



# Signal diversification in *Oecanthus* tree crickets is shaped by energetic, morphometric, and acoustic trade-offs

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Physiology, physics, and ecological interactions can generate trade-offs within species, but may also shape divergence among species. We tested whether signal divergence in *Oecanthus* tree crickets is shaped by acoustic, energetic, and behavioral trade-offs. We found that species with faster pulse rates, produced by opening and closing wings up to twice as many times per second, did not have higher metabolic costs of calling. The relatively constant energetic cost across species is explained by trade-offs between the duration and repetition rate of acoustic signals—species with fewer stridulatory teeth closed their wings more frequently such that the number of teeth struck per second of calling and the resulting duty cycle were relatively constant across species. Further trade-offs were evident in relationships between signals and body size. Calling was relatively inexpensive for small males, permitting them to call for much of the night, but at low amplitude. Large males produced much louder calls, reaching up to four times more area, but the energetic costs increased substantially with increasing size and the time spent calling dropped to only 20% of the night. These trade-offs indicate that the trait combinations that arise in these species represent a limited subset of conceivable trait combinations.

**KEY WORDS:** Biomechanics, performance constraints, sexual selection, signal divergence, speciation, trade-offs.

Despite the remarkable diversity of organisms, many combinations of traits do not arise due to constraints imposed by physics, physiology, and features of the environment. Often, constraints on organisms promote trade-offs, where increased expression of one trait is associated with decreased expression of other traits (Garland et al. 2002; Roff 2002; Dial et al. 2008). Trade-offs have long been central to life history theory and have received increased attention in fields such as in foraging and locomotion (Billerbeck et al. 2001; Van Damme et al. 2002; Blanco and Patek 2014). The prevalence and diversity of trade-offs suggests that they are integral to the evolution and divergence of populations. Trade-offs likely help to define the opportunity set of traits, the trait combinations that populations can utilize as they evolve on a changing fitness landscape (Wright 1970; Perrin and Travis 1992; Gavrillets 1997).

Trade-offs could be important in the production and evolution of sexual signals, but trade-offs are not consistently integrated into the study of signaling and signal divergence. The majority of studies have considered variation in sexual signals primarily as a reflection of signaler quality, with relatively little attention to the more complex possibilities arising from simultaneous optimization of multiple signal features in variable environments (Bro-Jørgensen 2010; Ward et al. 2013). The potential relevance of trade-offs in sexual signals seems even greater than before as evidence accumulates for the ubiquity of multicomponent and multimodal signals (Partan and Marler 1999; Candolin 2003; Hebets and Papaj 2005).

Intraspecific signaling trade-offs are increasingly well documented. Some of the most frequently studied signal combinations include the bandwidth and trill rate of acoustic signals (Podos

1997; Podos 2001; Pasch et al. 2011) as well as the interaction of signal duration and repetition rate (Taigen and Wells 1985; Reichert and Gerhardt 2012; Ward et al. 2013). The prevalence of multicomponent and multimodal signals raises the question of whether signals diverge as individual traits responding to individual selective pressures or via trade-offs within suites of traits that are linked by morphology, physiology, and physics (Derryberry et al. 2012). One possibility is that as lineages diversify and occupy different habitats and niches, selective forces are sufficiently different among lineages that trade-off relationships have different slopes (e.g., one trait becomes relatively more or less costly). Alternatively, the trade-offs that shape signals may be sufficiently fundamental that they transcend the biology of a single species and instead dictate the phenotypic pathways along which clades can diversify. Consequently, there may be a suite of comparably demanding signals that are related via trade-offs, with the specific variant that arises in a given taxon dictated by factors such as predation, population density, heterospecifics, and habitat characteristics. The result would be sets of trait combinations across which diversification occurs, but with many trait combinations that either have low fitness or cannot occur without major shifts in physiology, biomechanics, or behavior (Muller and Wagner 1991; Kuratani 2005; Theißen 2009).

Mating signals are often metabolically expensive. For example, acoustic signaling can increase metabolic rate by 10 to 15-fold in the spring peeper, *Hyla crucifer* (Taigen et al. 1985), and by 2 to 20-fold in calling insects (Prestwich and Walker 1981; Prestwich 1994; Wagner and Hoback 1999). If physiological, neurological, or morphological constraints (Ahtiainen et al. 2005) or ecological interactions limit the expression of signals, it could generate trade-offs among signal characteristics and lead to diverse investment strategies across individuals and species. For example, the variable field cricket (*Gryllus lineaticeps*) shows trade-offs among acoustic characteristic, both across field populations and within lab-reared families (Wagner et al. 2012). In the current study, we tested for the presence of trade-offs across species in *Oecanthus* crickets by measuring acoustic characteristics, metabolic cost, amplitude, and signaling time in congeners that employ different signals.

*Oecanthus* crickets occupy a wide range of habitats and vary in body size and life history (Walker 1962; Walker 1963; Walker and Moore 2013), making them well-suited to assessing how signal characteristics and metabolic cost varies across species. There is currently no molecular phylogeny for the group, but the species included in this study belong to two different clades as defined by morphology (Walker 1962; Walker 1963) and supported by preliminary sequencing results. The broad-winged *varicornis* group includes *O. varicornis*, *O. latipennis*, and *O. texensis* and the narrow-winged *nigricornis* group includes *O. argentinus*, *O. celerinicus*, *O. forbesi*, *O. nigricornis*, and *O. quadripunctatus*.

Across these species, the basic sound production mechanism is conserved, but the acoustic characteristics of the calls differ. We first examined sound production and metabolic cost of calling to assess how calling performance differs among species. We then assessed how the metabolic cost of calling is affected by specific features of the call and whether the features of the call covary to form performance thresholds.

Male tree crickets call by rubbing a file on one wing against a hardened vein (the scraper) on the other wing (Walker 1962; Walker 1963). Females respond by walking toward preferred sounds (Walker 1957). The major features of the call can be described by pulse rate, pulse duration, dominant frequency (perceived as pitch), and call amplitude. Each time a male closes his wings, he produces a pulse of sound: the number of times that wings are closed per second is synonymous with pulse rate. Pulse rate is known to be important for female response, with females responding to pulse rates at or above the male mean when response to faster pulse rates is not constrained by the presence of heterospecifics (Symes 2014). Pulse duration corresponds to the amount of time spent rubbing the file during a single wing closure; thus, if wings are closed at the same velocity across species, a longer file yields longer pulse durations. Dominant frequency is generated by the vibration pattern of the wings (Mhatre et al. 2011, 2012). There is mixed support for the hypothesis that larger crickets have lower dominant frequency (Brown et al. 1996; Symes 2013) and there is evidence that mating with larger males confers higher fitness on females (Brown 1997). When females are presented with a single call, they respond to a wide range of pulse durations and frequencies (Symes 2013), but it is not known whether females prefer longer pulse duration or lower frequency calls.

To test how call characteristics affected the energetic cost of signaling, we first compared signal costs across species with a variety of call pulse rates. Across species, tree cricket pulse rates vary from approximately 35–75 pulses per second at 25°C, meaning that some species close and open their wings more than twice as many times per second (Walker 1962; Walker 1963). If the costs per wing closure remain constant, faster pulsing species would tend to expend more energy. Alternatively, the energetic cost of calling could be constant across species, mediated by trade-offs between pulse rate and other call characteristics such as pulse duration (Prestwich and Walker 1981). If so, all species would incur similar metabolic costs when calling, but other call characteristics would experience tradeoffs with changes in pulse rate, maintaining consistent trade-offs across species.

Body size may also affect calling performance. Tree crickets vary in body mass within and among species. Basal metabolism of larger organisms typically requires more absolute energy but less energy per unit mass (Niven and Scharlemann 2005). Signaling costs could follow the pattern of resting metabolism in that

larger individuals pay a larger absolute cost, but benefit from an economy of scale (lower metabolic cost per unit mass). Alternatively, if the larger wings of calling males act as a longer lever arm (magnifying torque as the center of mass becomes farther from the pivot point), larger wings will cause an accelerating increase in energetic costs as wing length increases. Despite the inherent cost of calling with larger wings, the larger wings may act as larger resonators, resulting in a call that is louder and therefore audible over a greater area, with the potential to attract more mates (Bailey et al. 1990; Castellano et al. 2004; Costello and Symes 2014). To determine how mass affects calling energetics and call amplitude and whether these relationships were maintained across species, we measured the metabolic rate of insects at rest, the effects of calling on metabolic rate, and the effect of mass on resting metabolism and the costs of calling. In addition, we measured the call amplitude of crickets to determine the relationship between body mass, amplitude, and transmission distance. Trade-offs may be visible in instantaneous measures (such as bandwidth and trill rate), but may also arise over longer time scales (such as the total amount of time spent signaling in the course of a night). Accordingly, we also measured the total amount of time spent signaling to determine whether higher instantaneous investment in signaling was associated with decreased time spent signaling, a finding that would reveal the possibility of performance trade-offs acting over longer time periods, within or across species.

## Methods

All insects used in this study were wild-caught as nymphs and adults from sites across the Eastern United States between May and September, 2011. In captivity, crickets were held singly and fed a standard diet of Fluker's Cricket Chow (Port Allen, LA, USA). Eight species were examined for morphology, with five of these species used in the metabolic portion of this study: *O. argentinus* Saussure 1874 ( $N_{\text{Morph}} = 20$ ;  $N_{\text{Metab}} = 17$ ), *O. celerinictus* T. Walker 1963 ( $N_{\text{Morph}} = 8$ ), *O. forbesi* Titus 1903 ( $N_{\text{Morph}} = 39$ ;  $N_{\text{Metab}} = 8$ ), *O. latipennis* Riley 1881 ( $N_{\text{Morph}} = 18$ ;  $N_{\text{Metab}} = 6$ ), *O. nigricornis* F. Walker 1869 ( $N_{\text{Morph}} = 17$ ,  $N_{\text{Metab}} = 20$ ), *O. texensis* L. Symes and N. Collins 2013 ( $N_{\text{Morph}} = 8$ ), *O. quadripunctatus* Beutenmuller 1894 ( $N_{\text{Morph}} = 16$ ;  $N_{\text{Metab}} = 10$ ), and *O. varicornis* F. Walker 1869 ( $N_{\text{Morph}} = 10$ ).

Carbon dioxide is a byproduct of metabolic activity, with the volume of carbon dioxide produced being proportional to the energy used in aerobic metabolism (Prestwich 1994). Therefore, we measured carbon dioxide production in crickets to calculate the metabolic costs of calling. Individual crickets were placed in 180 mL airtight glass jars where they remained for 6–13 hours. Initial carbon dioxide levels were measured by using a syringe

to withdraw 5 mL of air through an airtight septum in the top of the jar and injecting the air into an infrared gas analyzer (CI-301 Gas Analyzer, CID Inc., Camas, WA). At the end of the trial, carbon dioxide concentration was measured a second time using the same technique. The difference between the initial and final measurement was used to calculate the total volume of carbon dioxide produced by the cricket during the duration of the trial. At the completion of the trial, crickets were weighed to the nearest milligram on a small portable balance (Sartorius AY123 M-PROVE, Bohemia, NY). Thus carbon dioxide production could be expressed as  $\mu\text{L}/\text{h}$  or  $\mu\text{L}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ .

During the metabolic trials, crickets were acoustically isolated from each other and were individually recorded with a small Dictaphone recorder on top of each jar. At the completion of the trial, we downloaded the sound files from each recorder and used Raven Pro 1.4 (Cornell University, Ithaca, NY) to calculate percentage of the trial spent calling. All respiration trials were conducted between 22 and 24°C between dusk and dawn. We used a general linear model to partition the variance in the dataset, allowing us to estimate the mass-specific resting metabolic cost and calling metabolic cost (Nelder and Baker 1972; SAS Institute 2003; Bolker 2008). We fit the data to:

$$R = \beta_0 + \beta_1 \cdot M + \beta_2 \cdot C + \varepsilon \quad (1)$$

where  $R$  = the experimentally measured respiration rate ( $\mu\text{L}$  of  $\text{CO}_2/\text{h}$ ),  $M$  = mass (mass of live insect in mg, expressed as residuals relative to the mean of all measured insects),  $C$  = calling time (as proportion of time during trial),  $\varepsilon$  represents the error term, and  $\beta_0$ ,  $\beta_1$ , and  $\beta_2$  were estimated from the data via maximum likelihood (SAS Proc Nlin) [ $\beta_0 = R - \beta_1 \cdot M - \beta_2 \cdot C$ ;  $\beta_1 = (R - \beta_0 - \beta_2 \cdot C)/M$ ;  $\beta_2 = (R - \beta_0 - \beta_1 \cdot M)/C$ ]. With this model formulation,  $\beta_0$  is interpretable as the resting respiration rate ( $C = 0$ ) for a cricket of average mass ( $M = 0$ ),  $\beta_1$  describes the effect of mass on respiration rate and  $\beta_2$  describes the specific cost of calling above and beyond that explained by body mass. We tested for differences among species in the resting respiration rate and in the cost of calling by comparing the model fit to equation (1) with models that allowed for differences in  $\beta_0$  or  $\beta_2$  among species. Models were compared using AIC scores and likelihood ratios (Burnham and Anderson 1998).

To determine the pulse rates of males, we created short high-quality recordings of individual males (recordings archived at the Cornell Lab of Ornithology). Adult male crickets were placed individually in mesh cages, and recorded using a Sennheiser ME62 omnidirectional microphone with K6 power module (Solrød Strand, Denmark) seated in a 43.2 cm Telinga parabolic reflector (Tobo, Sweden). Recordings were captured on a Marantz 661 solid state recorder (Mahwah, NJ) sampling at 96 kHz with

24 bit depth. We then used Raven Pro 1.4 to analyze the recordings and to count the number of pulses produced in two seconds, then divided by two to determine the number of pulses per second. Pulse rates in continuously calling males are generally quite stable through time, but are influenced by temperature (Walker 1957; Walker 1963). These high quality recordings were made at a variety of temperatures. Given that male call characteristics vary linearly with temperature over the biologically relevant range (Walker 1957; Walker 1963), we used site-specific curves to standardize male calls to 25°C, a temperature that occurs commonly in all sites. We used linear regression to test whether pulse rate explained interspecific variation in the metabolic cost of calling after standardizing for mass-related differences in calling cost. Recordings are archived at the Macaulay Library of Natural Sound (Cornell University).

Call amplitude was measured in two ways. For a set of 140 individuals, males were recorded under screen tents using a Telinga parabolic dish and Sennheiser microphone. These recordings revealed a qualitative relationship between mass and amplitude (Fig. S2). Therefore, we created calibrated recordings using an unshielded microphone and standardized orientation so that all crickets were directly on the axis of the microphone (Forrest 1991). To minimize the contribution of reflected sound, we recorded the crickets in suspended mesh containers. All calibrated recordings were made at a standard distance of 40 cm using a Sennheiser ME62 omnidirectional microphone with K6 power module. At the end of each recording, we inserted the microphone into the calibrator and recorded a standard 94 dB tone as an internal standard. These recordings were analyzed in Raven Pro. We used the known-amplitude tone as a reference point and then found the difference between the maximum amplitude of the call and the amplitude of the standard, allowing us to normalize the recorded amplitude and estimate the actual sound pressure level produced by the insect. For each recorded cricket, we photographed the stridulatory file under a microscope, and counted the number of teeth in the file.

## Results

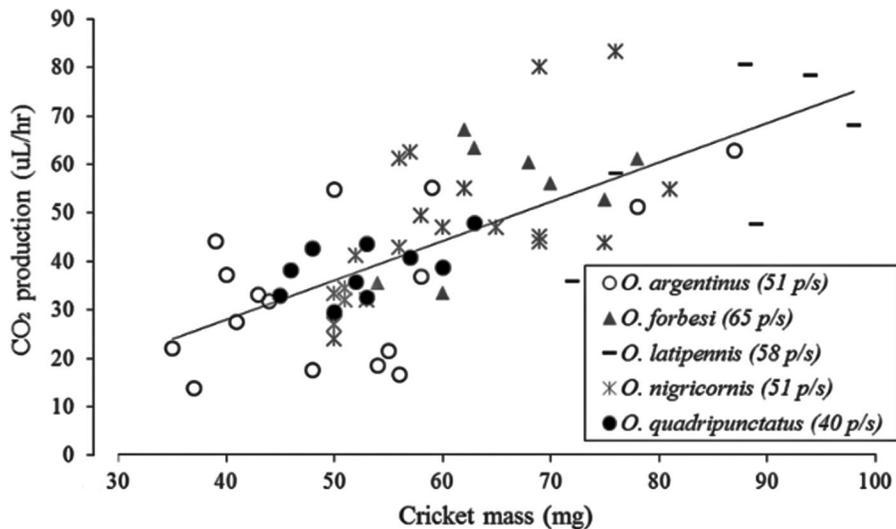
Cricket respiration rates were well described ( $r^2 = 0.78$ ) by equation (1) where  $\beta_0$  (average resting respiration rate  $\pm$  SE) =  $44.1 \pm 1.9 \mu\text{L CO}_2/\text{h}$ ;  $\beta_1$  (change in respiration rate per mg of body mass) =  $0.81 \pm 0.11 \mu\text{L CO}_2/\text{h}$  and  $\beta_2$  (change in respiration rate per time spent calling) =  $1.10 \pm 0.08 \mu\text{L CO}_2/\text{h}$ . Average resting respiration rate varied from about 25 to 75  $\mu\text{L CO}_2/\text{h}$  as cricket mass varied from 40 to 90 mg (Fig. 1). Consequently, the average mass-specific resting respiration rate was relatively constant across the range of body sizes at  $0.71\text{--}0.76 \mu\text{L CO}_2 \cdot \text{mg}^{-1} \text{h}^{-1}$ . Average respiration rate increased linearly from 44 to 128  $\mu\text{L CO}_2/\text{h}$  as percent time calling went from 0 to 75% (Fig. 2),

which indicates that continuous calling increases metabolic rate by about fourfold.

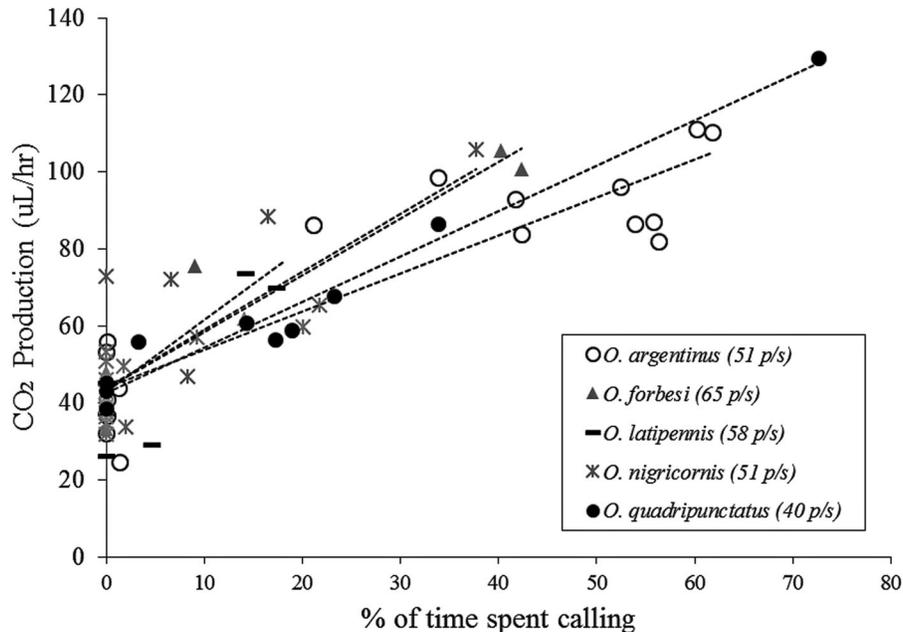
There was no support for differences among species in resting respiration rates beyond that which could be explained by differences in body size (the simple 3 parameter model (eq. (1) had more support than the more complex model in which  $\beta_0$  was allowed to vary among species (AIC = 547 vs. 552; likelihood ratio = 8.06,  $df = 5$ ,  $P = 0.15$ )). However, there was variation among species in the energetic cost of calling. A model in which  $\beta_2$  was allowed to vary among species (thin lines in Fig. 2) had better support than the simpler 3 parameter model (AIC = 547 vs. 543; likelihood ratio for  $Species \times C = 16.27$ ,  $P = 0.006$ ; Fig. 2). Furthermore, there was a very clear relationship between species-specific estimates for calling costs and body mass (Fig. 3, with  $\beta_2$  increasing approximately linearly with body mass), meaning that differences in mass among species explained nearly all the interspecific difference in the metabolic costs of calling, independent of species identity (Fig. 3,  $R^2 = 0.98$ ). The hypothesis that differences in pulse rate explained the small amount of residual difference in calling costs was rejected ( $F_{1,3} = 2.66$ ,  $P = 0.20$ ,  $R^2 = 0.47$  Fig. 3).

Insects with higher mass paid a higher cost of calling, independent of species identity or pulse rate (Fig. 3). We examined the underlying morphology to determine what explained the constant energy expenditure across pulse rates. There was a direct linear relationship between the number of teeth in the file and the duration of the sound pulse (Fig. S1). Each additional tooth strike increased the pulse duration by  $\sim 0.3$  milliseconds, with an intercept of approximately zero (Fig. S1). The number of teeth in the file was inversely related to the number of pulses produced per second (Fig. 4). That is, crickets that close their wings many times per second strike few teeth per wing closure while crickets that close their wings few times per second strike many teeth per wing closure. The product of teeth in the file and pulse rate was relatively constant across species at just under 2500 teeth/second. Striking a roughly constant number of teeth corresponded to a constant duty cycle across species of approximately 80% sound production per second (Fig. S1).

Insects of higher mass had a higher cost of calling, but also had louder calls (Fig. 5 upper panel: slope =  $0.12 \pm 0.04$  and  $P = 0.02$ , Fig. S2). As call amplitude increases, transmission distance increases, and the area that a male can reach with his call goes up exponentially (Fig. 6). Although call amplitude increased with male body size, the time spent calling decreased dramatically as body size increased (Fig. 5 lower panel: slope =  $-0.65 \pm 0.21$  and  $P = 0.005$ ), revealing an apparent trade-off between call amplitude and calling duration (Fig. 6). Larger size confers exponential gains in amplitude, but also exponential increases in metabolic costs.



**Figure 1.** Resting respiration rate increased linearly but not quite proportionately with the mass of the cricket:  $R = -3.65 + 0.81 M$ . Respiration rates were adjusted via equation (1) to that expected without calling ( $C = 0$ ).  $N = 61$ .

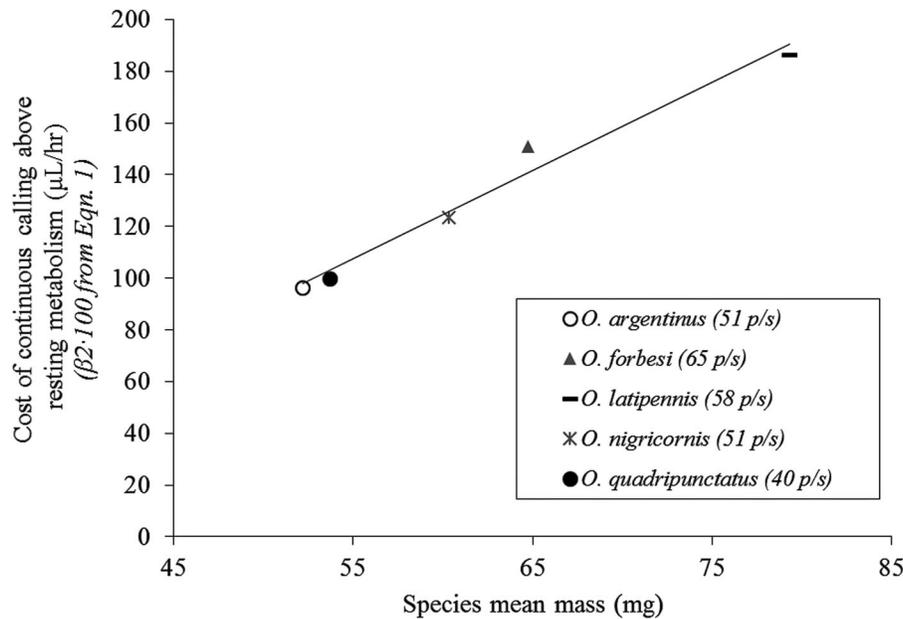


**Figure 2.** Tree cricket respiration rate as a function of percent time spent calling. Lines indicate best fit when  $\beta_2$  was allowed to vary among species. Respiration rates were adjusted via equation (1) to that expected for a cricket of average mass (via  $\beta_1$  in eq. (1)). Legend indicates species-specific pulse rates ( $N = 6$  to 19 per species).

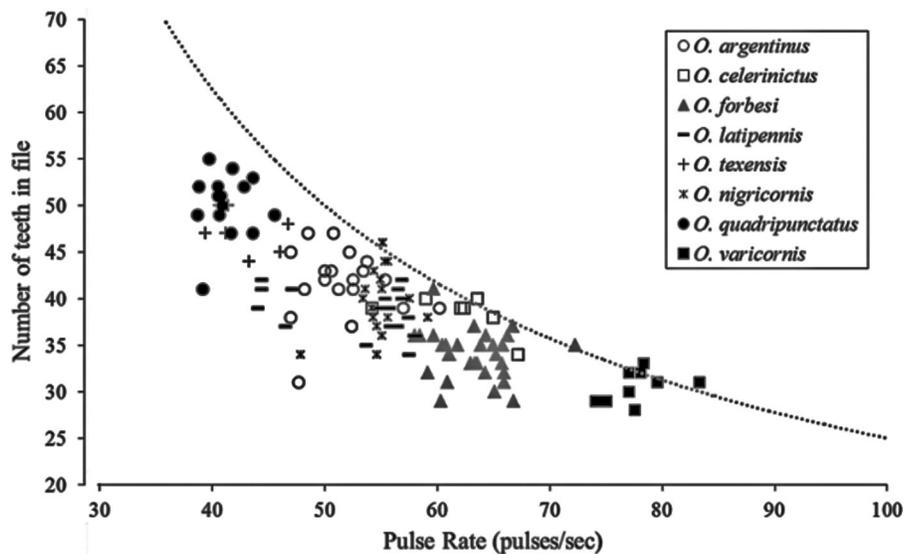
## Discussion

In continuously calling tree crickets, physics, physiology, and behavior interact in ways that appear to constrain and shape the diversification of mating signals. The combination of traits used by each species formed isoclines that were shared by all sampled species, meaning that signals did not diverge at random but moved back and forth along set mathematical relationships. Despite divergence in pulse rate among continuously calling species of *Oecanthus*, the mass-specific metabolic costs of calling are

relatively constant (Fig. 3). The energetic equivalence appears to be maintained by trade-offs between the number of times per second that the cricket closes its wings and the number of teeth that it strikes when it does so: teeth struck per second equals the product of wing closures per second and teeth struck per wing closure. Although wing closure rates and teeth struck per closure are variable across the genus, the product, teeth struck per second, is approximately constant, apparently explaining the constancy in costs of calling (Fig. 4, Fig. S1).



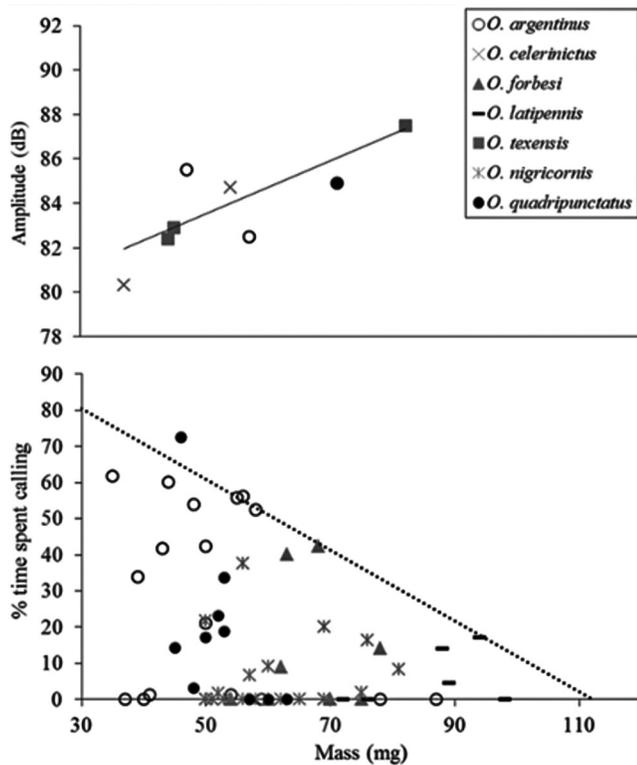
**Figure 3.** Calling cost ( $\beta_2$  from eq. (1)) increased with body size across species (slope  $\pm$  SE =  $0.034 \pm 0.003$ ,  $P = 0.002$ ) ( $N = 5$  species). Datapoints represent species-specific slopes from Figure 2. Interspecific variation in mass explained nearly all of the interspecific variation in calling cost.



**Figure 4.** There was a strong negative relationship between the number of teeth in the file and the pulse rate (number of tooth strikes per second) ( $N = 136$ ). The dotted line represents combinations that correspond to striking 2500 teeth per second.

There are several possible explanations for trade-offs among signaling traits (Podos 2001). In tree crickets, one possibility is that pulse rate and tooth number are mechanistically related—changing tooth number automatically changes the pulse rate. This may arise if a calling cricket closes its wings until it meets the resistance at the end of the file and then as a response reopens its wings for a subsequent closure. In this case, all species could be using the same musculature and neural system, with changes in the pulse rate being dictated by changes in the number of teeth

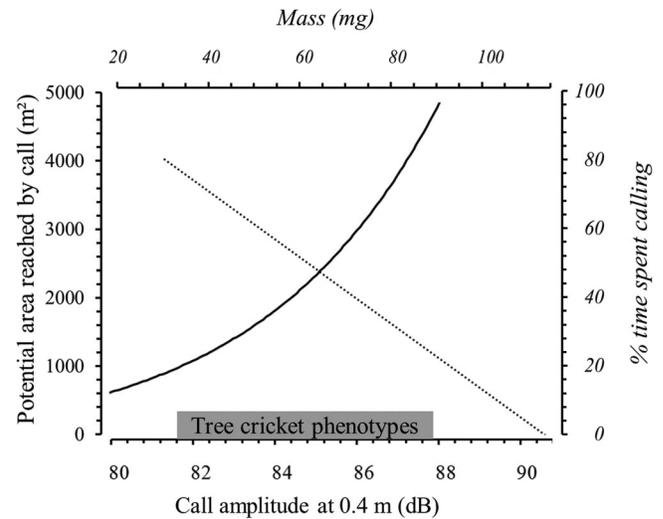
in the file (a case of pleiotropy). An alternative possibility is that when one trait changes, there is rapid corresponding evolution of other genes controlling the paired response (Wagner et al. 2012). This could arise, for example, if females exhibit strong preference for a high density of sound output (maximum time spent producing sound and minimal time spent reopening wings). If males are physiologically limited to a certain number of tooth strikes per second, decreases in pulse rate or tooth number may allow female preference to drive the other trait higher. Conversely, an increase



**Figure 5.** Across the genus, as the mass of the individual increased, the amplitude increased (upper panel,  $N = 8$ , Fig. S2), but time spent calling decreased (lower panel,  $N = 61$ ). Line in lower panel represents the quantile regression of the 95th percentile.

in one trait may enforce decreases in the other due to physiological limitation (Taigen and Wells 1985; Hebets and Papaj 2005; Ward et al. 2013). Constraints on male performance could arise through limits on vigor (sustained energetic output), limits on skill (the ability to produce carefully timed and coordinated movements), or interactions between vigor and skill (Nowicki et al. 2002; Byers et al. 2010). The relative importance of vigor and skill likely varies across taxonomic groups. The high but consistent metabolic costs of calling suggests that tree cricket performance is almost certainly limited by vigor and the ability of males to maintain calling for multiple hours. The fixed morphology used in sound production (file and scraper) may constrain the extent to which skill affects song production, but skill may be evident in call characteristics such as the variance in spacing of the pulses (with more skilled singers exhibiting less variation in the timing between pulses).

If there are physiological limitations on the number of teeth that a male can strike, females may be able to recognize high performance males as those with relatively high teeth strikes per second, which could be produced by relatively fast pulse rates or long pulse durations. In no-choice trials, females of all species regularly responded to signals with pulse rate-tooth strike products



**Figure 6.** Louder cricket calls can reach exponentially more area (solid line, amplitude values from Fig. 5). However, crickets with the large body size needed to produce loud calls pay greater than isometric costs for calling (Fig. 3) and spend less time calling than smaller individuals (dotted line and italicized axes). The gray box on the lower x-axis shows the distribution of phenotypes across the genus, with the majority of species having a mean mass of 50–70 mg. Under idealized conditions, every 6 dB increase in call amplitude doubles the transmission distance ( $r$ ), and the area that can be reached increases as  $\pi r^2$ . The radius of transmission was considered to be the distance at which the call volume amplitude would have dropped to 48 dB (typical ambient noise-level in a quiet site).

that fell both above and below the maximum tooth strike rate produced by conspecific males (Symes 2014), but it is not known how they would behave when choosing among multiple male calls. Apparently female preference is more complicated than a universal preference for high pulse rate-tooth strike products because female response functions varied in shape depending on the heterospecific assemblage. When males of the focal species had the highest pulse rate in the community, females responded readily to pulse rates higher than those offered by their potential mates, but this was not true when males of the focal species had pulse rates that were intermediate to co-occurring species (Symes 2014). It might be that females have an innate tendency to prefer signals with a high pulse rate-tooth strike product, but that this tendency can be countered in species-rich communities by selection against responding to heterospecifics (Pfennig 1998; Hankison and Morris 2002).

Both resting and calling metabolic rate scaled with mass such that larger individuals paid a higher metabolic cost for maintenance and for signaling (Figs. 1, 3). However, the mass-specific cost of maintenance decreased with size, while the mass-specific cost of calling increased. Thus a doubling of body mass did not

double maintenance costs, but more than doubled the cost of calling. However, amplitude output also scaled with mass; large *Oecanthus* called at an amplitude about 6 dB greater than small *Oecanthus* (Fig. 5) and can therefore be heard at about twice the distance. The calls produced by tree crickets have directionality, with higher amplitude produced in front of and behind the insect (Forrest 1991). This permits greater reach in any direction than would be possible if the output were omnidirectional. Furthermore, individuals typically turn and change the direction of their calling, akin to a light house with a rotating directional beam. While the exact area reached by a male's call will depend on the hearing threshold of the female and the physical structure, temperature, and humidity of the transmission environment, the total area that can be reached by a large male calling from a fixed location is approximately fourfold that of a small male (exponential function in Fig. 6).

Physiological and energetic scaling means that larger crickets can reach more area with their calls, but doing so costs more joules per minute of calling. The metabolic rate of the smallest species (*O. argentinus*, average 52 mg) increases from about 38 to 57  $\mu\text{L CO}_2/\text{h}$  as calling goes from 0 to 20% versus 56 to 93.2  $\mu\text{L CO}_2/\text{h}$  for the largest species (*O. latipennis*, average 79 mg). Thus the cost of calling for a small versus large cricket is about 0.54 versus 1.04 mW (assuming a respiratory quotient of 1 and an oxyenergetic equivalent of 20.08 kJ/L  $\text{O}_2$ ). This seems a likely explanation for the short calling times of the largest crickets (Fig. 5). Furthermore, this suggests that *Oecanthus* approach the maximum size at which they retain the ability to signal. At current sizes, it seems that males of large species, with energetically intensive calls, are constrained to call less during the night than males of small species, who can call for a greater proportion of the night, but cannot reach out as far with their lower energy signals. More generally, scaling constraints on the tree cricket sound production mechanism may also explain why tree crickets (and other Orthopterans that call with their wings fully perpendicular to their body) do not evolve the large body size of other calling insects that call using different sound production mechanisms or wing positions.

Trade-offs in the divergence of *Oecanthus* cricket signals could be driven by a variety of factors including underlying performance constraints, female preferences, predation, and ecological interactions (Taigen and Wells 1985; Podos 1997; Byers et al. 2010). Calling performance may be constrained by limitations on metabolism, neural firing rate, or wing structure (Byers et al. 2010). Experimental manipulations of partial pressure of  $\text{O}_2$ , temperature, body size, or file and wing structure could provide insight into the proximate drivers of this system of tradeoffs. The crickets used in this study were wild-caught, meaning that environmental variation could contribute to the observed patterns. However, most species were collected in multiple sites

and multiple species were often collected in the same site, indicating that these patterns are quite robust to environmental variation.

The signals of continuously calling tree crickets appear to diverge across constant energy isoclines. This is consistent with previous observations that calling metabolism was similar in two different species of *Oecanthus* (Prestwich and Walker 1981). Signal diversification across constant energy gradients may be a unique feature of sound production mechanisms in *Oecanthus* or may be a more fundamental feature of energy budgets and of signal divergence (Wagner et al. 2012). *Oecanthus* signals are metabolically demanding, but are not out of line with the costs of acoustic signaling in other organisms, indicating that the patterns seen in *Oecanthus* are not a unique feature resulting from exceptionally costly signals (Prestwich and Walker 1981; Taigen and Wells 1985; Prestwich 1994; Wagner and Hoback 1999). If signal energy is maintained as signals diverge, only a certain subset of signals may arise via predictable trade-offs among signal characteristics. Many signal combinations would never arise because they are physiologically infeasible (Podos 2001; Emlen 2001), demand too much mechanical skill (Byers et al. 2010), or are not capable of eliciting high levels of female response. If most divergence occurs within the framework of existing signal trade-offs, it implies that the available signal space in a community may be more tightly constrained than previously appreciated. Additionally, if sexual selection tends to push signals to a physiological maximum, the result would be a narrow set of trait combinations that are both feasible and effective.

If it is broadly true that signals diverge along constant energy isoclines, it suggests that there may be two types of divergence: divergence that moves species along existing trade-offs (as seen in this study) and divergence that generates new sets of trade-offs. New trade-off relationships may be important to radiation at higher taxonomic levels (such as the origination of genera and families) and could arise from events such as the evolution of a novel metabolic pathway that increases the rate at which energy can be mobilized while signaling or a novel sound production mechanism that shifts the intercept of the performance relationship. Divergence within the confines of relatively inflexible trade-off sets may be the rule for most speciation events, while mutations that fundamentally alter the trade-off relationships may facilitate radiations into new trait combinations.

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## DATA ARCHIVING

Sound files (Macaulay Library of Natural Sound); Specimens (In progress at California Academy of Natural Sciences); Respiration data (<http://datadryad.org/resource/doi:10.5061/dryad.fb48n>)

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### Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Figure S1.** There is a linear relationship between the number of teeth in the stridulatory file and the duration of the pulse produced by the cricket at 25°C (Panel A).

**Figure S2.** Larger crickets produced higher amplitude recordings.

**Figure S3.** Waveforms of *Oecanthus* cricket species.