# Divergent Mechanisms of Acoustic Mate Recognition Between Closely-Related Field Cricket Species (*Teleogryllus spp.*)

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# ABSTACT

Effective recognition of conspecific mating signals reduces the risk of maladaptive hybridisation. Dissecting the signal recognition algorithms that underlie preferences is a useful approach for testing whether closely related taxa evaluate the same or different signal features to achieve mate recognition. Such data provide information about potential constraints and targets of selection during evolutionary divergence. Using a series of mate choice trials, we tested whether closely-related, but genetically and phenotypically divergent, field cricket species (*Teleogryllus oceanicus* and *Teleogryllus commodus*) use shared or distinct recognition algorithms when evaluating acoustic male calling songs. These species overlap in sympatry, show premating isolation based on female discrimination of male calling songs, yet are capable of producing hybrid offspring. Unexpectedly, female selectivity for features of male song differed between the two species. We found that the two species use a combination of shared and unique signal filtering mechanisms, and we characterised how information about male carrier frequency, pulse rate and temporal patterning is integrated to achieve song recognition in each species. These results illustrate how comparatively few, simple modifications in key components of signal recognition algorithms can lead to striking interspecific discrimination among closely related taxa, despite apparent signal complexity. The finding that some steps during signal recognition and filtering are shared between the species, while others differ, can help to identify behavioural traits targeted by selection during evolutionary divergence.

*Keywords:* **acoustic communication, divergence, female preference, mate recognition, reproductive isolation, sexual selection, speciation, *Teleogryllus***

# INTRODUCTION

The decision-making processes that animals use to evaluate and select among potential mates can have an important influence on the evolutionary outcome of sexual selection (Bateson, 1983). For example, mismatches between populations in sexually-selected traits and preferences can generate reproductive isolation and promote speciation (West-Eberhard, 1983; Greenfield, 2002; Coyne & Orr, 2004; Mendelson & Shaw, 2005; Safran et al., 2013; Shaw & Mendelson, 2013). Understanding how individuals recognise different male signals is therefore a fundamental goal of sexual selection research (Bateson, 1983; Andersson, 1994; Ritchie, 2007; Chenoweth & McGuigan, 2010), and theoretical models of sexual selection in systems with female choice have predicted a key role for female responsiveness, preference and discrimination of such signals (Lande, 1981; Bateson, 1983; Mead & Arnold, 2004; Andersson & Simmons, 2006). Understanding the mechanistic bases of mating preferences and decision-making behaviours can help to answer questions about their function and evolution. For example, work on the genetic basis of mate choice in drosophilid fruit flies has illustrated an evolutionary link between ecological and mating traits (Chung et al., 2014), studies of the zebra finch *Taenopygia guttata* have clarified neural architecture that might control species difference in song preferences (MacDougall-Shackleton, Hulse, & Ball, 1998), and characterising perceptual tuning in the acoustically-signalling anuran *Physalaemus pustulosus* has shown how pre-existing sensory biases can facilitate evolution via sexual selection (Ryan et al., 1990).

One way to study the neurophysiological mechanisms underlying mate recognition is to treat the decision-making process as a computational algorithm, or series of operations used to evaluate incoming signals and transform that evaluation into a behavioural action such as a mating response (Ronacher, Hennig, & Clemens, 2015). Filters are integral components of such signal processing algorithms, and in animals, signal filters represent traits of the organism that exclude irrelevant information contained in incoming signals to focus reception upon important signal features. In acoustically-signalling organisms, for example, species can differ in the physical or mechanical properties of structures used to receive sounds, such as tympana, providing peripheral filtering of signals, and the central nervous system can also filter incoming signals depending on the configuration of neural pathways (Greenfield, 2002).

By designing tests that manipulate male signal components and assess female responses, it is possible to gain insight into which signal features females attend, which are filtered out, how different signal features might be traded off against one another during assessment, and which ones are possible targets of sexual selection (Kostarakos, Hartbauer, & Römer, 2008; Hedwig, 2006; Hennig, 2009; Henni, Heller, & Clemens, 2014). Much work examining signal recognition algorithms underlying female choice has focused on evaluations that females make among potential mating partners of the same species, and this has taken the form of measuring female preference functions (Wagner, 1998). However, it is less clear whether closely related taxa that risk coming into contact and producing low fitness hybrids use the same, different, or more complex algorithms when faced with the challenges of mate recognition. For instance, closely related species in the treefrog genus *Hyla* have been found to distinguish conspecific from heterospecific calls using different sets of temporal call features, reflecting divergence in signal recognition algorithms (Schul & Bush 2002). In addition to clarifying similarities and differences in the neural mechanisms underlying mate recognition in related species, such data can inform likely targets of sexual selection and constraints during the evolution of reproductive isolation and reinforcement (Coyne & Orr, 2004).

We tested whether the algorithms and filters underlying mate recognition differ between two closely related field cricket species, *Teleogryllus oceanicus* and *T. commodus*, which are a classic system in the study of acoustic signalling and reproductive isolation (e.g. Hoy & Paul, 1973; Hoy, 1974). These crickets are firmly established as separate species, and both attract mates using long-range acoustic signals that are clearly distinguishable at the phenotypic level (Otte & Alexander, 1983). Both species inhabit coastal regions of Australia, with *T. oceanicus* in the north and *T. commodus* in the south, and their distributions overlap for several hundred kilometres along the central eastern seaboard (Fig. 1a) (Otte & Alexander, 1983). The species readily hybridise in the lab, though hybrid females are almost always infertile, providing an unusual, reciprocal exception to Haldane's Rule (Hogan & Fontana, 1973; Moran, Ritchie, & Bailey, *in press*). Despite their known ability to interbreed, hybridisation is thought to be rare or absent in the wild (Hill, Loftus-Hills, & Gartside, 1972, though see Otte & Alexander 1983).

Long-range male advertisement songs of Australian *Teleogryllus* are unusual owing to a patterning complexity not normally observed in grylline crickets: the songs consist of two stereotyped elements, or phonemes: a higher-amplitude pulse train we refer as the "chirp" followed by a series of shorter, lower-amplitude pulses we refer to as "trills" (Figs. 1b, c). Both species also produce a similarly-structured, short-range courtship song which functions to release female mounting behaviour (Balakrishnan & Pollack, 1996), but here we focus on the long-range attraction signal given its known contribution to premating isolation (Hill, Loftus-Hills, & Gartside, 1972, Bailey & MacLeod 2014). Both species exhibit this two-part calling song pattern, although a distinguishing feature between them is that in *T. oceanicus,* the lower-amplitude trills following the initial chirp are comprised of paired pulses (with occasional triplets or, less frequently, higher pulse number trills), whereas the lower amplitude trills of *T. commodus* are comprised of a smaller number of longer-duration trill-like elements composed of a greater number of pulses (Fig. 1b, c). Average carrier frequencies are also higher for *T. oceanicus* (ca. 5 kHz) than for *T. commodus* (ca. 4 kHz) (Bailey & Macleod, 2014). This system therefore provided an opportunity to test whether recognition algorithms underlying female mate choice for conspecific vs. heterospecific songs rely on differential filtering of the same acoustical traits of male calling song, or whether females have diverged in the traits that their signal filters target. Put another way, are females of both species selective for the same or different song features when exerting preference?

Previous work illustrated the importance of pattern recognition for conspecific female phonotaxis in *T. commodus*, and suggested that a different balance of peripheral versus central nervous processing contributes to conspecific song recognition in each species (Hennig & Weber, 1997). After validating this result, we developed tests to examine the overall selectivity for con- and heterospecific song patterns and test the contributions of carrier frequency, pulse rate during chirps and trills, and trill pattern composition to song recognition and selectivity. We expected that both species use a combination of spectral and temporal filters (Hennig & Weber, 1997), but given that frequency differences may not definitively identify *T. commodus* and *T. oceanicus*, temporal patterns of song envelopes were expected to play an important role. Two main findings provide insight into divergence of mate recognition algorithms. First, closely related taxa do not necessarily employ the same filter types to differentiate individuals of the other taxon, and second, the strong divergence in mate recognition that this causes can reflect relatively few, minor shifts in the way signals are processed by the nervous system.

# METHODS

### *Cricket Rearing*

We used laboratory-reared adults from two allopatric populations located near Townsville, QLD (*T. oceanicus*) and Moss Vale, NSW (*T. commodus*). Otte & Alexander (1983) reported a single recording of *T. commodus* calling song during a field survey near Townsville. However, that specimen's reported carrier frequency was consistent with *T. oceanicus* (4.6 kHz compared to an average of 3.65 kHz for *T. commodus* reported by Otte & Alexander (1983)), and we observed no *T. commodus* in the field or among the laboratory-reared offspring of field-caught individuals from Townsville (Moran & Bailey, 2013). We therefore considered the populations used in this study to be allopatric. Prior to testing, the populations had been reared separately in common-garden conditions in the lab for at least one generation to mitigate maternal effects that could reflect field conditions. Stock crickets were kept in 16L translucent plastic containers at ca. 25 °C on a photo-reversed 12h:12h light:dark cycle. They were fed Supa Rabbit Excel Junior and Dwarf Rabbit nuggets *ad libitum*, and provisioned with cardboard egg cartons and moistened cotton pads. Sexually mature adult females (7 days or older) were tested.

### *Female Phonotaxis Tests*

Female phonotaxis responses were tested used a trackball system and a series of artificially-constructed song playbacks. Setup of the trackball and its operation followed Dahmen (1980) and Hedwig & Poulet (2004). The general protocol we used for phonotaxis assays has been described in detail elsewhere (e.g. Blankers, Hennig & Gray, 2015; Hennig, Blankers & Gray, 2016), so here we summarise the approach and highlight key differences in our experiments.

Females were suspended in a walking position over a hollow Styrofoam ball (100 mm diameter, weighing 1.2 to 1.8 g) positioned within a 50 x 50 x 50 cm box lined with acoustic foam. The ball floated on an airstream and its movements were recorded from the bottom by an optical sensor (Agilent ADNS-2051), or by two laterally-focused sensors (ADNS-5050, Avago Technologies) positioned perpendicular to one another. Each channel had a sampling rate of 10 kHz and signal was processed through an A/D-board (PCI-6221, National Instruments, Texas) with Labview v.7.1 or v.9 software. This enabled us to record longitudinal and lateral movements of the trackball when crickets responded during playbacks.

Playbacks with the required carrier frequencies and pulse characteristics (1 ms rise and fall) were constructed using LabView 7.0 and transmitted as described in Hennig, Blankers and Gray (2016). Briefly, songs were played back at 80 dB through two Piezo Horntweeter PH8 loudspeakers 25 cm away and 45º to the left and right of the trackball's upper surface. Speakers were calibrated by playing a 1s tone matching the required carrier frequency and assessing with a Bruel and Kjaer sound level meter and a condenser microphone on a fast reading relative to 2 x $10^{-5}$ Pa (Bruel and Kjaer 2231 and 4133, respectively). Test sessions were run at 25 ± 1 ºC, and for each, we performed one 45 s silent control at the beginning, one 45 s continuous tone control at the end, a positive control at the beginning and a positive control at the end (Fig. 2a, b), plus the 8 focal test signals in randomized order. Parameter values for test signals are provided in the figure captions for each species. Signal presentations were separated by 10 s silent intervals. Silent and tone controls allowed us to monitor and adjust for female motivation and selectivity. Positive controls represented the most attractive combination of song elements for each species (Fig. 2d: Positive controls for *T. oceanicus*: 5.0 kHz and TP1: chirp duration: 275 ms, pulse rate during chirp: 16 pulses per second, pps, pulse duty cycle 0.6; trill duration 960 ms composed of double pulses at pulse periods of 40 ms and 80 ms. *T. commodus* 4.0 kHz and TP3: chirp duration: 320 ms, pulse rate during chirp 18 pulses per second, pps, pulse duty cycle 0.65; trill duration 700 ms at a pulse rate of 35 pps followed by a pause of 200 ms). Here we consider female selectivity as the degree to which females discriminate trait values to which they respond most strongly (cf. ‘preference window’ in Butlin (1993), ‘discrimination’ in Bailey (2008), and ‘tolerance’ in Fowler-Finn & Rodríguez (2011)).

## *Phonotaxis Response Scores*

We calculated phonotaxis scores ($PS$) of 9-32 females for each species, for each 45 second test pattern, using females’ longitudinal forward ($X$) and lateral sideward deviations ($Y$) towards the playback. Both $X$ and $Y$ were normalised to the attractive controls, and female response relative to the two speakers was averaged to obtain a robust measure of response strength. The $PS$ was calculated using the formula:

$$PS = \left[ \frac{\left( \frac{X_T}{\bar{X}_{CP1,2}} \right) + \left( \frac{|Y_T|}{\bar{Y}_{CP1,2}} \right)}{2} \right] \times [sgn(Y_T)]$$

where $X_T$ and $Y_T$ represent the forward ($X$) and lateral ($Y$) walking components during the test, and $\bar{X}_{CP1,2}$ and $\bar{Y}_{CP1,2}$ represent forward ($X$) and lateral ($Y$) walking components averaged over positive controls at the beginning ($CP1$) and end ($CP2$) of a test session. Multiplication by the sign of the lateral walking component, $sgn(Y_T)$ (equivalent to turns away from the active speaker), ensured that the overall $PS$ could obtain negative values. Negative scores and scores larger than 1 could thus be obtained, although $PS$ typically ranged between 0 and 1. For example, $PS < 0$ could result if females turned away from the active speaker, and $PS > 1$ could result if during a test, females exhibited a turning response stronger than that which they exhibited during the control stimulus. In some of the presented data, responses of females were high but did not reach scores of 1.0 (e.g. Figs. 3c, 4a and 5a, b). This reduction was most likely due to suboptimal combinations of the large number of parameters that describe the song patterns of these species. If female $PS$ to the initial positive control of a test session fell below 0.5, the session was aborted. Females were also excluded from further analysis if their final positive control $PS$ was less than 50% of their initial positive control $PS$, or if they were highly responsive  during silent and tone controls, although the latter occurred infrequently (Fig. 2a, b).

Statistical comparisons of the turning responses to test patterns were performed using paired t-tests. Statistical significance was assessed at $\alpha = 0.05$. Unless otherwise specified, means and standard errors of the data are presented, and sample sizes ($n$) for each test series are given in the figure captions. Degrees of freedom ($df$) were calculated as $df = 2(n - 2)$. R v. 2.15.2 was used in construction of the map in Fig. 1 (R Core Team 2012; Becker & Wilks 2013a,b).

# RESULTS

## *Interspecific Variation in Female Selectivity*

Females of both species were tested for their ability to discriminate con- and heterospecific song types. As illustrated in Fig. 1, song structure is distinct between these species (see also: Otte & Alexander, 1983; Hennig & Weber, 1997; and Table S1 in Bailey & Macleod, 2014). For this test, song patterns were constructed that exhibited an initial chirp section typical for the respective species, plus a trill part that mimicked the song pattern with respect to pulse rate (TP1 for *T. oceanicus,* TP3 for *T. commodus* in Fig. 2d). Additionally, females were tested with patterns representing a fusion of otherwise separated trill pulses to longer blocks of sound (TP2, TP4 in Fig. 2d). The latter two test patterns were expected to be indicative of potential differences in selectivity for the trill part between both species. Each test pattern was presented at the con- and heterospecific carrier frequency (4.0 and 5.0 kHz in Fig. 2c). *T. commodus* females were highly selective for carrier frequency and temporal patterning elements, whereas *T. oceanicus* females were less selective for temporal pattern features (Fig. 2c). For instance, *T. oceanicus* accepted all test patterns, provided they were broadcast at 5.0 kHz. *T. oceanicus* responses were attenuated at 4.0 kHz. In contrast, females of *T. commodus* only responded if both the carrier frequency and the temporal pattern corresponded to the conspecific song. This distinction illustrates that *T. commodus* females only showed strong responses to song models with the lower species-specific 4 kHz carrier frequency when they were presented with an appropriate species-specific pulse pattern, whereas *T. oceanicus* females responded strongly to species-specific 5 kHz frequency playbacks regardless of the pulse pattern presented. Females of *T. commodus* were therefore more selective for the temporal pattern than females of *T. oceanicus* (Fig. 2).

### *Components of female selectivity*

In a further series of tests, females of both species were exposed to test patterns designed to dissect the contribution to the selectivity observed before of carrier frequency, pulse rates in chirp and trill, and trill composition (Fig. 2c). As predicted, responses to carrier frequency were differently tuned in the two species. *T. commodus* females showed a peak response to calling songs at 4.0 kHz, whereas *T. oceanicus* females preferred songs 4.5 kHz or higher in frequency (Fig. 3a). Female responses for pulse rate during chirps were broadly similar, with only a small difference in the most preferred pulse rate (*T. oceanicus*: 12 pps, *T. commodus* 16 -18 pps, Fig. 3b). However, *T. commodus* showed selectivity for a specific pulse rate of 32 pulses per second during the trill portion of the calling song, whereas *T. oceanicus* females only responded if the pulse rate during the trill part was the same as during the chirp part, that is at 12 pps (Fig. 3c, c.f. *T. oceanicus* in Fig. 3b). *T. commodus* thus exhibits different pulse rate selectivity for the two song phonemes, requiring two pulse rate filters, whereas the most preferred pulse rate (12 pps) is the same for each phoneme in *T. oceanicus*, for which a single pulse rate filter suffices. The addition of a separate filter for pulse rate selectivity suggests higher sensitivity to temporal pattern properties of calling song for *T. commodus* females than for *T. oceanicus*. Indeed, the preferred pulse rate of 12 pps by *T. oceanicus* in Fig. 3c indicated that females did not require a trill part for recognition and that the pulse rate of the chirp part alone sufficed.

The contribution of the trill composition in terms of pulses per trill and trill duration indicated broadly similar responses in both species (Fig. 4). *T. oceanicus* females accepted trills built from two or more pulses, whereas *T. commodus* accepted trills built from three pulses or more (Fig. 4). Longer trills were accepted by both species equally readily, although only females of *T. commodus* appeared to be selective for a particular pulse rate during this part (Fig. 3c).

To examine whether *T. oceanicus* simply ignored features of the trill part or whether they exhibited selectivity to other temporal cues, females were tested with patterns that varied the pulse duty cycle. Such patterns exhibit different amounts of sound energy independent of a particular pulse rate as illustrated in Fig. 5c, as the duty cycle is calculated from the pulse duration divided by the pulse period. *T. oceanicus* females exhibited a strong selectivity for all patterns with a pulse duty cycle higher than 0.5, which corresponded to patterns with high sound energy as they contained pulses longer than the pauses in between (Fig. 5).

# DISCUSSION

The origin and maintenance of mating barriers is a fundamental requirement for speciation to occur in situations where diverging populations could hybridise, or when secondary contact occurs between closely related taxa (Coyne & Orr, 2004). Divergence in signalling and mate recognition traits facilitates the establishment of such barriers. While changes in signalling traits and mate recognition at the phenotypic level have been well-characterised in a number of systems, less is known about whether the underlying physiological mechanisms that control such mate recognition are shared or not in such taxa. Because signals are typically multi-component and complex, divergence could occur as a result of changes in the same filtering mechanism in different species, such that different values of the same signal trait are preferred, or by establishment of new filters such that divergent taxa are tuned to different signal traits. We found a mixture of both scenarios in *T. oceanicus* and *T. commodus*, which we can illustrate by separating the different filter components of the processing algorithm much like a flow diagram (Fig. 6).

Our dissection of mate recognition algorithms in *Teleogryllus* showed that females of both species attended to frequency differences and showed sharply tuned filters that almost perfectly match the documented differences in carrier frequency of conspecific male calling songs, consistent with prior reports (Hennig & Weber, 1997; Bailey & Macleod, 2014). The majority of known examples of acoustic species recognition in insects, and particularly crickets and other ensiferan insects, focus on temporal patterning of male advertisement songs (e.g. Ritchie, 1991; Mendelson & Shaw, 2005; Meckenhäuser, Hennig, & Nawrot

2013; Kostarakos & Hedwig, 2015), and a longstanding assumption about the evolution of cricket calling songs is that there is unlikely to be significant variation in carrier frequency among closely related taxa, due to the mechanical constraints imposed by physical features of male forewings used in song production (Alexander, 1962). For example, neural recordings of responses to courtship song in a gryllid from the western hemisphere, *Gryllus assimilis*, indicate the importance of temporal song patterning compared to carrier frequency, with female auditory neurons exhibiting a broad frequency response spectrum ranging from 3.5 kHz to 14.5 kHz (Vedenina & Pollack, 2012), and early perceptual models for discrimination of acoustic signals in *T. oceanicus* suggested that the main frequency-based distinction this species makes is of a categorical nature, between low frequency and ultrasound (Wyttenbach, May & Hoy, 1998).

Nevertheless, our results confirm that both *T. oceanicus* and *T. commodus* share frequency filters, with the result that females of both species filter incoming male signals as a function of those signals' dominant carrier frequency. Selectivity for frequency indicated that peak female responses were only approximately 1kHz apart. However, this selectivity matches observed differences in frequency differences of males, both from these populations (Moran & Bailey, 2013) and from other populations of the same species (Bailey & Macleod, 2014). Such a shift in the frequency filter does not necessarily require evolutionary change in complex neural architecture or physiological processes, and could be underpinned by simple size scaling differences that have arisen during the evolutionary history of these two species. For example, a meta-analysis of 58 species of tettigoniids, an ensiferan group in which males sing using a forewing file and scraper mechanism, uncovered significant overall covariance between body size and carrier frequency (Montealegre-Z, 2009). *Teleogryllus commodus* are larger than *T. oceanicus* on average, and if male forewing structures and tympanal hearing organs scaled with body size in a correlated manner during divergence, corresponding frequency filters in females of each species could be selectively tuned to the dominant carrier frequency produced by conspecific males.

Both cricket species appear to share another filter, by which the pulse rate of the chirp portion of the song is evaluated. Pulse rate selectivity can arise from only a small network of neurons, in which the property of a rebound oscillation plays a crucial role (Weber & Thorson, 1989; Pollack, 2000; Clemens & Hennig, 2013; Schöneich, Kostarakos, & Hedwig, 2015). Notably, the preference functions for this song component were very similar in the two species (Fig. 3b, 6). This similarity is consistent with previous reports suggesting that pulse rate during the chirp is under stabilising selection in both species (Hennig & Weber, 1997). In contrast with the chirp filter, the species differ in selectivity of the trill portion of the song. *T. oceanicus* females appear to be unselective towards the trill pattern (Figs. 2, 3 and 4), but they preferred trill patterns with longer pulses and shorter pauses (Fig. 5). Taken together, this is indicative of duty cycle selectivity favouring patterns with higher energy. The particular timing of pulses as given by a pulse rate did not appear relevant, which contrasted distinctly with *T. commodus* females (Fig. 3c). Thus, female selectivity for pulse rate within the trill portion of calling song highlights a key difference between the species: *T. commodus* females are more highly selective of trill patterning, focusing on temporal aspects of trill pulses such as pulse rate, whereas *T. oceanicus* females attend to the pulse duty cycle of the trill irrespective of the patterning (Fig. 2, Fig. 6). *T. commodus* appears to be the rarer species in sympatry (Moran & Bailey, 2013), and it enters a diapause in more southern populations (Otte & Alexander, 1983). Both scenarios might favour enhanced female selectivity in *T. commodus* females: rarity would increase the chances of maladaptive hybridization, and introgression of genes that reduce or eliminate the tendency to enter diapause would be detrimental to *T. commodus* females.

The integration of similar signal recognition algorithms based on frequency filters with a different mechanism based on discrimination of pulse rate during the trill portion of the song contrasts with recent findings in several gryllids producing either short, chirp-like phonemes (Hennig, Blankers, & Gray, 2016) or long, trill-like phonemes (Blankers, Hennig, & Gray, 2015). The latter species show identical computational algorithms for evaluating acoustic signals based on pulse pattern and chirp/trill features. (Blankers, Hennig, & Gray, 2015; Hennig, Blankers, & Gray, 2016). Nevertheless, these species differ in their preference for a particular pulse rate or chirp/trill duty cycle. Some gryllid species show a transition from a pulse rate filter to a pulse duty cycle filter, consistent with what we have observed in *Teleogryllus* (Fig. 5) (Hennig, Blankers, & Gray, 2016). Our behavioural experiments cannot resolve how the algorithmic flow of information during phonotaxis or particular filter component is implemented in terms of physiological or neural activity. Nevertheless, physiological recordings from sensory cells in the tympanic ear (Imaizumi & Pollack, 1999) and brain neurons sensitive to pulse rate (Schöneich, Kostarakos & Hedwig, 2015) support the proposed sequential processing steps and filter properties illustrated in Fig. 6.

There are several illustrative differences between song pattern recognition in the gryllids mentioned above versus *Teleogryllus*, which suggest a more general, taxonomically-widespread pattern underlying the evolution of signals and signal recognition during diversification. For example, most gryllids produce a series of pulses grouped into chirps or trills, which are separated by variable durations of silence (Blankers, Hennig, & Gray, 2015; Hennig, Blankers, & Gray, 2016). In contrast, the *Teleogryllus* species we studied produce calling songs with a greater number of phonemes, as in the chirp and trill part (Fig. 1), although *Teleogryllus* species with simpler song patterns are known (Rothbart & Hennig, 2012). The tendency toward additional song pattern elements, or phonemes, can be even greater in other ensiferan taxa; certain species of the Tettigoniid genus *Amblycorypha* produce some of the most complex acoustic signals of any insect, with varied arrangements of up to four phonemes (Walker & Dew, 1972). A tempting prediction is that the signal recognition filters required to process complex incoming signals will be correspondingly complex, and may therefore provide a larger target for selection or drift to modify (Fig. 6) (Hebets & Papaj, 2005).

Despite the phenotypic differences in song recognition and apparently larger number of filters required for mate recognition in *Teleogryllus* (Fig. 6), the filters themselves are in principle similar or even identical to those described in other crickets. This observation suggests that the apparently derived situation in *Teleogryllus* builds on existing schemes of pattern recognition. Two important transitions are worth highlighting: first the duplication of a pulse rate filter, and second, the transformation of a pulse rate filter to a duty cycle filter (Fig. 6), the latter of which appears complicated at first but can be simply achieved by small changes of the filter template used for song recognition (Hennig, Heller, & Clemens, 2014). These observations also suggest that recognition of a complex song pattern such as the trill portion of *T. oceanicus* calling song (Fig. 1) does not necessarily evolve because of a more complex filter, but may arise in response to a relatively simple duty cycle filter (Figs. 5, 6). The combined effects of multiple, simple filters thus provide a parsimonious explanation for the multitude of different ways in which species-identifying signals can diverge alongside recognition mechanisms for those signals. In *T. oceanicus* and *T. commodus*, divergence in signal recognition appears to have arisen from a combination of different filters applied to the same signal features, plus the modification of filters to target distinct signal features. Changes in decision algorithms must ultimately reflect measurable physical changes in the structure or neural connections within the organism, and our results are consistent with the idea that such divergence will follow an evolutionary “path of least resistance”: apparent signal recognition complexity can arise from few, basic decision algorithms.

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**Figure captions**:

**Figure 1.** Cricket ranges and signals. (a) Approximate Australian distribution of *T. oceanicus* (light grey), *T. commodus* (dark grey), and region of sympatry (stripes). , based on Otte & Alexander (1983) and Moran & Bailey (2013). Locations of populations used in this study are indicated with arrows. Our field and laboratory observations are consistent with these being pure-species, allopatric populations (see main text for details). Male calling song diagrams are based on Bailey & Macleod (2014) and illustrate song features of interest for (b) *T. oceanicus* and (c) *T. commodus*. Different authors have historically used different terminology to describe elements of *T. oceanicus* calling song. Those employed in the present study are indicated with larger font, while alternative terms for the same song features are indicated with smaller font in parentheses to ease comparison with prior work.

**Figure 2.** Female selectivity for male calling song models that varied in carrier frequency and temporal patterning. Phonotaxis scores are shown for *T. oceanicus* females (black bars, n = 15) and *T. commodus* females (open bars, n = 13). (a,b) Female response to positive (attractive stimuli) and negative controls (unattractive stimuli) during a test session (CP1, 2 positive controls at the beginning and end of a test session, CS: silent control, CT tone control). (c,d) Females were presented with test patterns (shown in (d)) similar to a *T. oceanicus* (TP1,2) or *T. commodus* (TP3,4) calling song. Each test pattern was presented at 4.0 and 5.0 kHz, corresponding to the carrier frequency of the song of both species. Responses in (c) marked with '#' were not significantly different from the positive controls in (a) and (b), and the response marked with '*' was significantly ($p<0.05$, t-test) different from the negative controls in (a) and (b). Means and standard errors are presented in (a)-(c).

**Figure 3**. Preferences (means and standard errors) for calling song features exerted by females of each species: (a) carrier frequency (*T. oceanicus* n = 15; *T. commodus* n = 13), (b) pulse rate in the chirp portion of the song, holding trill pulse rate constant (*T. oceanicus* n = 9; *T. commodus* n = 10), and (c) pulse rate in the trill portion of the song, holding chirp pulse rate constant (*T. oceanicus* n = 12; *T. commodus* n = 41). Pulse rates are given in pulses per second. Response levels higher than 0.7 were not significantly different from the positive controls, response levels below 0.3 were not significantly different from the negative controls (c.f. Fig. 2A, B). Test patterns in (a) corresponded to conspecific songs as in Fig. 2D (TP1 for *T. oceanicus* and TP3 for *T. commodus*). Test patterns in (b) corresponded to continuous pulse trains with variable pulse rate for *T. oceanicus* and variable pulse rate during the part with a continuous pulse train during the trill for *T. commodus* as for TP3 in Fig. 2D. Test patterns in (c) had a constant chirp part as TP1 and TP3 in Fig. 2D and a continuous trill part with variable pulse rate as TP3 in Fig. 2D. Typical trait values for the calling song signal of both species are available from Bentley & Hoy (1972), Hill, Loftus-Hills & Gartside (1972), and Hennig & Weber (1997). (For *T. commodus//T. oceanicus*, respectively: carrier frequency: 3.5-3.8//4.5-4.9, pulse rate within chirp: 19-20//15-16, pulse rate within trill: 31.5-31.6//24-26).

**Figure 4**. Preferences for overall trill composition. (a) Phonotaxis scores (means and standard errors) for *T. oceanicus* (n = 11) and *T. commodus* females (n = 23). (b) Diagram of test patterns in which the number of pulses was varied during the trill portion, thereby changing the trill duration. Pulse periods were set to 40 ms, and pulse periods between groups of pulses were set to 80 ms. Phonotaxis scores higher than 0.3 were significantly different from the negative controls ($p < 0.05$, c.f. Fig. 2a,b).

**Figure 5**. (a) Selectivity for temporal cues during the trill part containing more sound energy by females of *T. oceanicus*. Numbers refer to test patterns in (c). The open circle at the center refers to phonotaxis score to the positive control pattern. Diameter of circles indicates strength of phonotaxis score which was 1.0 for the positive control. (b) Females exhibit selectivity for the pulse duty cycle in the trill portion of the calling song (means and standard errors are presented). The curves correspond to transects through (a) from upper left to lower right at different pulse periods, as indicated. (c) Selected test patterns, as indicated in (a), with a constant chirp part (TP1 in Fig. 2D) and a varied trill section. Numbers to the right refer to the pulse duty cycle (pdc) of each pattern. Response levels higher than 0.3 were significantly different from the negative controls ($p< 0.05$, c.f. Fig. 2a,b).

**Figure 6**. Flow diagram describing differential processing for processing for signal recognition in *Teleogryllus* species. (Top): representative song signals for each species. (First recognition level): sensitivity to carrier frequency given by the frequency response of the tympanic ear and sensory cells depicted as tuning curves. (Middle level): processing of the pulse pattern within the phonemes of chirp and trill depicted by sensory templates for pulse rate and integration of sound energy for duty cycle evaluation. (Bottom level): integration of processing across time scales of both phonemes of chirp and trill. Common filters for carrier frequency of the song are differently tuned in the two species, leading to quantitative differences in female responses (grey boxes: brown lines indicate preferences for lower carrier frequencies by *T. commodus*, blue lines for higher carrier frequencies by *T. oceanicus*). Both species also share similar filters for the pulse rate during the chirp portion (greyboxes: black rectangles indicate sound pulses, brown lines (*T. commodus*) and blue lines (*T. oceanicus*) indicate sensory templates with rebound properties that will respond best to the given pulse rate in the chirp pattern (Schöneich, Kostarakos, & Hedwig, 2015)). A

qualitative difference is a more selective pulse rate filter in *T. commodus* for pulse rates during the trill part of a song, while *T. oceanicus* remain largely unselective for the trill pattern provided sound energy remains high (i.e. sensitivity for high duty cycle, yellow boxes: filters for trill pulse rate are symbolised by a rebound oscillation of the sensory template, filters for pulse duty cycle by an integration). Separate streams of information about chirp and trill features are finally integrated similarly for song recognition and discrimination in both species. In aggregate, while females of both species might employ similar algorithms to process incoming signals on the basis of carrier frequency and chirp pulse rate ( grey boxes), they show divergent filter properties for the trill part (yellow boxes), for which *T. commodus* females are more selective.

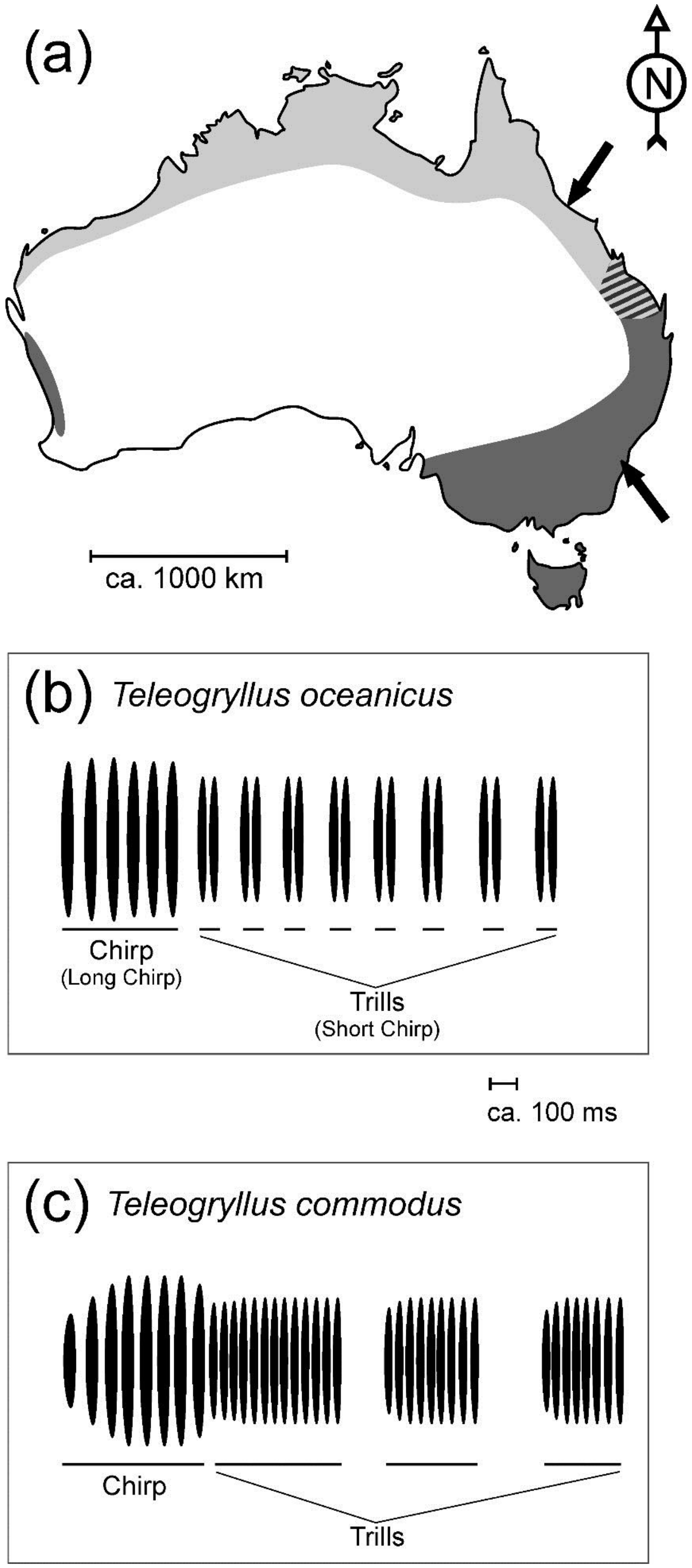
(a)
N
ca. 1000 km
(b) *Teleogryllus oceanicus*
Chirp
(Long Chirp)
Trills
(Short Chirp)
ca. 100 ms
(c) *Teleogryllus commodus*
Chirp
Trills

**Figure 1**

Fig. 2

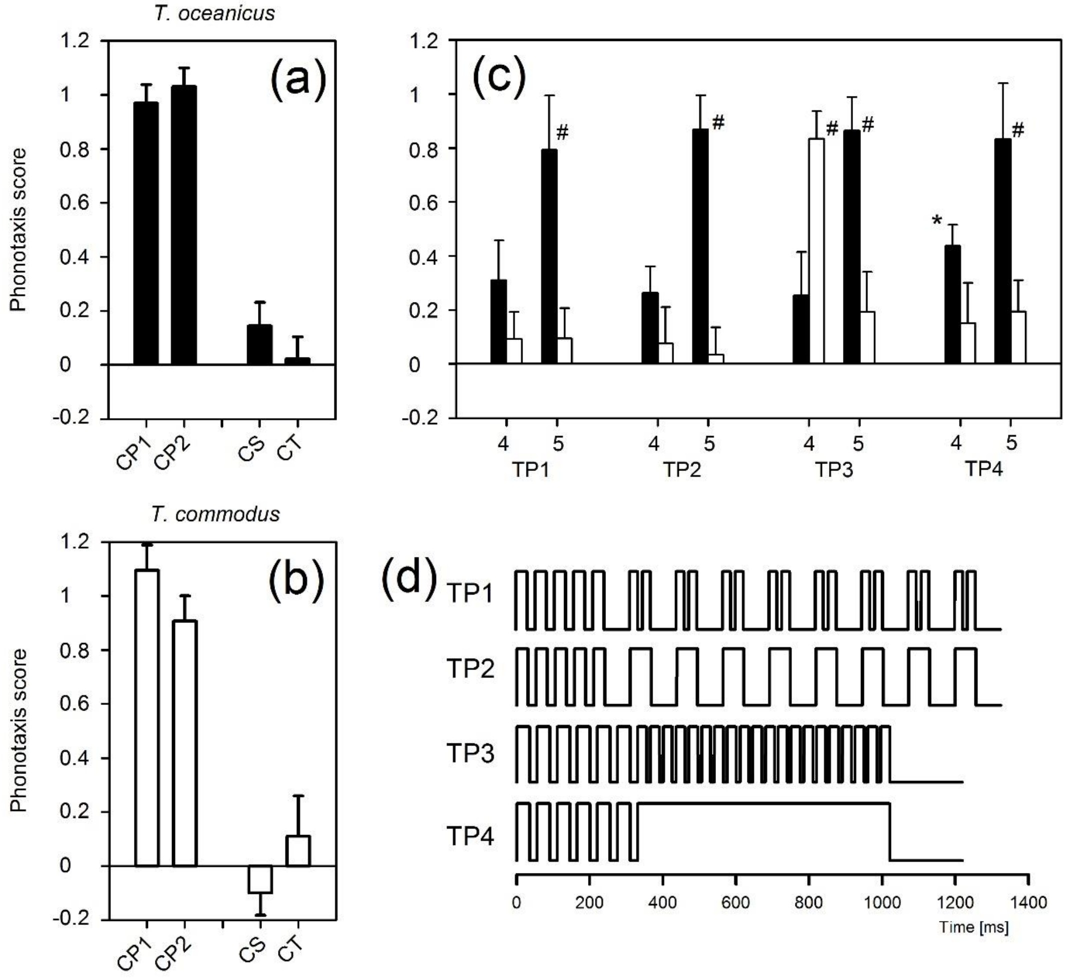

**Figure 2**

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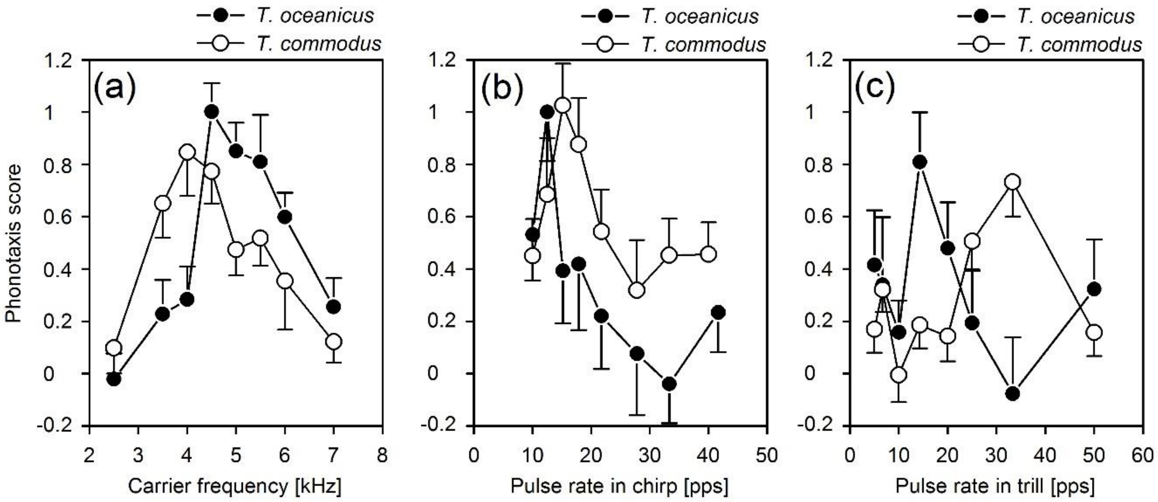
Fig. 3



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632 **Figure 3**

Fig. 4

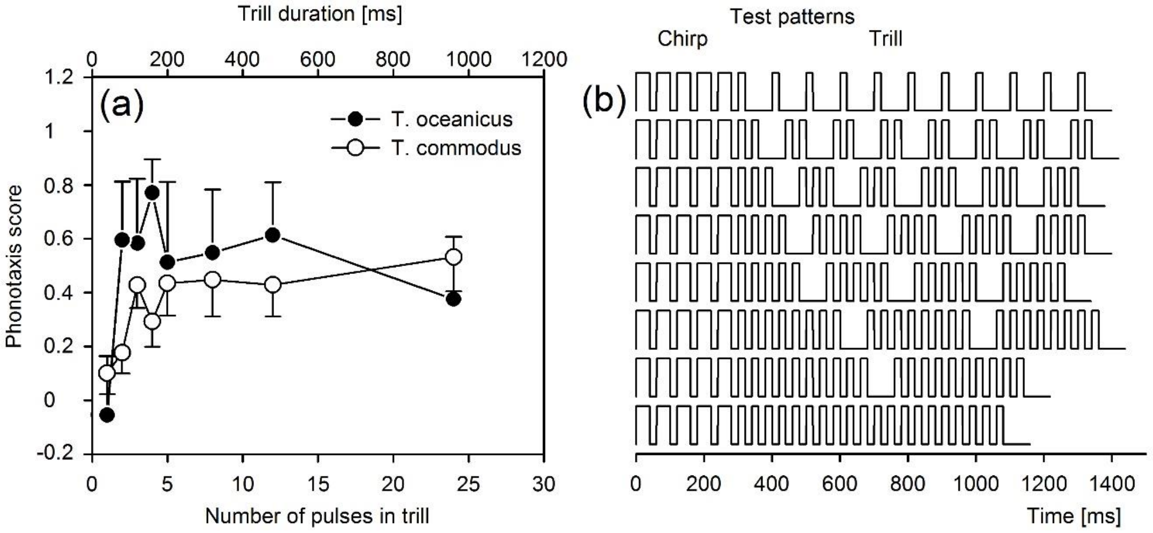

**Figure 4**

Fig. 5

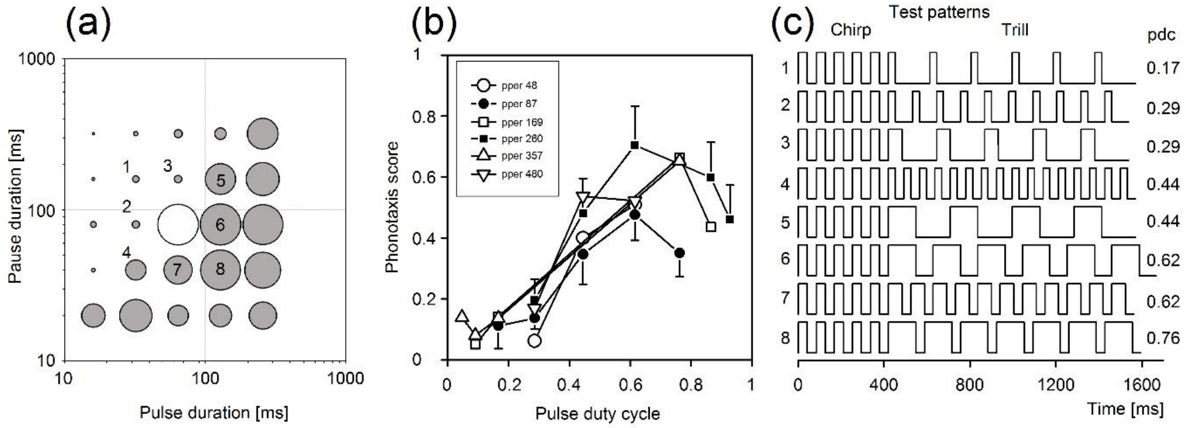

**Figure 5**

Fig. 6

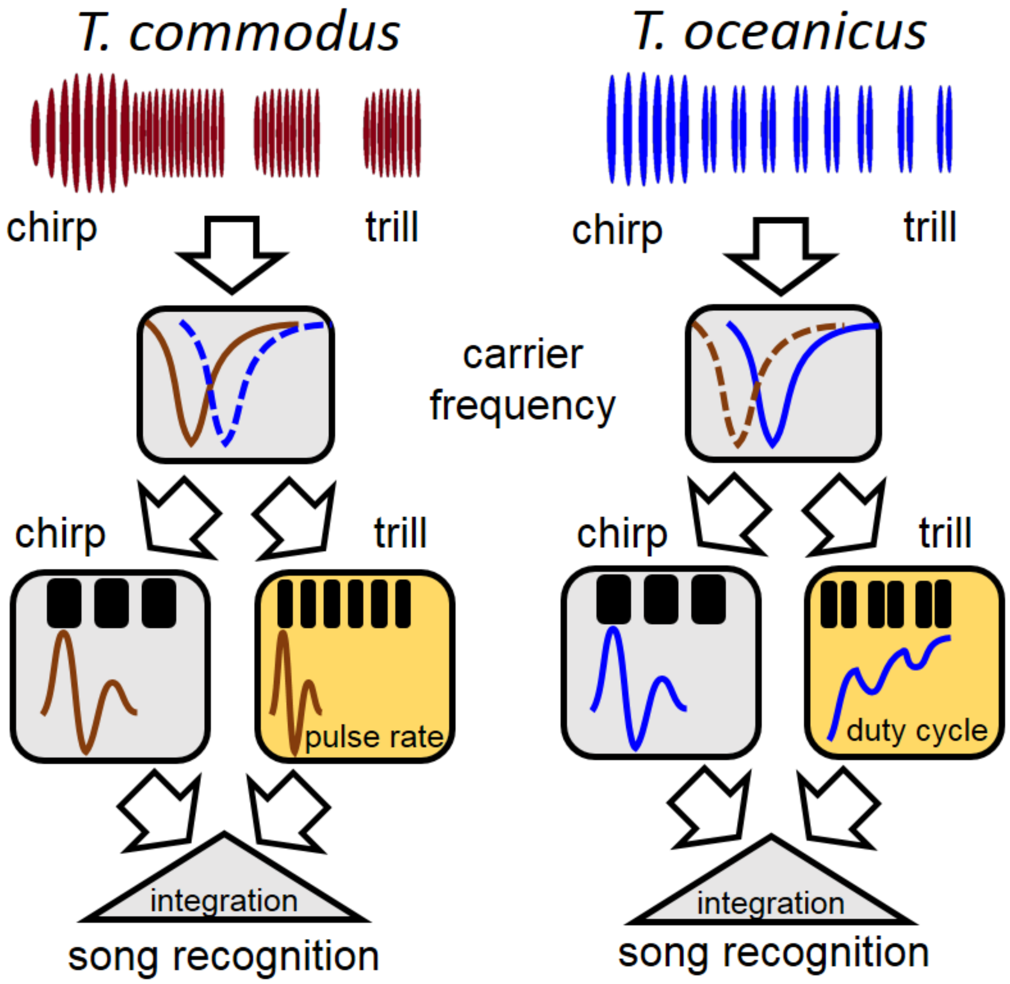

**Figure 6**