Quantitative genetic variation in courtship song and its covariation with immune function and sperm quality in the field cricket *Teleogryllus oceanicus*

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Acoustic signals used by males to attract females are among the most prominent examples of secondary sexual traits, yet we have only limited understanding of their genetic architecture. Male crickets produce a calling song to attract females and then switch to a courtship song that incites mounting by females once they are at close range. Although we know much about the genetics of cricket calling song, no study has yet examined the quantitative genetics of courtship song. Here, we conduct a quantitative genetic analysis of courtship song using the Australian cricket *Teleogryllus oceanicus*. We find substantial levels of additive genetic variation in courtship song parameters and genetic covariances between courtship song parameters that reflect female preferences. We also found evidence for a negative relationship between the amount of trill in the courtship song and the ability of males to mount an immune response and to produce an ejaculate containing a high proportion of viable sperm. Trade-offs between immunity and sexual signaling are a prerequisite for immunocompetence handicap models of preference evolution, whereas a trade-off between gaining matings and fertilizations is a fundamental assumption underlying sperm competition theory. These life-history trade-offs are likely to constrain evolutionary responses to selection from female choice, and to maintain additive genetic variance in courtship song. *Key words:* courtship song, ejaculate investment, heritability, immunity, trade-off. *[Behav Ecol 21:1330–1336 (2010)]*

Acoustic signals are one of the most prominent and widely studied examples of male secondary sexual traits. The males of many animal species use acoustic signals to compete for access to resources, including territories and mates, and females choose among potential mates based on information contained within male calls (Andersson 1994; Gerhardt and Huber 2002). Theoretical models of female preference evolution have been built around the notion that females obtain genetic benefits from their choice of mates (Kokko et al. 2006) so that male acoustic displays are predicted to be reliable indicators of male genetic quality. Thus, numerous studies of acoustically signaling animals have sought associations between the properties of male calls and the potential benefits that females might accrue from mate choice. For example, evidence suggests that energetically expensive acoustic displays can reveal the phenotypic quality of the signaling male (e.g., Alatalo et al. 1990; Forstmeier et al. 2002; Scheuber et al. 2003; Garamszegi et al. 2004). However, few studies have examined the quantitative genetics of male acoustic displays, and those studies that have come predominantly from acoustically signaling insects (e.g., Webb and Roff 1992; Ritchie and Kyriacou 1994; for a rare study on zebra finch, see Forstmeier et al. 2009).

Field crickets (Orthoptera; Gryllidae) have proved to be remarkably useful model organisms for studying the evolution of male acoustic signals and female choice. Male crickets produce 3 types of acoustic signals. The aggressive chirp is used in aggressive interactions with other males, whereas the calling song attracts females from a distance and the courtship song induces them to mount the male for spermatophore transfer (Alexander 1961). Male crickets produce sound by stridulation, drawing the plectrum of the left tegmen over the file of the right, and generating a single sound pulse with each closure of the tegmen (Koch et al. 1988). Pulses of sound are organized into the chirps and/or trills that characterize a species' song. The calling song of male crickets has been studied extensively. Female preferences have been found for a variety of call parameters, ranging from the duration of calling bouts (Hedrick 1986) to the carrier frequency (Simmons and Ritchie 1996) and temporal properties of the pulses of sound that make up the chirps and trills of the calling song (Pollack and Hoy 1981; Weber and Thorson 1989; Hennig and Weber 1997). Some parameters of calling song appear to be under stabilizing selection (Brooks et al. 2005; Hunt et al. 2007) and function in prezygotic reproductive isolation (Gray and Cade 2000; Honda-Sumi 2005), whereas others may reveal male phenotypic quality (Simmons and Ritchie 1996; Wagner and Hoback 1999; Ryder and Siva-Jothy 2000; Scheuber et al. 2003; Hunt et al. 2005; Simmons et al. 2005; Fedorka and Mousseau 2007), allowing females the opportunity to choose among potential mating partners. Quantitative genetic studies have revealed significant levels of additive genetic variation in some calling song parameters (Hedrick 1988; Webb and Roff 1992; Gray and Cade 1999; Simmons 2004; Hunt et al. 2007), suggesting that some aspects of calling song have the potential to respond to selection imposed by female choice. Much less work has focused on courtship song.

Males switch to courtship song only after they have attracted a female. That male crickets should adopt 2 very different

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acoustic signals for mate attraction is unusual, leading Zuk et al. (2008) to propose that calling song may be more important in species recognition and courtship song more likely to contain information about an individual males quality. In their study of *Teleogryllus oceanicus*, Zuk et al. (2008) found courtship song to be far more phenotypically variable than calling song. Courtship song is considerably more energetically expensive for males to produce than calling song (Hack 1998), and as such it should be more revealing of male condition than calling song. However, evidence for condition dependence in courtship song is scarce. Gray and Eckhardt (2001) found no influence of dietary manipulation during nymphal development on male courtship displays in *Gryllus texensis*, despite the fact that crickets reared on low-quality diets had lower body condition and reduced energetic fat reserves (for a similar study on *G. lineaticeps*, see Wagner and Reiser 2000). In contrast, Rantala and Kortet (2003) found a positive association between one measure of immunity, the encapsulation response, and the rate of high- and low-frequency ticks within the courtship song of *G. bimaculatus*. Tregenza et al. (2006) found little evidence for such an association in *T. oceanicus*, although when the male immune system was challenged experimentally, males were less likely to produce courtship song, and if they did, the temporal properties of their courtship song were altered relative to unchallenged males.

Regardless of whether courtship song exhibits condition dependence at the phenotypic level, females do show mating preferences based on the spectral and temporal properties of a male's courtship song (Wagner and Reiser 2000; Rantala and Kortet 2003; Tregenza et al. 2006). In *T. oceanicus*, females are more likely to mount and accept a spermatophore from a male whose courtship song contains more sound per unit time (courtship songs characterized by long pulses of sound and short intervals between pulses, within both chirp and trill elements of the song) (Rebar et al. 2009). Most parameters of the courtship song of *T. oceanicus* were found to be repeatable, both within a given bout of courtship singing and across bouts separated by several days (Rebar et al. 2009). Repeatability of courtship song elements suggests that courtship song may be a reliable signal of male genetic quality on which females can base their choice of mates. However, whether females can obtain indirect genetic benefits for their offspring from their mating preferences depends largely on the genetic basis of male courtship song. No study has yet quantified the amount of additive genetic variation in courtship song for any cricket species.

Here, we use a half-sib breeding design to examine the quantitative genetics of courtship song in the Australian field cricket *T. oceanicus*. We also look for evidence of costs of courtship song for males within our quantitative genetic framework, by examining the covariation between courtship song parameters and 2 important life-history traits, the ability to mount an immune response and sperm viability (an important determinant of a male's competitive fertilization success, García-González and Simmons 2005). Understanding the quantitative genetic basis and potential costs of courtship song are important because these parameters can shed light on the reliability of the male courtship song as a condition dependent signal, and on the evolution and maintenance of the female preference for courtship song.

MATERIALS AND METHODS

Crickets used in this study were sourced from a plantation in Carnarvon, Western Australia. Forty-one adult females were collected on a single night and placed into individual boxes (7 × 7 × 5 cm) supplied with cat chow and a moist pad of cotton wool, which served both as a source of water and an oviposition substrate. All crickets were maintained in a constant temperature room at 25 °C with a 12:12 h light:dark photoperiod. Cotton wool pads were collected after 7 days and incubated at 25 °C until all nymphs had emerged. These half-sibling families (females collected from the field are expected to have mated with several different males, Simmons and Beveridge 2010) were reared in groups of 25, within 5-l containers supplied with cat chow, moist cotton wool, and cardboard egg carton for shelter. Sexes were separated at the final instar.

At 10 days of age, a single adult male from each family was housed with 4 unmated females. One female was taken from each of 4 different families, and all female families differed from that of the male. Crickets were left to mate for 7 days, following which each female was housed in an individual box and allowed to oviposit. Nymphs derived from each female were reared to adulthood in 2 replicate groups of 25 as described above. Not all females produced offspring, and not all families survived to adult maturity. Of the 41 sires, each produced half-sib adult offspring with between 2 and 4 dams (mean ± standard error [SE]: 2.6 ± 0.1 dams per sire). In the current study, we sampled male offspring from 52 of these dam families distributed across 19 of the 41 sires (range: 2–4, median 3, mean ± SE 2.7 ± 0.2 dams per sire), in order to estimate the levels of additive genetic variation in courtship song.

On the day of adult eclosion, males were housed in individual boxes. We recorded courtship song when males were 10 days of age. Males were placed, in their boxes, into a controlled temperature anechoic room, 1 h before the start of the dark cycle. The room was set at 25 °C and lit by a single red incandescent bulb. Recordings were made during the first 3 h of the dark cycle. A single female, selected haphazardly from bulk stock, was placed into a male's box, which was closed using a lid fitted with wire flyscreen. As soon as the male began to court the female, we started audio recording. Recordings were made using a Sony Pro-walkman with a Sennheiser directional microphone. We attempted to record at least 10 complete courtship songs (Figure 1). If a female mounted the male before a complete recording was obtained, we interrupted the pair by gently tapping the container. We were thus able to obtain courtship songs from 211 males, distributed across our 52 dam and 19 sire families (mean number of sons per dam, 4.1 ± 1.7, range: 1–7; mean number of sons per sire, 11.1 ± 0.9, range: 5–18).

Songs were digitized at 22 kHz and analyzed with the Raven 1.3 software package (Cornell Laboratory of Ornithology, New York). Songs were filtered to remove noise at <3.5 kHz and >6 kHz. We did not measure song amplitude because this variable is sensitive to the orientation of the singing cricket and the distance between microphone and cricket, neither of which could be controlled during recording. We measured a set of temporal parameters of the courtship song, which can be divided into chirp and trill elements: the length of the chirp (CL), the interval between pulses within the chirp (CPI), the length of the chirp pulses (CPL), the total number of pulses in the chirp element (P/C), the interval between the chirp and the onset of the trill (CTI), the total length of the trill (TL), the length of the trill to the first break (TBk), the duration of the break in the trill (BD), the length of the pulses in the trill (TPL), the interval between the pulses in the trill (TPI), and the total number of pulses in the trill element (P/T) (Figure 1). A break in the trill was defined as 2 or more missing pulses. If the trill was continuous, without a break, the length of the break was scored as zero. For CPI, we measured the interval between the penultimate and last pulse of the chirp, and for CPL, we measured the final pulse in the chirp. For TPL, we measured the first and second pulse of the trill and calculated an average. For TPI, we measured the intervals
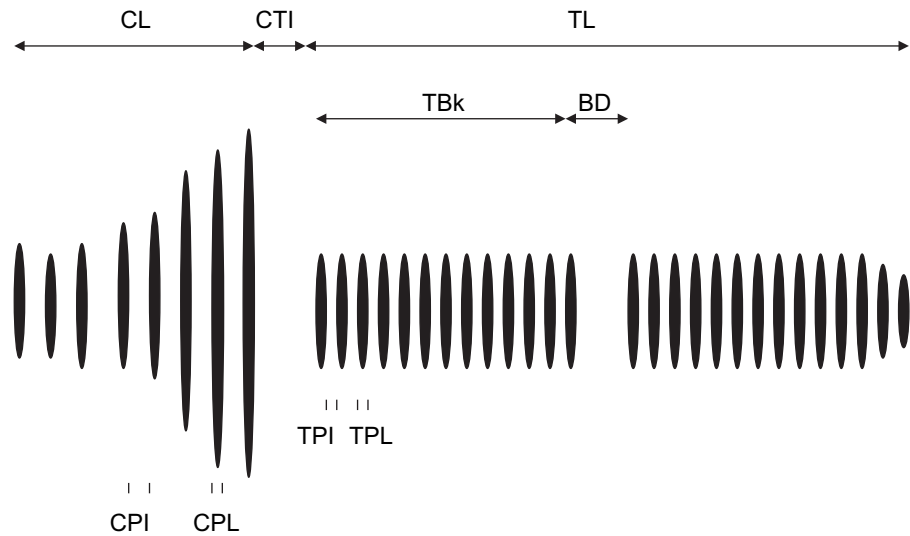
Figure 1
Stylized sonogram of the court-ship song of *Teleogryllus ocean-icus,* illustrating the song parameters measured. The mean durations of the various song elements are presented in Table 1. CL: chirp length; CPI: chirp pulse interval; CPL: chirp pulse length; CTI: chirp trill interval; TL: trill length; TBk: trill to break; BD: break duration; TPI: trill pulse interval; TPL: trill pulse length.

between the first and second and the second and third pulses and calculated an average. We analyzed 3 songs per male and calculated average song parameters across the 3 songs for use in our quantitative genetic analyses. To further minimize measurement error, all song analysis was conducted by the same person (R.M.T).

Quantitative genetic analyses were conducted using nested analyses of variance, with dams nested within sires (Lynch and Walsh 1998). Because the design was not balanced, we used a restricted maximum likelihood approach to estimate variance components. The statistical significance of sire effects were tested using dam nested within sire mean squares as the denominator, and heritability estimates and their standard errors were generated by jackknifing across sires. All analyses were conducted using the S-Plus code provided by Roff (2008). Coefficients of additive genetic and residual variation were calculated following Houle (1992).

The set of 19 half-sib families used in the currant study were included in a previous study in which the entire set of 41 half-sib families were used to assess the levels of additive genetic variation in 3 immune function parameters (the ability of males to encapsulate a nylon implant, antimicrobial lysozyme–like activity, and hemocyte count) and in sperm viability (the proportion of live sperm in a male's ejaculate) (for detailed methodology and results, see Simmons and Roberts 2005). Thus, for the

19 half-sib families used in the currant study, we have published information on levels of additive genetic variation in immuno-competence and ejaculate quality. Here, we use family means (method 1 of Via 1984) to estimate the genetic correlations between courtship song parameters measured from the males in this study with life-history traits measured in their brothers by Simmons and Roberts (2005).

RESULTS

Descriptive statistics, estimates of quantitative genetic variances, and the heritabilities of courtship song parameters are provided in Table 1. We found significant variance due to sires in 8 of the 11 song parameters measured. Heritabilities were moderate to high for both chirp and trill elements of the courtship song. The break in the trill element that characterizes many, though not all, songs had very high residual variation and zero additive genetic variance, indicating that the breaks most likely represent environmental variation. Therefore, we do not consider trill breaks in subsequent analyses.

Table 2 provides the matrix of sire family mean correlations between courtship song elements. Sire family mean correlations provide tests for the presence and direction of genetic correlations between traits, although the magnitude of those

Table 1

Means, standard deviations, coefficients of genetic variation, and narrow sense heritabilities (h^2) of courtship song parameters of the field cricket, *Teleogryllus oceanicus*

Song parameter (s)	Mean ± SD	CV_A	CV_R	$F_{18,33}$ (P)	$h^2 \pm SE$
Chirp length (CL)	0.536 ± 0.120	14.5	19.7	1.85 (0.062)	0.45 ± 0.31
Chirp pulse interval (CPI)	0.032 ± 0.006	14.4	17.9	1.97 (0.045)	0.48 ± 0.36
Chirp pulse length (CPL)	0.037 ± 0.005	3.4	14.3	1.14 (0.358)	0.06 ± 0.09
Pulses/chirp (P/C)	9 ± 2	14.8	17.4	2.31 (0.018)	0.57 ± 0.29
Chirp–trill interval (CTI)	0.067 ± 0.032	34.1	44.3	2.29 (0.019)	0.52 ± 0.24
Trill length (TL)	3.332 ± 1.205	17.9	35.1	1.82 (0.067)	0.26 ± 0.17
Trill to first break (TBk)	1.388 ± 0.983	0.00	67.4	0.84 (0.642)	
Trill break duration (BD)	0.083 ± 0.080	0.00	94.7	0.48 (0.951)	
Trill pulse interval (TPI)	0.009 ± 0.002	12.1	20.5	2.16 (0.027)	0.32 ± 0.19
Trill pulse length (TPL)	0.015 ± 0.003	12.8	15.6	2.68 (0.007)	0.60 ± 0.31
Pulses/trill (P/T)	106 ± 38	20.5	34.1	1.87 (0.059)	0.45 ± 0.19

CV_A, coefficient of additive genetic variation; CV_R, coefficient of residual variation (calculated following Houle 1992). *F* ratios and probability values provide the statistical significance of the additive genetic variance due to sires. Heritabilities were only calculated for song parameters with sire variances greater than zero; SD, standard deviation.

Table 2

Sire family[a] mean correlation matrix of courtship song parameters of the field cricket *Teleogryllus oceanicus*

Song parameter[b]	CL	CPI	CPL	CTI	TL	TPI	TPL	P/C
CPI	−0.407							
CPL	0.390	**−0.508**						
CTI	−0.097	−0.050	0.324					
TL	0.155	0.036	0.007	−0.264				
TPI	−0.095	0.163	0.028	0.072	0.343			
TPL	−0.380	−0.034	0.212	0.040	−0.264	−0.459		
P/C	**0.968**	**−0.580**	0.370	−0.129	0.172	−0.121	−0.385	
P/T	0.046	0.319	−0.209	−0.210	**0.856**	0.204	−0.392	0.007

[a] Nineteen sire families; values in bold exceed the critical value of $r_{18} = 0.444$ with $P = 0.05$.

[b] See Table 1 for full song parameter names.

correlations may be inflated when family sizes are small (Astles et al. 2006). The matrix of correlations provides an indication of the genetic architecture of courtship song. Increased length of chirps and trills was associated with the addition of sound pulses rather than the lengthening of the pulses or the intervals between them (Table 2). Increased length of chirp pulses or an increased number of pulses within a chirp were associated with a decrease in the intervals between chirp pulses (Table 2).

The males used in this study came from a larger study in which their brothers were assessed for immune function and sperm quality (Simmons and Roberts 2005). Sire family mean correlations between the courtship song parameters measured here and the life-history parameters measured in their brothers by Simmons and Roberts (2005) allow us to assess potential costs of courtship song within a quantitative genetic framework. The sire family mean correlations in Table 3 indicate trade-offs between the total amount of sound per unit time and sperm viability and 2 measures of immune function, hemocyte load and encapsulation ability (Figure 2). Sire families with more pulses per trill showed reduced sperm viability as well as lowered encapsulation ability and hemocyte loads (Table 3). The same pattern of correlation was present for the total length of trills, though these correlations were not statistically significant (Table 3). The 95% confidence intervals on these sire family mean correlations indicate that they are robust estimates of the effect sizes. Chirp elements of the courtship song showed little evidence of association with the life-history traits measured.

DISCUSSION

We show that the temporal properties of courtship song in a field cricket, *T. oceanicus*, harbor significant levels of additive

genetic variation. Coefficients of additive genetic variation (CV_A) ranged from 0% to 34%; the average CV_A for chirp elements of the song was ~11% and for trill elements of the song ~10%. The average heritabilities for these traits were both 0.41. The CV_{AS} and heritabilities are of a magnitude typically reported for sexually selected traits (Pomiankowski and Møller 1995) and for life-history traits that contribute most to fitness (Houle 1992). They contrast with the levels of additive genetic variation reported for *Drosophila* courtship song parameters, where heritabilities were found not to differ significantly from zero (Aspi and Hoikkala 1993; Ritchie and Kyriacou 1994). The heritabilities of courtship song found here are also higher than estimates of heritability for the calling song of *T. oceanicus*, which were ~0.08 and ~0.13 for populations sampled from northern Queensland and the Hawaiian island of Oahu, respectively (Simmons 2004). However, the lower heritabilities for calling song traits seem to be due to greater residual variability rather than lower levels of additive genetic variance (cf. CVAs and CVRs for courtship song reported here and for calling song in Simmons 2004).

Previously, Rebar et al. (2009) showed that females were more likely to mount males whose courtship songs contained longer chirps and trills that were composed of longer pulses and shorter intervals between pulses. These song parameters were all found to harbor significant additive genetic variation. Moreover, the genetic architecture of courtship song appeared to reflect these female preferences. Thus, we found evidence for negative genetic correlations between the length of sound pulses and the intervals between pulses, and positive genetic correlations between the lengths of chirp and trill elements of the song and the number of pulses, contained within these song elements. The genetic architecture thereby

Table 3

Sire family mean[a] correlations (lower, upper 95% CI) between courtship song parameters, sperm quality, and immune function parameters of the field cricket, *Teleogryllus oceanicus*

Song trait	Sperm viability	Encapsulation	Hemocyte load	Lysozyme
CL	0.064 (0.040, 0.088)	0.092 (0.067, 0.118)	0.162 (0.140, 0.186)	0.132 (0.093, 0.178)
CPI	−0.252 (−0.272, −0.233)	−0.215 (−0.229, −0.201)	−0.179 (−0.194, −0.163)	−0.273 (−0.302, −0.241)
CPL	0.251 (0.223, 0.278)	−0.022 (−0.053, 0.008)	0.041 (0.003, 0.075)	0.312 (0.282, 0.344)
P/C	0.049 (0.026, 0.075)	0.114 (0.089, 0.140)	0.149 (0.125, 0.174)	0.182 (0.141, 0.228)
CTI	0.255 (0.234, 0.275)	−0.007 (−0.039, 0.023)	0.052 (0.020, 0.081)	−0.024 (−0.048, 0.003)
TL	**−0.481** (**−0.499, −0.461**)	−0.380 (−0.404, −0.356)	−0.430 (**−0.449, −0.409**)	0.169 (0.150, 0.190)
TPI	−0.301 (−0.317, −0.286)	−0.118 (−0.129, −0.023)	−0.243 (−0.257, −0.207)	−0.180 (−0.287, −0.218)
TPL	0.272 (0.237, 0.304)	0.234 (0.090, 0.206)	0.331 (0.220, 0.301)	−0.019 (−0.017, 0.109)
P/T	**−0.516** (**−0.535, −0.496**)	**−0.471** (**−0.490, −0.449**)	**−0.483** (**−0.501, −0.463**)	0.015 (−0.016, 0.046)

[a] Nineteen sire families; values in bold exceed the critical value of $r_{18} = 0.444$ where $P = 0.05$; 95% confidence intervals calculated by jackknifing across sires.
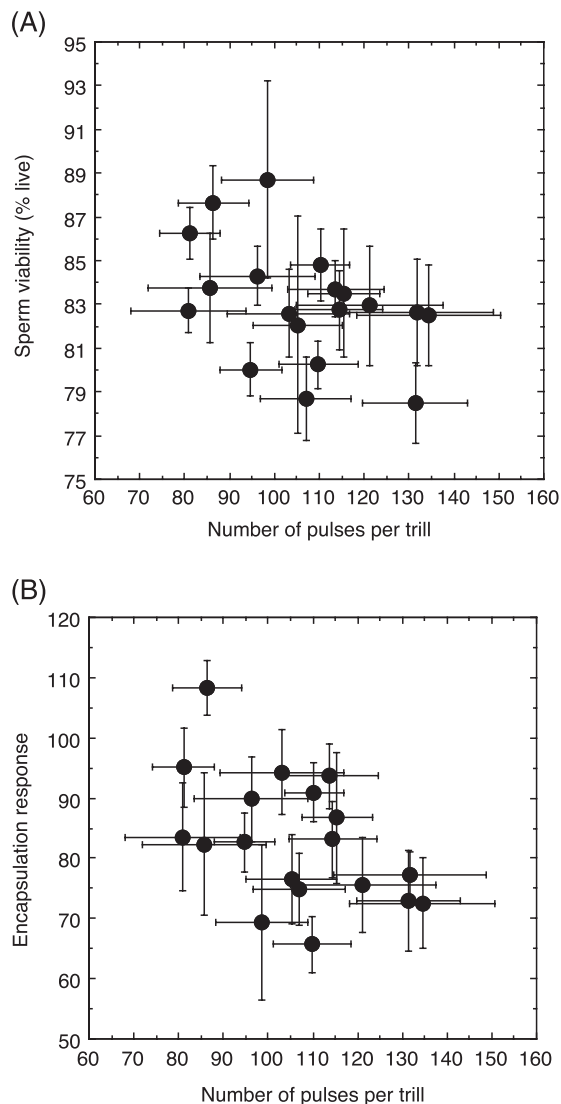
Figure 2

Plots of the sire family mean ($\pm$SE) number of pulses per trill against (A) sire family mean ($\pm$SE) sperm viability (percentage of live sperm in the ejaculate) and (B) sire family mean ($\pm$SE) encapsulation response (see Simmons and Roberts (2005) for detailed methods for estimating sperm viability and encapsulation response).

suggests that female choice has shaped the evolution of male courtship song in this species.

Genetic variation in male traits and female preferences are explicit assumptions underlying theoretical models of preference evolution (Kokko et al. 2006). All else being equal, heritable variation in courtship song structure might imply that female *T. oceanicus* can gain indirect genetic benefits in the form of more attractive sons. Indeed, studies of other cricket species suggest that attractive fathers can sire attractive sons (Wedell and Tregenza 1999; Head et al. 2005). Nevertheless, although female *T. oceanicus* clearly exert selection on male courtship song, the form of that selection remains largely unknown. Courtship song is a complex multivariate trait, and female choice is unlikely to act on the different song parameters independently of one another. Indeed, in the congeneric species *T. commodus*, females exercise postmating female choice based on the quality of courtship song delivered prior to mounting, by removing the spermatophores of males with unattractive courtship songs before sperm have been trans-

ferred to their sperm stores. Multivariate selection analysis suggests that in *T. commodus*, female choice favors a courtship song with a combination of intermediate values for trill length and interval between chirp pulses, and extreme combinations of trill lengths, and chirp–trill and chirp pulse intervals (Hall et al. 2008). A similar analysis of the calling song of *T. commodus* shows that calling song is also subject to stabilizing selection for intermediate combinations of song parameter values (Brooks et al. 2005; Bentsen et al. 2006). Importantly, although there is significant additive genetic variation in individual calling song parameters, there remains little additive genetic variation in the multivariate calling song trait combinations that females find most attractive (Hunt et al. 2007). Future work on *T. oceanicus* should therefore examine the form of selection acting on courtship song in multivariate trait space.

Zuk et al. (2008) suggested that courtship song might be particularly revealing of male quality because it is energetically more expensive to produce than calling song (Hack 1998). We found evidence to suggest that male *T. oceanicus* do trade energetically expensive courtship song for other important life-history traits, including investment in immunity and in sperm quality. Interestingly, these trade-offs occurred predominantly with the trill element of the courtship song; specifically the number of pulses in the trill had negative genetic correlations with hemocyte load and encapsulation response, and with sperm viability. For cricket songs generally, the order of magnitude greater wing stroke rate required to produce trills compared with chirps translates into an order of magnitude difference in the energetic costs of these song elements (Prestwich and Walker 1981). The trill element of *T. oceanicus* courtship song should therefore be more energetically expensive than the preceding chirp, represent a greater draw on a male's available resources, and therefore should be particularly revealing of male condition. Interestingly, studies of cricket calling songs have also found associations between calling song structure and a male's ability to mount an immune response (Ryder and Siva-Jothy 2000; Simmons et al. 2005). In their study of *G. campestris*, Jacot et al. (2004) found that the experimental induction of an immune response resulted in a decline in daily calling rate that was both temporary and less severe for males given access to a supplemental food supply. Work with *G. campestris* thereby provides support for the notion that resource allocation trade-offs underlie the negative relationships between immune responses and call production frequently observed in crickets, and our finding for *T. oceanicus* courtship song suggests that this life-history trade-off may have the genetic basis necessary for immunocompetence handicap models of female choice (Folstad and Karter 1992; Sheldon and Verhulst 1996).

We also found evidence of a trade-off between the viability of sperm within a male's ejaculate and the number of pulses in the trill element of the courtship song. Such a trade-off is a fundamental assumption underlying sperm competition theory; males are assumed to have a fixed resource pool with which to invest both in attracting females and in gaining fertilizations (Parker 1998). The theory predicts that males should increase their investment in the ejaculate, at the cost of investment in gaining additional matings, when the risk of sperm competition is increased. Male *T. oceanicus* exhibit the phenotypic plasticity in ejaculate expenditure predicted by sperm competition game theory, producing ejaculates with a greater proportion of viable sperm under elevated risk of sperm competition and ejaculates with a decreasing proportion of viable sperm as the number of males in competition increases (Simmons et al. 2007; Thomas and Simmons 2007, 2008). Here, we show that one cost of increasing sperm viability might be a reduction in an element of courtship song

that females find attractive. Interestingly, support for this fundamental trade-off also comes from studies of *T. oceanicus* that have examined the role of cuticular hydrocarbons (CHCs) in female choice; males that invest more heavily in the CHCs that females find attractive do so at the cost of a reduction in the viability of their sperm and ultimately in their competitive fertilization success (Thomas and Simmons 2009).

Our finding of a negative relationship between a courtship trait preferred by females and sperm quality does not support the phenotype linked fertility hypothesis (Sheldon 1994). Under this hypothesis, exaggerated secondary sexual traits are proposed to be positively correlated with male fertility so that female choice provides the direct benefits of mating with a fertile male. Some evidence for this hypothesis comes from studies of vertebrates (Peters et al. 2004; Malo et al. 2005; Locatello et al. 2006; Pitcher et al. 2007). In species with sexual coloration, the relationship between ornamentation and fertility may arise because of the dual effect of antioxidants on male coloration and the protection of sperm from oxidative stress (Helfenstein et al. 2010; Pike et al. 2010). However, negative associations between secondary sexual traits and sperm quality have also been reported (Pitcher et al. 2009), suggesting that in some species males may trade secondary sexual traits for fertility. In stalk-eyed flies, juvenile hormone promotes the growth of the sexually selected eyestalks but reduces the growth of testes, and ultimately the numbers of sperm produced, illustrating the hormonal regulation for this life-history trade-off (Fry 2006). The trade-off between courtship song and sperm quality in *T. oceanicus* means that females will not obtain fertility benefits from their choice of mating partners.

In summary, we have reported significant levels of additive genetic variation in the parameters of male courtship song that females find attractive. Some of these courtship song parameters appear to have significant trade-offs with a male's ability to defend himself against infection and to compete for fertilizations in the face of sperm competition. These important life-history trade-offs may constrain any evolutionary response to selection via female choice and provide a mechanism for the evolutionary maintenance of additive genetic variation in courtship song.

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