

# Individual variation and repeatability in calling song of the field cricket *Loxoblemmus equestris* (Orthoptera: Gryllidae)

Takashi KURIWADA\*

(Received 25 October, 2016)

## Abstract

Individual variation in the calling songs of field cricket species provides important information for the study of sexual selection, speciation, and inter-specific interaction among other cricket species. This study investigated individual variation in, and repeatability of song parameters of the field cricket *Loxoblemmus equestris*. High variations in nightly calling duration and the number of chirps per phrase were detected, whereas dominant frequency exhibited the lowest variations. Repeatability of all song parameters within individuals was significant, and the results showed that calling song of *L. equestris* can be considered a stereotyped signal. Repeatability of song parameters within families was also significant for all parameters except one. Although the repeatability within families was smaller than that within individuals, the results suggest that the calling song was heritable.

**Key words:** acoustic communication, calling effort, field cricket, individual variation, repeatability

---

\* Associate Professor of Kagoshima University, Faculty of Education

## Introduction

In most field cricket species, males emit “songs” by rubbing their forewings together as the signal. The acoustic signals function in species recognition, localization of males relative to other individuals, display of territoriality to other males, and advertisement of male quality (Zuk & Simmons, 1997; Gerhardt & Huber 2002). Honda-Sumi (2005) showed that the male calling songs of three *Teleogryllus* cricket species serve an important role in premating reproductive isolation. Because sound waves interfere with each other, the communication systems can be hindered by the acoustic signals of other species. Therefore, inter-specific acoustic interactions among crickets have been examined (e.g., Schmidt et al., 2011). In tropical crickets, for example, signal interference decreases recognition and localization of conspecific individuals, resulting in partitioning in the spectral domain of the calling frequencies (Schmidt & Römer, 2013). As stated above, variation in song parameters serves an important role in the study of sexual selection, speciation, and inter-specific interaction.

To understand inter-specific acoustic interaction, ranges in parameters of acoustic signals of each species should be investigated. Therefore, variation in the song parameters of each species is fundamental to the study of inter-specific acoustic interaction. A variety of field cricket species are distributed on Amami Oshima Island in Japan (Orthopteran Society of Japan, 2006; Murai & Ito, 2011), including *Teleogryllus occipitalis* (Serville, 1838) and *Loxoblemmus equestris* Saussure, 1877, which cohabit the small area (T. Kuriwada, personal observation). To investigate the coexistence mechanism of these two species, data related to variation in their calling songs will need to be collected. Characteristics of calling song and female preference for the song of *T. occipitalis* have been closely investigated (Honda-Sumi, 2004; 2005, originally cited as *T. taiwanemma*), but little information pertaining to the calling song of *L. equestris* is available (but see Lian & Li 2001).

This study investigated individual variation in temporal features and repeatability of calling song in *L. equestris*. Repeatability of calling song can provide genetic information because repeatability corresponds with the upper limit of heritability (Falconer, 1989). Body size is often correlated with calling parameters in some field crickets (e.g., *Gryllus bimaculatus*: Simmons & Zuk, 1992; *Acheta domesticus*: Gray, 1997), this study also examined the relationships between body size and parameters of calling song for *L. equestris*. Since male *L. equestris* exhibits a flat head shape, the experiment tested whether the head size and pronotum width were correlated with calling song parameters.

## Materials & Methods

### Insects

The field cricket *L. equestris* (Orthoptera: Gryllidae) is distributed throughout the low grasslands and farmlands of Nansei Islands in Japan and Southeast Asia (Orthopteran Society of Japan, 2006). All

individuals used in this experiment were collected from Amami Oshima Island in Japan (28° 19' N, 129° 22' E) in July 2015. Collected mated adult females were individually contained in cups (diameter=10.5cm; height=5.0cm) at 24–26°C for a 16:8 L:D photoperiod (light cycle: 0300–1900). The crickets were supplied with egg cartons for shelter, damp soil for oviposition, and unlimited amounts of commercial rabbit food and cat food. They were allowed to oviposit for approximately 20 days, after which time they were removed. Hatched nymphs were collected within 24 h, and then 25–30 nymphs were placed in a container (diameter=9cm; height=10cm) with paper filter shelter, excess food, and a cotton plugged water vial. After 40 days, the nymphs were placed in a larger container (29.9cm×19.2cm×20.1cm). Last-instar nymphs were individually placed in separate containers (diameter=10.5cm; height=5.0cm). Five to 6 full-sib virgin males were collected from each family (N=7), and they were used for the following experiment (total sample size=37).

#### Measurements of calling effort and repeatability

The calling song was recorded in an uncompressed format with a digital IC recorder (Panasonic RR-XS455, Osaka, Japan) with a 44.1-kHz sampling rate and 16-bit dynamic range. Recordings were stored as wav files. The first set of recordings were taken 12–14 days after the final molt (young age). The second set of measurements were made at 22–24 days after the final molt (old age). Each male's container (diameter=10.0cm; height=3.0cm) was placed with the IC recorder in a two-ply corrugated fibreboard box (interior dimensions: 23cm×17cm×17cm, exterior dimensions: 28cm×23cm×23cm) where it was physically, visually, and acoustically isolated from other crickets. The distance between the recorder and the cricket container was 1cm. All recordings were conducted at  $25 \pm 1^\circ\text{C}$  during the 1900–0300 dark cycle. Five components of the calling song were analysed using Audacity 2.0.5® Free Digital Audio Editor

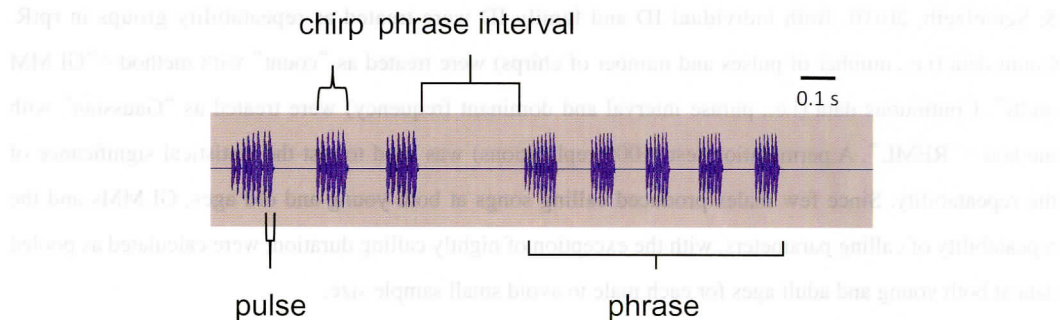


Fig. 1

**Fig.1.** Oscillogram of the male *Loxoblemmus equestris* calling song. The head chirp per phrase was longer than the follow on the chirps.

(Dominic Mazzoni, CA, USA). These included nightly calling duration (min / 8 h), number of pulses per chirp, number of chirps per phrase, phrase interval, and dominant frequency, as indicated in Fig. 1. Ten randomly selected phrases from each male were analysed.

### Measurements of body size

Male status (dead or alive) was recorded daily. After males died, they were frozen at  $-10^{\circ}\text{C}$  for 24 h. Crickets were photographed using a scanner (Epson GT-S650, Japan) connected to a personal computer using Image J 1.49 (National Institute of Health, USA) with a 24-bit colour image type at a resolution of 1200 dpi. The size of body parts was measured using these photos. Pronotum width was defined as the distance between right and left edges of the pronotum. Head width was defined as the distance from the tip of the right side to the tip of left side of the head.

### Statistical Analyses

All statistical calculations were conducted using R 3.0.3 software (R Core Team, 2014). Generalized linear mixed models (GLMMs) were used to examine the relationships between calling parameters and body size (Bolker et al., 2009). The arithmetical mean of the calling parameters at young and old ages was used as a response variable, and identity link and Gaussian error were used to analyse the calling parameters. Family ID was treated as a random effect. A Wald test was also used to examine the statistical significance of each coefficient in the models. For the analyses of nightly calling duration, however, the statistical models could not be calculated, thus the data could not be fitted to the GLMMs. When nightly calling duration was response variable, generalized linear models (GLMs) with inverse link and gamma error were used. To examine the statistical significance, a likelihood ratio test was used. Here, family ID was treated as an explanatory variable. Repeatability of calling parameters was calculated using R package: rptR (Nakagawa & Schielzeth, 2010). Both individual ID and family ID were treated as repeatability groups in rptR. Count data (i.e., number of pulses and number of chirps) were treated as “count” with method = “GLMM multi”. Continuous data (i.e., phrase interval and dominant frequency) were treated as “Gaussian” with method = “REML”. A permutation test (1000 replications) was used to test the statistical significance of the repeatability. Since few males produced calling songs at both young and old ages, GLMMs and the repeatability of calling parameters, with the exception of nightly calling duration, were calculated as pooled data at both young and adult ages for each male to avoid small sample size.

**Results**

Mean, standard deviation (SD), and coefficient of variation (CV) are shown in Table 1. Calling duration per night exhibited the highest variance among calling parameters, with dominant frequency exhibiting the lowest variance. Few if any males in the four families produced calling songs at either young or adult ages (Fig. 2). The calling parameters among individuals and within families were significantly repeatable, except for the number

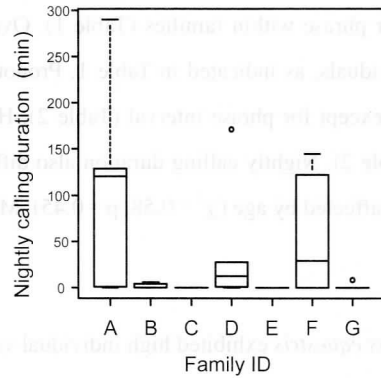


Fig. 2

**Fig.2.** Nightly calling duration across families. The horizontal bar in the middle of the box denotes the median value. The top and bottom of the box denote the 75th and 25th percentile, respectively. Whiskers indicate the maximum and the minimum values, excluding the outliers. Outliers were defined as the values that were more than 1.5 times the interquartile range above the 75th quartile or below the 25th quartile (circles).

**Table1.** Descriptive statistics value and repeatability of song parameters.

Song parameters	mean (SD)	C.V. (%)	Repeatability (SE) within male	Repeatability (SE) within family
Nightly calling duration (min)	29.08 (77.03)	265	<b>0.41 ( 0.14)</b>	<b>0.20 ( 0.13)</b>
No. pulse/chirp	10.07 ( 2.05)	20	<b>0.25 (0.071)</b>	<b>0.12 (0.056)</b>
No. chirp/phrase	5.88 ( 2.18)	37	<b>0.31 ( 0.12)</b>	0.00 (0.011)
Phrase interval (s)	1.39 ( 0.27)	19	<b>0.46 ( 0.11)</b>	<b>0.31 ( 0.15)</b>

Statistically significant effects are highlighted in bold type (P<0.05).

**Table 2.** The results of GLMMs test of the effects of body sizes on song parameters.

Song parameters	Body parts	$\beta$ (SE)	Statistics	p-value
Nightly calling duration (min)	head	0.0074 (0.033)	$\chi^2 = 0.053$	0.82
	pronotum	0.012 (0.041)	$\chi^2 = 0.091$	0.76
No. pulse/chirp	head	-0.37 (0.21)	$z = 1.72$	0.086
	pronotum	0.077 (0.28)	$z = 0.28$	0.78
No. chirp/phrase	head	0.50 (0.64)	$z = 0.78$	0.44
	pronotum	-0.48 (0.84)	$z = 0.57$	0.57
Phrase interval (s)	head	<b>0.73 (0.28)</b>	<b><math>t_{174} = 2.57</math></b>	<b>0.011</b>
	pronotum	0.048 (0.39)	$t_{174} = 0.12$	0.90
Dominant frequency (Hz)	head	101.92 (229.87)	$t_{174} = 0.44$	0.66
	pronotum	32.75 (329.14)	$t_{174} = 0.10$	0.92

Statistically significant effect are highlighted in bold type

of chirps per phrase within families (Table 1). Overall, repeatability within families was lower than that among individuals, as indicated in Table 1. Pronotum and head width did not significantly affect calling parameters, except for phrase interval (Table 2). Here, males with wider heads exhibited a longer phrase interval (Table 2). Nightly calling duration also differed depending on family ID ( $\chi^2 = 309.55$ ,  $p < 0.001$ ), and was not affected by age ( $\chi^2 = 0.58$ ,  $p = 0.45$ ). Male longevity was  $67.01 \pm 18.73$  days (mean  $\pm$  SD).

## Discussion

*Loxoblemmus equestris* exhibited high individual variation in calling song similar to that found in previous studies using other cricket species (e.g., *Teleogryllus oceanicus*: Kolluru, 1999; *Acheta domesticus*: Ryder, 2000; *Gryllus pennsylvanicus*: Harrison et al., 2013). Some calling parameters such as nightly calling duration and the number of chirps per phrase exhibited high variation, whereas dominant frequency exhibited the lowest variation. In general, call properties showing relatively low variation provide evidence for stabilizing selection and serve as key factors for species recognition, although there are some counter-examples (Gerhardt & Huber, 2002). In contrast, properties with high variation support directional selection, and are expected to correlate with male quality (Gerhardt & Huber, 2002).

No significant relationships were found between calling parameters and body size, with the exception of one parameter. Sample size was small in this study because calling males were unexpectedly few; as a result, statistical power may be low. Additionally, pronotum and head width may be unsuitable for determining a male's condition. Instead, body weight and residual mass (i.e., residuals of a regression of body weight on body size) may be more suitable fitness indicators (e.g., Harrison et al., 2013). Unfortunately, I did not measure the body weight of the cricket.

Males exhibiting wider head produced longer phrase interval songs. As a general rule, higher calling rates (i.e., short inter-phrase duration) incur higher energetic costs, increased predation risks, or both (for review Gerhardt & Huber, 2002). Because females often prefer this type of calling song in many orthopteran species (for review, Gerhardt & Huber, 2002), males with wider heads and longer phrase intervals may not be preferred by females of these species. To examine the relative importance of larger body size and preferred song in relation to the reproductive success of males, a playback experiment featuring quantitative analysis of male-male contests and mating behaviour, will be needed.

In two families, none of the males produced calling songs, whereas in the other two families, few males produced calling songs at both young and old ages. These non-calling males might adopt satellite strategy (Davies et al., 2012). Because repeatability of calling duration within families was significant, caller and non-caller may be heritable in *L. equestris*. Indeed, nightly calling duration is heritable in some other field cricket species (e.g., *G. integer*: Cade, 1981; *T. commodus*: Hunt et al., 2006; *G. texensis*: Bertram et al., 2007).

Repeatability of all calling parameters among individuals is significant (range: 0.25 to 0.65), indicating that calling song constitutes a male-associated phenotype. Repeatability within families was lower than that among individuals. The result suggest that non-additive genetic and environmental effects affect the male calling song (Greenway & Shuker, 2015). However, calling parameters for this species can be considered a heritable trait because almost all of the calling parameters within families were significantly repeatable. In order to more strictly examine the genetic basis of the song, more detailed analyses such as full-sib experiments and parent-offspring regressions are needed.

### Acknowledgments

I would like to thank the staff of Kagoshima University Research Center for the Pacific Islands for their assistance with this project. This study was also supported in part by Special Budget of MEXT in 2015 (Establishment of Research and Education Network on Biodiversity and Its Conservation in the Satsunan Islands) and KAKENHI (No. 16K21244 ).

### References

- Bertram SM, Kemp DJ, Johnson JS, Orozco SX, Gorelick R (2007) Heritability of acoustic signaling time in the Texas field cricket, *Gryllus texensis*. *Evolutionary Ecology Research* **9**, 975–986.
- Bolker BM, Brooks ME, Clark CJ, *et al.* (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**, 127–135.
- Cade WH (1981) Alternative male strategies: genetic differences in crickets. *Science*, **212**, 563–564.
- Davies NB, Krebs JR, West SA (2012) *An Introduction to Behavioural Ecology*. Wiley-Blackwell, UK.
- Falconer DS (1989) *Introduction to Quantitative Genetics*. Third Edition. Longman London, UK.
- Gerhardt HC, Huber F (2002) *Acoustic Communication in Insects and Anurans*. The University of Chicago Press, Chicago, IL.
- Gray DA (1997) Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Animal Behaviour* **54**, 1553–1562.
- Greenway EG, Shuker DM (2015) The repeatability of mating failure in a polyandrous bug. *Journal of Evolutionary Biology* **28**, 1578–1582.
- Harrison SJ, Thomson IR, Grantem, Bertram SM (2013) Calling, courtship, and condition in the fall field cricket, *Gryllus pennsylvanicus*. *PLoS ONE* **8**, e60356.
- Honda-Sumi E (2004) Female recognition of trills in the male calling song of the field cricket, *Teleogryllus taiwanemma*. *Journal of Ethology* **22**, 135–141.
- Honda-Sumi E (2005) Difference in calling song of three field crickets of the genus *Teleogryllus*: the role in premating isolation. *Animal Behaviour* **69**, 881–889.
- Hunt J, Jennions MD, Spyrou N, Brooks R (2006) Artificial selection on male longevity influences age - dependent reproductive effort in the Black field cricket *Teleogryllus commodus*. *The American Naturalist* **168**, E72–E86.
- Kolluru GR (1999) Variation and repeatability of calling behavior in crickets subject to a phonotactic parasitoid fly. *Journal of Insect Behavior* **12**, 611–626.
- Lian Z, Li K (2001) The comparative studies on the common sound types of crickets (Orthoptera: Grylloidea). *Entomotaxonomia* **24**, 45–51.
- Murai T, Ito F (2011) *A Field Guide to the Orthoptera of Japan*. Hokkaido University Press, Sapporo, Japan (In Japanese).

- Nakagawa S, Schielzeth H (2010). Repeatability for Gaussian and non - Gaussian data: a practical guide for biologists. *Biological Reviews* **85**, 935–956.
- Orthopterological Society of Japan (2006) *Orthoptera of the Japanese Archipelago in Color*. Hokkaido University Press, Sapporo, Japan (In Japanese).
- R Core Team (2014). R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Ryder JJ (2000) Male calling song provides a reliable signal of immune function in a cricket. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, 1171–1175.
- Schmidt AKD, Römer H (2011) Solutions to the cocktail party problem in insects: selective filters, spatial release from masking and gain control in tropical crickets. *PLoS ONE* **6**, e28593
- Schmidt AK, Römer H, Riede K (2013) Spectral niche segregation and community organization in a tropical cricket assemblage. *Behavioral Ecology* **24**, 470–480.
- Simmons LW, Zuk M (1992) Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. *Animal Behaviour* **44**, 1145–1152.
- Zuk M, Simmons LW (1997) Reproductive strategies of the crickets (Orthoptera: Gryllidae). In: Choe JC, Crespi BJ (eds) *The Evolution of Mating Systems in Insects and Arachnids*, pp. 89–109. Cambridge University Press, Cambridge.