimulus response. The vertical axis is normalized-rank stimulus response averaged across populations and chirp-rate responses. Error bars are omitted for clarity. Chirp duration of -2 SD = \circ , -1 SD = \square , 0 SD = $-$, $+1$ SD = \blacksquare , $+2$ SD = \bullet .

as stabilizing or directional (Lande, 1981), preferences of many species have both stabilizing and directional components (e.g. Gerhardt, 1991; Ritchie, 1996; Simmons, Zuk & Rotenberry, 2001).

Previous studies have reported geographical variation in female mating preferences (Houde, 1988; Ryan & Wilczynski, 1988; Houde & Endler, 1990; Ritchie, 1991; Simmons & Zuk & Rotenberry, 2001). Variation among populations can result from evolved genetic and/or environmental differences. Our study used a 'common garden' approach to examine variation among populations in female mating preferences, i.e. testing laboratory-reared offspring of field-caught females. Because test subjects were reared under common environmental conditions, the observed variation among populations in chirp-duration preferences may have a strong genetic basis, though non-genetic maternal effects, i.e. caused by differences in the environments experienced by the field-caught dams, can account for some or all of this variation. Female fecundity may also have varied among populations, and the variation in population density among family containers used in rearing offspring of field-caught dams may have affected female responses to mating calls. Future work should determine the degree to which observed differences among populations in chirp-duration preferences result from purely genetic vs. purely environmental differences.

Doherty & Howard (1996) reported that female *A. socius* failed to discriminate conspecific calls from those of *A. fasciatus*, a congener with which it naturally hybridizes. To test for female preferences, Doherty & Howard (1996) constructed synthetic calls that varied only in chirp rate (i.e. chirp period was varied but chirp duration remained the same for the two

species) or dominant frequency. Our results can explain this documented absence of female discrimination: while displaying call preferences based on chirp duration, which is not known to differ between the species, *A. socius* females appear unresponsive to variation in chirp rate (\sim chirp period in Doherty & Howard, 1996), which indeed differs between the species (Veech, Benedix & Howard, 1996; Mousseau & Howard, 1998).

One strength of our experimental approach was the use of synthetic signals in which we could independently vary two male signalling traits. Only a few studies have explicitly examined the relative importance of different male traits to female mate choice, and many of these used live males and/or natural stimuli as models, which often precludes independent manipulation of different traits (but see Zuk, Ligon & Thornhill, 1992 and Basolo, 1998). In contrast, numerous studies have examined female preferences based on multiple male traits using independent choice tests in which all but one trait was held constant (e.g. Gerhardt, 1991; Moller, 1992; Ritchie *et al.*, 2001). While such tests can identify targets of female mating preferences, they also preclude direct comparison of the relative effect of each trait on female mating decisions.

Why female *A. socius* responded more strongly to variation in chirp duration than in chirp rate remains unclear. Both chirp rate and chirp duration are common targets of female mating preferences in orthopteran producing chirped calls (e.g. Pollack & Hoy, 1981; Simmons, 1988; Stout & McGhee, 1988; Wagner, 1996). In *A. socius*, chirp duration – and not chirp rate – may provide information about mating benefits. One possible pathway through which females might bene-

fit from their chirp-duration preferences is through 'age effects' on male calling behaviour. Because older males may appear better at acquiring and defending resources (Marchetti & Price, 1989), age may advertise superior physiological condition such that females mating with older males might obtain greater direct benefits. Some studies have found correlations between sexual display and age (Zuk, 1988; Petrie, Halliday & Sanders, 1991; Brown *et al.*, 1996). However, our preliminary correlation estimates for field-caught but laboratory-matured males yielded a significant chirp duration-by-age correlation in only one of the five study populations (TC population, $N = 46$, partial $r^S = -0.37$, two-tailed $t = -2.264$, $P = 0.0114$). Moreover, the direction of this correlation suggests that TC females actually preferred consorting with younger – not older – males (Fig. 4). Further investigation into the information content of sexual signals is warranted.

INDIVIDUAL VARIATION IN RESPONSIVENESS AND PREFERENCE

Overall, variation among females within populations accounted for 19.4% of the total variation in female response to mating call (Table 2). Females might differ in responsiveness for several reasons: a female might have low motivation to respond, or else respond very strongly and consistently to only one stimulus or to a few stimuli. The determinants of female responsiveness should be investigated in greater detail.

Phenotypic variation makes possible selection on preferences and evolutionary response to selection. While showing no variation in chirp-rate responses, *A. socius* females within populations displayed preferences for different values of call chirp duration (Fig. 3). Absence of individual variation in chirp-rate preferences, combined with the population-level result, suggests that *A. socius* females uniformly ignore variation in chirp rate, an unexpected result given previous studies reporting individual variation in preferences (e.g. Stout & McGhee, 1988; Godin & Dugatkin, 1995; Wagner *et al.*, 1995; Brooks & Endler, 2001a, b; but see Boake, 1989 and Ritchie, 1992). One explanation for not finding variation in chirp-rate responses might be insufficient statistical power to detect individual preferences. However, our success in documenting among-female variation in chirp-duration preferences (small sample size notwithstanding) renders such an explanation unlikely. Indeed, if there is no phenotypic variation in chirp-rate preferences, then contemporary selection cannot act on this preference, at least not to the salient degree with which it can act on chirp-duration preferences.

Females tended to prefer above-average values of chirp duration, but within populations they varied in

their chirp-duration preferences: except for the DP population, no consistent pattern emerged to indicate an optimal chirp duration (Fig. 3). Among-female variation in chirp-duration preferences suggests that some of the observed variation is genetically based, and that selection can act on this preference. Whether or not among-population differences reflect geographical variation in the nature of selection is unknown. Our documentation of among- and within-population variation in call preferences, however, suggests this as a productive avenue of research.

NO INTERACTION BETWEEN CHIRP RATE AND CHIRP DURATION

The nature of interactions between attractiveness of male traits can have important consequences for the evolution of sexual displays. First, a male that produces preferred values of one trait may benefit little in producing preferred values of another, even when females prefer both traits. Under such conditions, males should only produce preferred values for one of the traits, particularly when producing preferred trait values proves costly. Second, a male may only benefit from producing preferred values of one trait when it produces preferred values of another. Under such conditions, males that can sustain the costs of producing preferred traits should produce preferred values for both traits.

Our experimental design yielded no interaction between chirp-rate and chirp-duration responses. Moreover, there was no variation among populations in the nature of interaction between female responses to these two traits (i.e. no three-way interaction among population origin, chirp rate and chirp duration), suggesting that female response to chirp rate has no bearing on chirp-duration response, and vice versa. The absence of such interaction may not necessarily arise from the lack of population-level chirp-rate preference: there might be no overall effect of chirp rate on (average) mating responses of females, yet individual female responses based on chirp rate might still vary depending on chirp duration.

When the interaction between two male traits affects female mating responses, long-term sexual selection should affect the pattern of phenotypic correlation among male traits. Males producing more preferred values of one trait, for example, may receive only marginal benefits when producing preferred values of another trait, thus favouring a negative phenotypic correlation between the two male traits. Alternatively, males may benefit substantially from producing more preferred values of one trait when they produce preferred values of another, a scenario favouring a positive phenotypic correlation between the two traits. Our analysis of mating calls from field-

collected adult males indicated no significant phenotypic correlation between chirp rate and chirp duration within the five study populations (Fig. 1), a finding that remains consistent with the absence of interaction between the two calling traits on female phonotaxis.

EFFECT OF AGE ON FEMALE MATING PREFERENCES

Adult age explained a small proportion of the total variance in female phonotaxis (Table 2). Because phonotactic females necessarily travelled a large distance towards the stimulus area, the age-related decline in female responsiveness to chirp duration (Fig. 4) might be due to several physiological changes, including deterioration of auditory perceptiveness and of neuromotor skills (Kodric-Brown & Nicoletto, 2001 and references therein). Such a decline in female somatic condition implies increasingly costly preference with age. However, the extent to which physiological fitness affects female mating preferences over the reproductive life of *A. socius* remains to be demonstrated.

CONCLUSIONS

In summary, *A. socius* females uniformly ignored variation in the chirp rate of male calls. In contrast, conspecific calls with long chirps attracted females; there was both among- and within-population variation for chirp-duration preference. There was no interaction between female preference for chirp rate and that for chirp duration, suggesting no influence of chirp rate (of calling males) on chirp-duration preferences of *A. socius* females. Lastly, female responsiveness to mating calls with average to above-average chirp duration appeared to decline with adult age.

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