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Sounds different: inbreeding depression in sexually selected traits in the cricket *Teleogryllus commodus*

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Abstract

If male sexual signalling is honest because it captures genetic variation in condition then traits that are important mate choice cues should be disproportionately affected by inbreeding relative to other traits. To test this, we investigated the effect of brother–sister mating on advertisement calling by male field crickets *Teleogryllus commodus*. We quantified the effect of one generation of inbreeding on nightly calling effort and five finer-scale aspects of call structure that have been shown to influence attractiveness. We also quantified inbreeding depression on six life history traits and one morphological trait. Inbreeding significantly reduced hatching success, nymph survival and adult lifespan but had no detectable effect on hatching rate, developmental rate or adult body mass. The effect of inbreeding on sexually selected traits was equivocal. There was no decline in calling effort (seconds of sound production/night) by inbred males, but there were highly significant changes in three of five finer-scale call parameters. Sexually selected traits clearly vary in their susceptibility to inbreeding depression.

Introduction

Models for the evolution of adaptive female mating preferences require that males possess sexual traits that are reliable signals of their genetic quality (Tomkins *et al.*, 2004; Kokko *et al.*, 2006). The handicap principle is the most widely invoked mechanism responsible for maintaining honest signalling (Zahavi, 1975; Grafen, 1990). It is usually interpreted to mean that low quality individuals must pay greater marginal costs for a given increase in signalling, however, it is actually possible for the exact opposite to be the case (Getty, 2006). The more general requirement for honest signalling is simply that high quality males are more efficient at converting advertisements into fitness. Efficiency seems to be related to the resources that males have access to because males in better condition tend to produce more attractive sexual signals, leading to condition-dependent signals. In general, males that are better able to accumulate resources (i.e. are in good condition) can allocate more to sexual signalling than those that are less efficient (Rowe & Houle, 1996). Males can differ in condition as a result of external environmental factors (e.g. food availability), biotic factors (e.g. pathogens) and/or because genotypes vary in how they affect resource acquisition and assimilation ability (Rowe & Houle, 1996; Hunt *et al.*, 2004a). Consequently, females choosing males that have invested more into condition-dependent sexual signals might gain genetic benefits for their offspring if some of the variation in signalling is because of additive genetic variation in condition (Tomkins *et al.*, 2004).

To test the extent to which sexual traits are condition-dependent, many experimental studies have attempted to vary male condition by altering environmental quality (e.g. by altering diet, Hunt *et al.*, 2004b) or by adjusting parasite loads (e.g. Zuk *et al.*, 1990). The bias towards manipulating condition in this way is due to the comparative ease with which environmental quality can be adjusted. In contrast, it is extremely difficult to manipulate the genetic basis of differences in male condition because one needs to create *a priori* categories of males that are

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assumed to vary in their condition (Cotton *et al.*, 2004). In the absence of detailed knowledge of the genes responsible for variation in condition one possible solution is to compare males that vary in their level of inbreeding. Sexual signals can then be compared between categories of males that should predictably vary in condition if it is subject to inbreeding depression.

Inbreeding increases homozygosity and results in a decline in fitness known as inbreeding depression. This reduction in fitness is thought to arise from either or both the unmasking of recessive deleterious alleles and the loss of advantageous heterozygosity (Charlesworth & Charlesworth, 1987). In addition, epistatic interactions between loci may also play a role in some species (van Oosterhout *et al.*, 2003). Inbreeding depression in sexually selected traits might be the result of effects of changes in heterozygosity at loci that directly code for sexual traits. If sexual traits are condition-dependent, however, it is highly likely that this will lead to genic capture of negative effects of inbreeding at the many loci that affect the general ability of males to acquire and assimilate resources (Rowe & Houle, 1996).

If condition dependence of sexual signals is commonplace then they should show very strong inbreeding depression relative to other traits because they will capture the genetic effects of inbreeding at many loci. Some studies have documented a reduction in male mating success with inbreeding (e.g. Sharp, 1984; Joron & Brakefield, 2003), but they have not investigated whether this was caused by reduced investment in specific morphological traits, lower courtship or decreased competitive ability during male–male interactions. To date, surprisingly few studies have examined in detail the effects of inbreeding on sexual traits involved in mate choice. Sheridan & Pomiankowski (1997) investigated the effect of inbreeding on several nonsexual morphological traits and male coloration in two populations of the guppy *Poecilia reticulata*. Morphological traits showed very little inbreeding depression, whereas sexual coloration showed a substantial reduction. Interestingly, the pigments that showed the greatest inbreeding depression differed between the populations and appeared to correspond to the colouration that most strongly affects female choice in each population (Sheridan & Pomiankowski, 1997). Similarly, Aspi (2000) compared male courtship song between inbred and outbred strains of *Drosophila montana*. Females prefer courtship songs with a high carrier frequency, which is associated with greater offspring viability. After 20 generations of brother–sister mating, the frequency of the courtship song was the only call trait measured that exhibited significant inbreeding depression. Both of these studies found that male sexually selected traits that are important to female choice were sensitive to the genetic effects of inbreeding. A few other studies on guppies (van Oosterhout *et al.*, 2003; Mariette *et al.*, 2006), *Drosophila* (Miller *et al.*, 1993) and bulb mites (Konior *et al.*, 2005) have also shown that some sexually selected traits show substantial inbreeding depression; however, we still lack sufficient data to know whether sexual traits show greater levels of inbreeding depression than other traits (van Oosterhout *et al.*, 2003).

Here, we tested the prediction that traits that are important mate choice cues will be disproportionately affected by inbreeding in the black field cricket *Teleogryllus commodus*. Males produce a long-distance advertisement call to attract sexually receptive females (Campbell & Shipp, 1979; Evans, 1988). We have already shown that female choice exerts strong positive directional sexual selection for greater calling effort and stabilizing and directional multivariate sexual selection on finer-scale features of advertisement calls (Brooks *et al.*, 2005; Bentsen *et al.*, 2006). Furthermore, through diet manipulations, we have shown that calling effort (calls/night) is condition dependent (Hunt *et al.*, 2004b). Here we quantify the effect of one generation of brother–sister mating (inbreeding coefficient $F = 0.25$) on nightly calling effort and five finerscale components of call structure. To provide a comparison, we also quantified the effect of inbreeding on six life history traits (egg hatching success, days taken to hatch, nymph survival, development time and male and female adult lifespan), and a morphological trait (mass at maturity). Previous studies show that even one generation of brother–sister mating can lead to substantial declines (e.g. > 30%) in life history traits in both wild and captive animals (Crnokrak & Roff, 1999).

Methods

Stock populations

The stock population was established from > 100 naturally mated wild-caught females collected in Canberra, Australia, during March 2003. Each new generation was bred from more than 100 pairs and housed in 6–8 large communal tanks with dry cat food (Kite Kat Krunch, Uncle Ben's, Raglan, Australia) and water provided *ad libitum*. Immature females were separated from males as soon as their ovipositor became visible to ensure their virginity. The stock population, experimental crickets and egg pads (see below) were maintained at 26–28 °C with a 12 : 12 light : dark photoperiod. For the current study, 20 full sibling families were obtained by mating randomly chosen males and females from the stock population in late 2003. Each full sibling family was then reared separately. Emerging nymphs were initially housed in $7 \times 16 \times 12$ cm plastic containers with food and water provided *ad libitum*. As the nymphs grew, each family was split into three larger tubs ($20 \times 15 \times 13$ cm) and males and females were then separated before their final moult.

Generation of inbred and outbred individuals

The 20 full-sibling families were divided into five sets of four families. For each set, we crossed all 16 possible male–

	Females				
Males	Family	A	B	C	D
	A ♂1♂2♂3♂4♂5♂6	♀1♀2♀3♀4♀5♀6	♀1♀2♀3♀4♀5♀6	♀1♀2♀3♀4♀5♀6	♀1♀2♀3♀4♀5♀6
	B ♂1♂2…♂♂♂	♀7♀8…♀♀♀♀	♀7♀8…♀♀♀♀	♀7♀8…♀♀♀♀	♀7♀8…♀♀♀♀
	C ♂♂♂♂♂♂	♀♀♀♀♀♀	♀♀♀♀♀♀	♀♀♀♀♀♀	♀♀♀♀♀♀
	D ♂♂♂♂♂♂	♀♀♀♀♀♀…24	♀♀♀♀♀♀…24	♀♀♀♀♀♀…24	♀♀♀♀♀♀…24

Fig. 1 The mating design used to obtain inbred and outbred individuals. The first set, which comprises families A, B, C and D, is shown. Six females were used for each cross, equating to 24 females from each family per set. Six males per family were used for each set. Males were mated with four females, one from their own family and one from each of the three remaining families. For example, male 1 from family A mated with female 1 from families A, B, C and D. Likewise, male 1 from family B mated with female 7 from families A, B, C and D and so on.

female combinations of the families, generating four inbred crosses and 12 outbred crosses (Fig. 1). We attempted to replicate each cross six times. Female *T. commodus* store sperm and consequently they could only be used in a single cross. Accordingly, six females were used for each cross. Females from each family were crossed with males from their own family and three other families, equating to 24 females from each family per set. Males, on the other hand, can be mated several times in several crosses, allowing for more accurate replication of male genotype among crosses between the four families than was possible for females. Six males from each family were mated with four females, one from their own family and one from each of the three remaining families (Fig. 1). The order of mating was randomized to control for any effect of male mating history on ejaculate production. Matings were carried out in 9 × 9 × 5 cm plastic containers. Where possible, females were mated twice to ensure successful spermatophore transfer ($N = 210$ females). In other cases, females were mated only once to maximize the number of matings obtained from a male before it died ($N = 135$ females). The number of matings a female engaged in was recorded, and included in the initial model for egg hatching success. [Although a previous study showed that the number of matings a female receives from a single male does not affect egg hatching success if successful insemination occurs (M.D. Jennions, unpublished).] Males that died before mating to their four designated females were replaced with a brother. Matings were carried out from March to May 2004.

Life history and morphological traits

Hatching success and days taken to hatch

Following the completion of mating, females were housed individually in 9 × 9 × 5 cm plastic containers with food and a dish of moist sand in which to oviposit. After a week, or earlier if it was obvious that the female had laid, we removed eggs from the sand (Jennions *et al.*, 2004). To measure hatching success we then placed a known number of eggs (96.8 ± 10.0 SD, $N = 282$ egg pads) onto moist cotton wool in a large Petri dish ('egg pads'), covered it with a lid and placed it in a sealed plastic container. These egg pads were then checked every second day for more than 150 days to count hatching nymphs, and record the days taken for eggs to hatch (i.e. the time from when the eggs were placed onto the cotton wool until hatching).

Nymph survival, development time, mass at maturity and adult lifespan

A subset of the emerging nymphs from the hatching success trials were retained and reared to adulthood to quantify the effect of one generation of inbreeding on nymph survival (the proportion of nymphs surviving to adulthood), development time (days from hatching to final moult), mass at maturity (mass at final moult) and adult lifespan (days from final moult to death). From each of the five sets, we attempted to rear 72 inbred nymphs and 72 outbred nymphs to adulthood. Nymphs were chosen to obtain the same representation of family genotypes in the inbred and outbred nymphs. For every set, six nymphs (mean = 5.3 ± 1.7) were kept from each of 12 females that had mated with their brother (three females per family), and six nymphs (mean = 5.8 ± 1.1) were kept from each of 12 females that mated to an unrelated male (one female from each of the 12 outbred crosses) (Fig. 1).

Nymphs were reared individually to eliminate any effects resulting from social interactions that would have been difficult to standardize. After hatching, each nymph was placed in a 9 × 9 × 5 cm plastic container with a water tube (a small plastic tube containing water and plugged with cotton wool at the open end), a piece of cat food and a cardboard egg cup for shelter. These were kept at 26–28 °C at a photoperiod of 12 : 12 light : dark. Each week, food and water tubes were changed and any deaths were recorded. When nymphs approached maturation, they were checked daily. The development time,

sex and mass at final moult were recorded. Adults were then checked daily for mortality.

Calling effort and call structure of males

Male crickets produce calls using a stridulatory apparatus consisting of a file and scraper on both elytra. To produce sound the scraper on the upper side of one wing is rubbed against the stridulatory teeth of the file of the lower side of the other wing. Sound is only produced during the closing movements of the wings (Gerhardt & Huber, 2002). The most basic unit of a song is the pulse. Each pulse is produced by closing the wings once so the pulse repetition rate is equal to the wing stroke rate (Kavanagh, 1987). In the advertisement call of *T. commodus* there are two types of sound pulses. The first are longer and more intense and grouped into chirps. The second are shorter and softer and grouped into trills with a faster pulse rate than that of a chirp. Chirps and trills are arranged into a repeating phrase that consists of a single chirp followed by a variable number of trills (Bentley & Hoy, 1972; Hill *et al.*, 1972) (Fig. 2).

We quantified the nightly calling effort and the call structure of both inbred and outbred males that survived to adulthood using the protocol of Hunt *et al.* (2004b). In brief, call effort was measured using a custom-built electronic monitoring device that can monitor up to 64 males per night. The device consisted of 64 recording chambers (5 × 5 × 5 cm), each with a condenser microphone mounted in the lid. Each chamber housed one male and was placed in a separate closed Styrofoam container (15 × 10 × 10 cm), keeping males acoustically isolated. During sampling, one microphone was activated at a time. If the sound level produced by the individual male is 10 dB or more above the background noise, a '1' representing that the male is calling is recorded. Conversely, if the received signal is < 10 dB above the background noise a '0' representing no calling is recorded. The microphone is then de-activated and the next microphone in the series activated, so that only one microphone is sampling at a given time. We set the sampling speed so that each of the 64 recording chambers was sampled 10 times per second. If a male was recorded as calling for any of the 10 sampling events per second, he was recorded as singing for that second. The nightly calling effort is total number of seconds that a male was recorded as calling per night. Sampling was carried out from 18:00 to 09:00 at a constant 28 °C. The nightly calling effort of males was recorded at both 10 and 15 days post-maturation.

On nights following the assessment of calling effort, males were again set up in a constant temperature room (28 °C) in containers with condenser microphones mounted in the lids. Calls were recorded opportunistically (i.e. if a male was calling during the nightly 2-h sampling period) using a Sony Professional Walkman (Sony, Japan). Five random calls per recording were digitized and we measured the following parameters of the advertisement call using RAVEN software (version 1) (Cornell lab of ornithology, Ithaca, NY, USA): dominant frequency of the call (DF), the interval between the last trill pulse of one phrase and the first chirp pulse of the next, i.e. the intercall interval duration (ICD), the number of pulses per chirp (CPN), the duration of the interval between pulses in the chirp (CIPD) and the number of trills in a phrase (TN) (Fig. 2). These were the same five parameters used by Brooks *et al.* (2005) and Bentsen *et al.* (2006) to quantify multivariate selection on call structure in this species. The reader is referred to Hunt *et al.* (2004b) and Brooks *et al.* (2005) for a more detailed description of call parameters and full information on the apparatus used.

Statistics

Life history and morphological traits

To test for an effect of inbreeding on hatching success, days taken to hatch, nymph survival, development time,

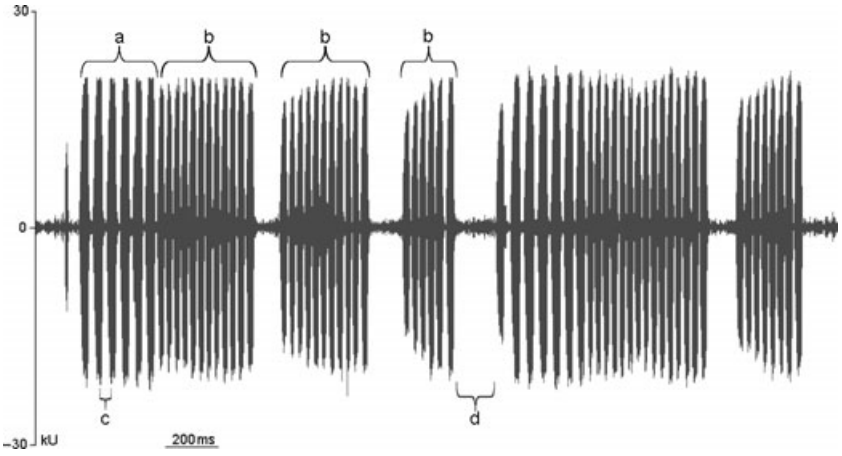

Fig. 2 The advertisement call of male *Teleogryllus commodus*. Louder pulses repeated at a lower rate are grouped into chirps (a) and softer pulses repeated at a higher rate are grouped into trills (b). Each call consists of a single chirp followed by a variable number of trills. We measured the dominant frequency, the number of pulses per chirp, the chirp interpulse interval duration (c), the intercall interval duration (d), and the number of trills in a phrase. The horizontal axis is time (ms), and the vertical is amplitude (kU).

adult lifespan and mass at maturity, we ran separate linear mixed models estimated using REML in S-PLUS 7.0 (Insightful Corp., Seattle, USA). Where necessary, variables were transformed to ensure that residuals were normally distributed and showed homoscedasticity. Maternal family was treated as a random factor and inbreeding as a fixed factor. Offspring sex was included as a fixed factor in some analyses (e.g. adult lifespan). The number of matings that a female engaged in was included as a fixed factor in the model for hatching success, but had no significant effect ($t_{221} = 1.51$, $P = 0.13$). The significance of random terms was assessed using likelihood ratio tests (deletion of random term). However, to be conservative, we did not remove nonsignificant random terms as they were part of the experimental design (their removal did not change the results). We used a model simplification approach, initially fitting a full model and then removing fixed terms until the final model only contained significant terms ($P < 0.05$) (Crawley, 2002). The significance of fixed terms and their interactions were assessed by determining whether model parameter estimates differed significantly from zero using conditional *t*-tests (see Pinheiro & Bates, 2000, p. 87).

Call effort

Calling effort was not normally distributed due to an excess of zero values. We therefore analysed the effect of inbreeding on calling on days 10 and 15 using Mann–Whitney *U*-tests. We also calculated the nonparametric correlation (r_s) between calling effort and male mass, adult lifespan or development rate to test whether these life history variables influenced calling effort and might therefore confound our analysis of the effect of inbreeding on calling.

Call parameters

Of the five call parameters, CIPD, ICD and TN were transformed to fit the requirements of parametric tests. However, DF and CPN could not be transformed successfully and were analysed using nonparametric tests. The repeatability of each call parameter among males was assessed using a one-way ANOVA or Kruskal–Wallis test ($N = 5$ calls/male). All call parameters showed high repeatability (see Table 2). To test for an effect of inbreeding we ran separate linear mixed models for each call parameter with male identity (because we measured five calls per male) and maternal family as random factors and inbreeding as a fixed factor. For DF and CPN we ran separate Mann–Whitney *U*-tests using the mean value per male. All tests are two tailed unless otherwise stated. For life history traits (Table 1), one-tailed tests are based on the strong *a priori* prediction that inbreeding will reduce the value of traits. Summary statistics are presented as mean ± standard error. When two values are presented for a sample size, the first value is the sample size of the inbred group, and the second is that of the outbred group. We calculated the power to detect a significant effect at $\alpha = 0.05$ (two-tailed) given a medium strength effect (*sensu* Cohen, 1988 for *t*-tests and correlation coefficients). Finally, to compare traits, we calculated the standardized coefficient of inbreeding δ (Lande & Schemske, 1985) which is the percentage change with inbreeding calculated as the difference in fitness of inbred progeny relative to outbred progeny divided by the fitness of outbred progeny. A negative value indicates that inbred individuals had a larger value for the trait, interpretation of which depends on the direction of selection on the trait.

Results

Life history and morphological traits

Summary statistics for the effect of inbreeding on life history traits and the single morphological trait are presented in Table 1. One generation of brother–sister mating significantly reduced both hatching success and nymph survival. Inbreeding also significantly reduced both male and female adult lifespan. In addition, within

Table 1 The effect of inbreeding and offspring sex on life history traits.

	Effect of inbreeding			Effect of sex			Mean ± SE		δ (% change
Trait	d.f	*t*	*P*	d.f.	*t*	*P*	Inbred	Outbred	with inbreeding)
Hatching success (%)	222	1.96	**0.026**	–	–	–	42.0 ± 3.2	47.7 ± 2.0	11.9
Days taken to hatch	222	0.27	0.393	–	–	–	23.8 ± 1.9	22.7 ± 0.9	−4.8
Nymph survival (%)	17	1.91	**0.037**	–	–	–	28.6 ± 4.6	42.8 ± 4.4	33.2
Development time (days)	171	0.02	0.492	171	1.83	0.070	♂: 105.9 ± 3.7	♂: 106.5 ± 2.8	0.6
							♀: 110.9 ± 6.7	♀: 105.4 ± 2.8	−5.2
Mass at maturity (mg)	162	0.61	0.271	162	0.26	0.794	♂: 480.9 ± 20.3	♂: 529.8 ± 13.8	9.2
							♀: 567.3 ± 33.0	♀: 505.2 ± 20.6	−12.3
Adult lifespan (days)	122	2.58	**0.006**	122	4.75	**< 0.0001**	♂: 43.3 ± 4.8	♂: 50.0 ± 3.4	13.4
							♀: 52.3 ± 8.7	♀: 76.3 ± 4.0	31.5

P-values for the effects of inbreeding are all one-tailed, whereas those for the effect of sex are two-tailed. Significant *P*-values at the 0.05 level are in bold.

inbred and outbred groups, females lived longer than males. There was no significant effect of inbreeding on the days eggs took to hatch, development time or mass at maturity. Males and females did not differ significantly in development time or mass at maturity (Table 1).

Calling effort

There was no difference between the calling effort on days 10 and 15 (Wilcoxon test: $Z = 1.09$, $N = 75$ males, $P = 0.276$) and these two measures of calling efforts were positively correlated ($r_s = 0.338$, $N = 75$, $P = 0.003$). We therefore compared the mean nightly calling effort of inbred and outbred males and found no significant difference (Mann–Whitney test: $Z = 0.463$, $N = 26$, 51, $P = 0.643$, inbred males = 1957.7 ± 776.1, outbred males = 1865.0 ± 554.2, seconds of calling/night; power ≈ 53%; $\delta = -5.0\%$). Mean calling effort was not correlated with male mass ($r_s = 0.009$, $P = 0.937$), adult lifespan ($r_s = 0.041$, $P = 0.722$) or development time ($r_s = 0.148$, $P = 0.199$) so these variables did not confound our analysis (all $N = 77$; power ≈ 76%).

Call parameters

The measures of all five call parameters were highly repeatable among males (Table 2). For both TN and CIPD the inclusion of both the random terms maternal family and male identity significantly improved the fit of the model (TN maternal family: $\chi^2_1 = 6.85$, $P = 0.009$, male id: $\chi^2_1 = 145.31$, $P < 0.0001$, CIPD maternal family: $\chi^2_1 = 4.75$, $P = 0.029$, male id: $\chi^2_1 = 137.04$, $P < 0.0001$). For the ICD, male identity significantly improved the fit of the model ($\chi^2_1 = 171.54$, $P < 0.0001$) but maternal family did not ($\chi^2_1 = 0.00$, $P = 0.999$).

Inbreeding significantly increased the CIPD ($t_{37} = 7.41$, $P < 0.0001$, inbred: 25.25 ± 0.88 ms, outbred: 18.79 ± 0.36 ms; $\delta = -34.4\%$) and ICD ($t_{37} = 8.95$, $P < 0.0001$, inbred: 678.90 ± 118.92 ms, outbred: 150.59 ± 6.29 ms; $\delta = -350.8\%$) as well as the CPN (Mann–Whitney test: $Z = 3.204$, $P = 0.001$, inbred: 7.04 ± 0.29, outbred: 5.97 ± 0.15; $\delta = -17.9\%$) (Fig. 3). Inbreeding had no detectable effect on TN ($t_{37} = 0.37$, $P = 0.717$, inbred: 2.82 ± 0.28, outbred: 2.83 ± 0.16; $\delta = 0.4\%$) or dominant frequency (DF) (Mann–Whitney test: $Z = 0.370$, $P = 0.711$, inbred: 4.06 ± 0.04 kHz, outbred: 4.06 ± 0.03 kHz; $\delta = 0$) (all tests $N = 17$, 38; power ≈ 17% but the mean estimated effect sizes for DF and TN are close to zero). The effect of inbreeding on CIPD, ICD and CPN remained significant at $P < 0.01$ after Bonferroni correction.

Table 2 The repeatability of call parameters among males (five calls per male) ($N = 55$ males).

Call parameter	Test statistic	*P*-value	r_I
CIPD	$F_{54,220} = 29.79$*	< 0.001	0.939
ICD	$F_{54,220} = 31.10$*	< 0.001	0.937
TN	$F_{54,220} = 18.42$*	< 0.001	0.923
DF	$\chi^2_{54} = 269.96$†	< 0.001	–
CPN	$\chi^2_{54} = 199.17$†	< 0.001	–

DF, dominant frequency; CPN, the number of pulses per chirp; CIPD, chirp interpulse interval duration; ICD, intercall interval duration; TN, number of trills in a phrase; r_I, intra-class correlation coefficient. *One-way ANOVA, †Kruskal–Wallis test.

Discussion

Life history and morphological traits

It is predicted that traits closely related to fitness (i.e. life history traits) will often show greater levels of inbreeding depression than those less closely related to fitness (i.e. morphological traits) (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Inbreeding depression is largely due to the effects of dominance interactions (Charlesworth & Charlesworth, 1987) and life history traits are therefore predicted to show higher levels of dominance variance than morphological traits. This is based on the assumption that they will have reduced additive genetic variance because they are more closely associated with fitness and are therefore under stronger selection which depletes variation (Mousseau & Roff,

Fig. 3 The waveform of the advertisement call of an outbred male (top) and an inbred male (bottom) generated using RAVEN (version 1) software. The horizontal axis is time (ms), and the vertical is amplitude (kU).

1987; DeRose & Roff, 1999). Six of the traits that we measured (hatching success, days to hatching, nymph survival, development time and sex-specific adult lifespan) are generally defined as life history traits (Mousseau & Roff, 1987; Roff, 1998), whereas mass at maturity is generally defined as a morphological trait (Lynch & Walsh, 1998). Male and female adult lifespan were treated as independent traits because lifespan differed significantly between the sexes (see also Hunt *et al.*, 2004b). Consistent with the above prediction, we found substantial inbreeding depression in four life history traits [hatching success (11.9% decline), nymphal survival (33.2% decline) and male and female adult life span (13.4% and 31.5% decline)], whereas no significant inbreeding depression was detected for either male or female mass at maturity (9.2% decline and 12.3% increase). Similar results have recently been reported in another insect species (Fox & Scheibly, 2006) and, more generally, the values of inbreeding depression we report here fall within the range found for full sibling matings ($F = 0.25$) in other animals across a range of taxa (review: Crnokrak & Roff, 1999). However, we found no inbreeding depression for two other life history traits [days to hatching (4.8% increase) and male or female development rate (0.6% decline and 5.2% increase)]. Interestingly, similarly small inbreeding effects for development rate and time to hatching were seen in another cricket, *Gryllus firmus* (Roff, 1998). This might indicate low levels of dominance, suggesting that these traits are under weaker selection than other life history traits.

Sexually selected traits: calling effort

Based on the limited published data, sexually selected traits seem to show strong inbreeding depression (e.g. Sheridan & Pomiankowski, 1997; van Oosterhout *et al.*, 2003; Mariette *et al.*, 2006). In *T. commodus* call rate is a key predictor of male attractiveness to females (Bentsen *et al.*, 2006) and we have shown by dietary manipulation that calling effort can reliably signal male condition (Hunt *et al.*, 2004b). We therefore predicted that inbreeding would cause a large reduction in nightly calling effort (sound production/second) due to greater homozygosity at loci that affect condition. Calling is energetically costly for crickets (Prestwich & Walker, 1981; Kavanagh, 1987), and it is plausible that inbreeding will decrease efficiency in acquiring and/or processing energetic resources. Contrary to our prediction, however, inbred males actually showed a nonsignificant 5% increase in calling effort. There are several possible explanations for this finding. First, it might simply be the case that call rate is a trait that is not subject to inbreeding depression. This seems unlikely given the general effect of inbreeding on condition in most taxa and the fact that call rate is condition dependent. Secondly, the very high mortality of inbred nymphs might have selectively eliminated males so that those surviving to maturity were those with genotypes that did not reduce the ability to allocate resources to calling. Thirdly, the effect of inbreeding might only be detectable under harsher conditions. Males had *ad libitum* access to high quality food which might obscure any effect of inbreeding on calling. Finally, due to the high mortality rate for inbred nymphs, the final sample sizes for calling males were smaller than expected. This led to modest statistical power to detect an effect of inbreeding on calling effort (power: 53% to detect a medium strength effect at the 0.05 level; Cohen, 1988). The small difference in observed mean call rates suggests, however, that even with a larger sample size the effect of inbreeding will still be weak.

Another possible reason for the absence of an inbreeding effect on our estimate of call effort could be attributable to the observed structural changes in calling. First, the calls of inbred males had an 18% increase in the number of pulses per chirp. In *T. commodus* the pulse rate of chirps is slower than that of trills, corresponding to a lower wing stroke rate (Hill *et al.*, 1972; Kavanagh, 1987). Chirps are therefore cheaper to produce because the energetic cost associated with calling increases with wing stroke rate (Prestwich & Walker, 1981). By increasing the number of pulses per chirp, inbred males might be able to increase call duration by biasing their investment into a less costly call component. More importantly, both chirp interpulse interval and intercall duration increased for inbred males (34% and 351% respectively, Fig. 3). Inspection of Fig. 3 shows that by producing a longer call (chirp and trills) with a longer interval between successive calls, an inbred male could produce sound for as many seconds per night as an outbred male. Our event recorder sampled each male 10 times per second and, if a male was recorded as producing sound at any of those 10 sampling events, it was recorded as calling in that second. Because of space constraints, our data collection protocol did not retain information at the 0.1 s scale. Consequently, a male that produced sound nine times per second will receive the same measure of calling effort as a male that produced only one sound per second, even though the former male is clearly producing more sound energy. The unknown biological factor is clearly how females sample sound production.

It is therefore possible that inbred males could register the same calling effort as outbred males despite producing fewer calls. To investigate this possibility we assumed (based on the sampling rate of 10 times per second) that a bout of continuous calling by an inbred or outbred male would register the same number of seconds called per night. We then calculated how few calls an inbred and outbred male would need to produce to obtain the observed values for seconds of sound production per night. We used the mean values of the four fine-scale call parameters reported here for inbred and

outbred males and the values for other traits were taken from Brooks *et al.* (2005) (e.g. pulse duration). On average, an inbred male takes 2.279 s to complete one call cycle, whereas an outbred male takes 1.663 s. To achieve an average calling effort of 1957.7 s per night, an inbred male would have to produce 858.7 calls, whereas an outbred male would have to produce 1121.5 calls to register a calling effort of 1865.0 s per night. This corresponds to a 23.4% decrease in the number of calls per night produced by inbred males relative to outbred males. This result has to be viewed with caution, however, as it relies on assumptions about the consistency in other call parameters and call bout frequency. We are currently refining our call analysis software to record both seconds of sound production and calls produced per night.

Sexually selected traits: other call parameters and net attractiveness

It is unlikely that the significant increase in chirp interpulse interval and greater intercall duration of inbred males is caused by lower male condition. These traits do not seem to be condition dependent in *T. commodus* as reducing dietary protein content had no effect on either call parameter (Hunt *et al.*, 2004b). This suggests that there are other, as yet unknown, mechanisms responsible for some of the effect of inbreeding on fine-scale call structure. Consequently, inbreeding depression on call structure may indicate substantial dominance variance for these underlying traits. Likewise, the lack of inbreeding depression for dominant frequency (no change) and trill number (0.4% decline) might indicate low dominance variance for these traits (Roff, 1998).

We did not directly test whether females prefer the calls of inbred or outbred males, so we can only infer how the observed changes affect attractiveness. A recent field playback experiment showed strong directional selection for greater calling effort by *T. commodus* males (measured as the number of calls in a bout repeated ever 5 min) when females had to move > 10 m to locate a male (Bentsen *et al.*, 2006). Studies with other crickets also show that females prefer males with higher calling effort (Hedrick, 1986; French & Cade, 1989; Crnokrak & Roff, 1995; Holzer *et al.*, 2003). Female *T. commodus* are expected to benefit by preferring such males because calling is condition dependent and might therefore signal genetic quality or direct benefits (Hunt *et al.*, 2004b). It might also simply be easier to locate males that call consistently for longer periods (Bentsen *et al.*, 2006). Although we do not know the underlying mechanism (direct inbreeding effects or a phenotypically plastic response to changes in energy budgets), longer interpulse duration and intercall interval will lengthen the calls of inbred males. In the field experiment, selection favoured a longer intercall interval, presumably because this gives males more 'time on air' during which a female can move towards them using phonotaxis. Furthermore, females seemed to prefer chirps with more pulses and longer interpulse intervals than the average, although multivariate selection on call parameters was ultimately stabilizing (Bentsen *et al.*, 2006). Intriguingly, inbred males had more pulses per chirp and longer chirp interpulse intervals. The findings of Bentsen *et al.* (2006) suggest that inbred males will not suffer a reduction in call attractiveness if they call at the same intensity as outbred males.

Short-range attraction (< 0.55 m) to advertisement calls has also been tested in the laboratory. In contrast to the field study, females preferred calls with shorter intercall intervals (Brooks *et al.*, 2005). Over this distance, inbred males' calls would thus be less attractive. Female mate choice therefore depends on the distance females must travel towards a calling male and on intermale spacing patterns. Furthermore, once a female has reached a male, the latter produces a courtship song to induce the female to mate. It is currently unknown what effect inbreeding has on courtship song. There is, however, evidence that courtship calls are affected by male condition as male *T. oceanicus* produced a different courtship calls after suffering an immune challenge (Tregenza *et al.*, 2006). Consequently, even if inbred males attract females from a distance, they might still have lower mating success if they fail to successfully court females, or compete with nearby males over shorter distances.

To resolve the issue of the effect of inbreeding on male attractiveness it is necessary to directly test the phonotactic response of females to the advertisement call of inbred and outbred males in the field to determine whether the structural changes in the advertisement calls of inbred males we report here reduce attractiveness. In addition, it will be necessary to quantify the effect of inbreeding on courtship song. It will also be worthwhile to examine female choice for kin vs. non-kin given the detrimental effects of inbreeding that we report here for life history traits. Studies with another cricket have shown that females can discriminate against related males (Simmons, 1989, 1991). Our next goal is to directly test these predictions. Nonetheless, the data presented here adds to the small but growing evidence that inbreeding can substantially alter trait values of sexually selected traits.

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