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ORIGINAL PAPER

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Temperature dependence of cicada songs (Homoptera, Cicadoidea)

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Abstract The songs of male Portuguese cicadas *Tettigetta argentata*, *T. josei* and *Tympanistalna gastrica* were recorded at five to seven temperatures within the range 24–38.5°C. To investigate the temperature dependence of the neuromuscular apparatus involved in song production, different temporal elements of the calling songs were measured. We report a strong temperature dependence for the syllable and the echeme rates in *T. josei* and *Ty. gastrica*. This suggests that in these species the neuromuscular structures involved in the timbal cycle and in generating the echeme succession of the song are strongly temperature dependent. In *T. argentata*, the syllable rate was again significantly temperature dependent; the echeme rate, however, increased between 25.5°C and 33.5°C but decreased with the highest temperature. This indicates that at least in *T. argentata* two separate neuronal networks control both song parameters. Other temporal elements of the song with potential behavioural significance were also measured and found to be temperature dependent (e.g. echeme duration and interval). The possible implications for intraspecific communication are discussed. We also demonstrate that the temperature of these small cicadas is not significantly influenced by the muscle activity involved in song production. On the other hand, exposure to sunlight can be used by these cicadas to elevate their body temperature by more than 10°C.

Keywords *Tympanistalna gastrica* · *Tettigetta josei* · *Tettigetta argentata* · Cicada calling song · Temperature

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Abbreviations *ER* echeme delivery rate · *PR* phase rate · *SD* syllable duration · *SR* syllable rate

Introduction

Cicada males use acoustic signals with characteristic temporal patterns for intraspecific communication. On the other hand, cicadas are ectotherms or partially endothermic insects (Sanborn et al. 1992) and therefore fluctuations in environmental temperature might influence physiological parameters of the nerve and muscle systems that determine the temporal patterns of the song. Indeed, evidence from other insect groups supports this hypothesis (Walker 1962, 1975; Doherty 1985; Bauer and Helversen 1987; Pires and Hoy 1992; see also Willmer 1982 for a review of temperature dependence in insects). To our knowledge, in cicadas only one study has addressed this question. Josephson and Young (1979) have reported that the timbal cycle of *Cystosoma saundersii* is influenced by temperature. At present, the temperature dependence of cicada songs is poorly known.

The Portuguese cicadas *Tettigetta argentata*, *T. josei* and *Tympanistalna gastrica* are active during the day in June and July, usually singing at ambient air temperatures of 22–35°C. Those species often call from small shrubs or grasses and especially *T. argentata* can share the same habitat with the two other species. All three cicadas produce broad band signals overlapping in frequency, but the temporal properties of the songs are species-specific (Fonseca 1991). Hence, the temporal parameters may play an important role in species recognition but are also likely to be affected by temperature variations. Here, we investigated the following questions: (1) is the body temperature of these small cicadas influenced both by singing activity and by solar radiation?; (2) are the temporal elements of the calling songs temperature dependent?; (3) what can be said about the neuronal generator(s) involved in the song pattern production, based on the temperature dependence of the different song elements?; and (4) what implications might arise for intraspecific communication?

Materials and methods

Animals and experimental set-up for song recordings

Males of the species *T. argentata*, *T. josei* and *Ty. gastrica* were collected in central and southern Portugal. The cicadas were transported to the lab and kept on a feeding plant at about 10°C. The songs of three animals of each species were successfully recorded in a wide range of temperatures. The experiments were performed within 2 days of capture.

Each animal was individually allowed to sing on a plant stem at the centre of a temperature-controlled (precision ± 0.2°C) cubic chamber (30 cm height). To reduce echoes the chamber was lined with sound absorbing material. The temperature was monitored with a thermocouple (resolution of ± 0.1°C) placed close to the cicada.

The temperatures within the chamber ranged from 18°C to 40°C. The temperatures used varied by 2.5–3°C. At each temperature a playback of the conspecific song was used to stimulate the cicada to sing. When a calling song was elicited, the stimulus was turned off and the song was recorded for 90 s on tape (UHER 4200 report) via a Sennheiser MKE-2 microphone within 10 cm of the animal. After singing the temperature was varied and allowed to stabilise. A 10-min interval was then allowed in order to equalise the animal's temperature before the cicada was again stimulated to sing. Since only the temporal parameters were to be analysed, the microphone position close to the singing cicada, allowed for a better signal-to-noise ratio.

Song analysis

The sound signals were sampled at 50 kHz offline (Data Translation 2821-F-8di) with 12-bit resolution and analysed with NEUROLAB (Hedwig and Knepper 1992).

The elements of the calling song analysed were the syllable, the echeme and the phrase (see Fig. 1 for details on nomenclature; cf. Broughton 1976 for a definition of echeme). The rate of production for each of these elements was measured to investigate the temperature dependence of the neuromuscular apparatus involved in song production. Other song parameters with potential behavioural significance for intraspecific communication were also measured, namely the duration of echemes and phrases, and their respective intervals. For all parameters except the phrase, 30 measurements per animal were performed within the last 10 s of the 90 s of song recording. For the phrase rate, duration and interval all 90 s of the song were analysed.

A regression analysis was performed to estimate the statistical significance of the temperature dependence of the song parameters. In Tables 1 and 2 the coefficient of determination (r^2) and the probability associated with the observed F statistic are presented for the exponential regression. The exponential model was preferred to the linear model since the former revealed the best fit to the present data. Indeed, a goodness of fit analysis showed that for 17 of 26 regressions calculated, the exponential function reduced on average 27% of the error sum of squares obtained with the linear function (comparisons were between $\sum(y-\hat{y}_1)^2$ and $\sum(y-\hat{y}_e)^2$, for the linear and exponential regressions, respectively; see Walker 1975). For the remaining nine data sets in which the linear model proved the best fit, the error sum of squares was reduced on average by 29% when compared to the exponential function. Elsewhere this function has also been found to better describe the temperature effects on invertebrate song production (e.g. Skovmand and Pedersen 1983; Doherty 1985).

Effects of singing activity and exposure to sunligh in the body temperature

Muscle activity involved in song production generates heat. In order to ensure that the temperature inside the chamber reflected closely the cicada body temperature, it was necessary to evaluate the variation in the body temperature caused by singing activity. Moreover, this allowed us to investigate whether the timbal muscle activity during singing does significantly change the body temperature in free-living animals. The timbal muscle temperature as well as the temperature inside the thorax (flight muscles) were measured with home made thermocouples (< 0.2 mm) while *T. argentata* ($n=2$) and *Ty. gastrica* ($n=3$) were singing under electrical brain stimulation. This method evokes a singing pattern similar to the calling song (for details on brain stimulation see Fonseca and Hennig 1996). The thermocouple was inserted in the thorax through small holes in the integument made with an insect needle.

Since the body temperature of free-living cicadas may be influenced by exposure to solar radiation (Hastings and Toolson 1991) we assessed the importance of this effect in these small cicadas by monitoring the thoracic temperature of one *T. josei* male while exposed to sunlight. This allowed us to evaluate the extent to

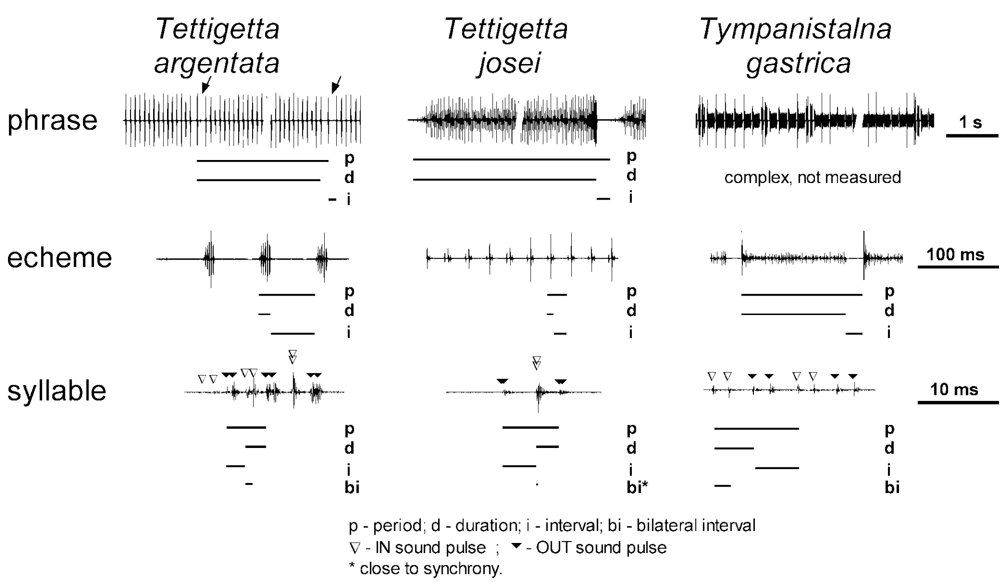

Fig. 1 Oscillograms of the calling songs of *Tettigetta argentata*, *T. josei* and *Tympanistalna gastrica* showing the nomenclature of temporal song elements used in this paper. The phrase of *T. argentata* is recognised by the very stereotyped longer pauses between echemes shown by *arrows* in the figure

Table 1 Syllable (SR), echeme (ER) and phrase rate (PR) variation with temperature in the three cicada species. For each species, the songs of three males were analysed for each temperature. Variability is expressed as ± inter-individual standard deviation (t_i initial temperature; t_f final temperature; r^2 coefficient of determination for the exponential regression; P probability level associated with the calculated F statistic). Degrees of freedom (*df*)for the F statistic: 4 (*T. argentata*, except PR in which $df = 3$); 5 (*T. josei*); and 3 (*Ty. gastrica*)

Song parameter	*Tettigetta argentata*				*Tettigetta josei*				*Tympanistalna gastrica*			
	t_i (25.5°C)	t_f (38.0°C)	r^2	P	t_i (24.0°C)	t_f (38.5°C)	r^2	P	t_i (24.0°C)	t_f (34.0°C)	r^2	P
SR (s^{-1})	149.9 ± 0.9	215.3 ± 7.8	0.95	< 0.001	112.7 ± 6.3	196.3 ± 0.4	0.99	< 0.001	74.9 ± 7.8	108.7 ± 3.0	0.95	0.004
ER (s^{-1})	10.9 ± 0.3	12.4 ± 1.6	0.45	ns	31.6 ± 1.6	46.7 ± 0.6	0.99	< 0.001	4.1 ± 0.5	6.2 ± 0.4	0.98	0.002
PR (min^{-1})	11.4 ± 6.4	15.6 ± 0.3[a]	0.43	ns	3.3 ± 1.9	8.7 ± 0.9	0.69	0.02	–	–	–	–

[a]In *T. argentata* the t_f for PR was 35.5°C

Table 2 Variation of the echeme duration and interval with temperature. Again, for each species the songs of three males singing at different temperature were analysed. Abbreviations as in Table 1. *df* for the F statistic: 4 (*T. argentata*), 5 (*T. josei*), and 3 (*Ty. gastrica*)

Song parameter	*Tettigetta argentata*				*Tettigetta josei*				*Tympanistalna gastrica*			
	t_i (25.5°C)	t_f (38.0°C)	r^2	P	t_i (24.0°C)	t_f (38.5°C)	r^2	P	t_i (24.0°C)	t_f (34.0°C)	r^2	P
Echeme duration (ms)	19.8 ± 1.0	13.4 ± 0.8	0.94	0.001	13.4 ± 1.0	7.3 ± 0.3	0.99	< 0.001	197.1 ± 23.4	126.0 ± 3.7	0.95	0.002
Echeme interval (ms)	71.9 ± 2.7	68.7 ± 8.1	0.09	ns	18.3 ± 1.0	14.1 ± 0.5	0.99	< 0.001	49.8 ± 5.9	35.6 ± 8.6	0.88	0.01

which these animals can use solar radiation to modify their body temperature.

Results

Does singing activity and exposure to sunlight influence the temperature of these small cicadas?

Neither the timbal muscle nor the thoracic temperature were significantly affected by singing activity (Fig. 2). In fact we only recorded an increase by about 1°C on the timbal muscle (maximum of 2°C in one of six measurements in five cicadas) and less than 0.5°C inside the thorax during up to 10 min of singing activity (examples in Fig. 2a, b, c).

Solar radiation, however, significantly influenced the animals' body temperature, with an increase of over 10°C relative to that of the air when directly exposed to sunlight (Fig. 2d). Therefore, since external sources of energy were not available in the experimental set-up and the cicadas did not show flight activity, the recorded air temperature in subsequent experiments closely (± 1°C) reflects the actual body temperature of the animal.

Are the temporal elements of the calling songs temperature dependent?

The songs of three animals of each species were successfully recorded at five to seven temperatures within a range of 10–15°C. Most cicadas did not sing below 24°C. At temperatures of 38–40°C few animals sang, the song structure became irregular and the animals started to feed on the plant stem.

The activity of the neuromuscular apparatus involved in syllable production (i.e. timbal cycle) was affected by temperature in all three species (Table 1). Indeed, the syllable rate (SR) increased as the temperature inside the chamber was raised. At the highest temperature, the SR increased on average by 65 (44%), 84 (75%) and 45 (60%) syllables s^{-1} in *T. argentata*, *T. josei* and *Ty. gastrica*, respectively. The duration of the syllable (not shown), which reflects the buckling kinetics of the timbal apparatus, was also found to depend significantly on temperature ($P < 0.005$, for the three species), decreasing with increasing temperature. At higher temperatures, the timbal apparatus recovered faster from the initial deformation than at lower temperatures.

In these cicadas the syllables are grouped into echemes creating a higher level of complexity in the song pattern (cf. Fig. 1). The echeme delivery rate (ER) increased with temperature, indicating that the neuronal song generator determining the echeme onset is temperature dependent. Unlike that found in *T. josei* and *Ty. gastrica*, the ER in *T. argentata* increased regularly between 25.5°C and 33.5°C but decreased at the final temperature (Fig. 3a, 38°C). As a consequence, only in this species the syllable rate and the echeme rate were not correlated (Fig. 3a, b, c). The syllable rate increase found at 33.5°C and 38°C was not associated with an echeme rate increase in the three animals tested. This difference in temperature dependence suggests that in *T. argentata* different neuronal

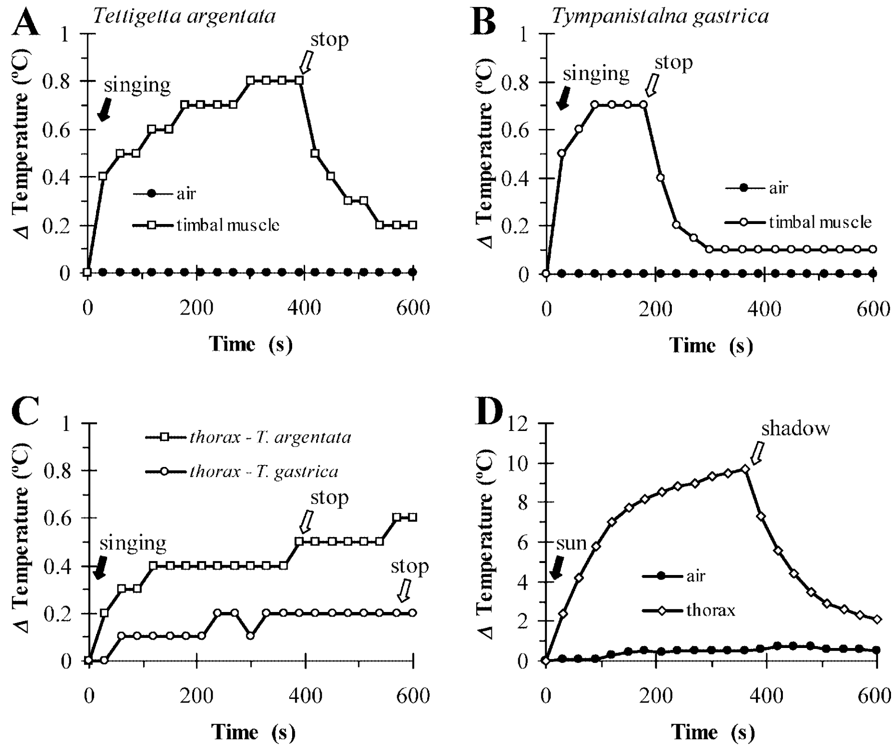

Fig. 2 A–D Examples of the effect of singing activity and solar radiation on body temperature. Temperature variation in the timbal muscle (**a** *T. argentata*; **b** *Tympanistalna gastrica*) and in the thorax (**c**) of *T. argentata* and *Ty. gastrica* during and after singing. **d** Temperature change in the thorax of *T. josei* during and after exposure to direct sunlight

generators are involved in the regulation of both the echeme and the syllable rates.

The phrase rate was characterised by a significant intra-individual variability (ca. 40%). Still, this parameter presented the largest increment with temperature for both *T. argentata* and *T. josei* (37% and 164%, respectively; Table 1). In *Ty. gastrica* the complex phrase structure prevented this analysis.

Other parameters such as the echeme duration and the echeme interval, which can be of behavioural significance for intraspecific communication, were also found to be temperature dependent (Table 2). In *T. argentata*, the echeme interval decreased between 25.5°C and 33.5°C but increased at still higher temperature. In contrast, in *T. josei* and *Ty. gastrica* both parameters decreased regularly as the temperature was raised. The phrase duration and interval (not shown) again presented a large intra-individual variability and were not found to be temperature dependent for both *T. argentata* and *T. josei*.

Discussion

Singing activity, solar radiation and body temperature

We first investigated whether singing activity and solar radiation influenced the temperature of these small cicadas. The action of the timbal muscles involved in song production had a small effect on the body temperature of the singing animal, as measured on the timbal muscle (+2°C) and inside the thorax (+0.5°C). Hence, unlike that described for larger cicada species (Josephson and Young 1979), singing activity cannot contribute significantly to vary the internal temperature of these smaller species. Other behavioural activities, namely flight or exposure to solar radiation, are more likely to influence the body temperature. Indeed, when exposed to direct sunlight the body temperature was seen to increase by up to 10°C in 4 min.

Temperature dependence of calling songs

Since these cicadas can be observed to sing under a wide range of temperatures in the field, we sought to understand (1) the temperature effects on the song temporal elements, and (2) the temperature dependence of the neuronal generator(s) involved in song production.

The syllable rate was found to depend significantly on temperature for the three species. The magnitude of variation within the temperature limits used (44–74% increase, 10–15°C range) reflects a strong temperature dependence of the neuromuscular system generating the most elementary song pattern. Similarly, the timbal cycle rate of *Cystosoma saudersii* was shown to increase as the timbal muscle temperature rises (Josephson and Young 1979). However, the variation presented here clearly exceeds the variation described for *C. saundersii* (ca. 24% increase, 12–17°C range). This difference might be explained because the temperature increase in the body temperature of *C. saundersii* was likely to be less than recorded in the timbal muscle, thus preventing a larger temperature effect on the timbal cycle observed.

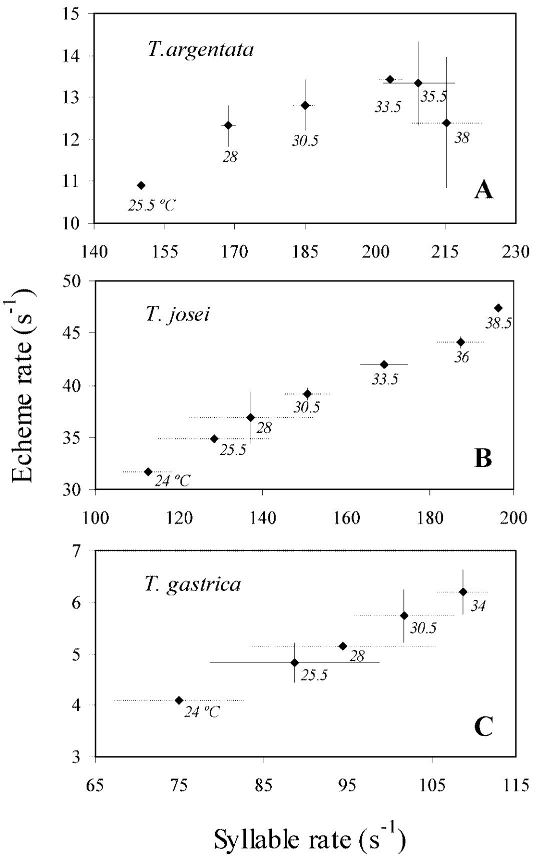

Fig. 3 Echeme and syllable rate variation with increasing temperature. Three males for each species were recorded and the songs analysed. Temperature was varied inside the chamber between 25.5°C and 38.0°C in *T. argentata* (six temperature levels), between 24.0°C and 38.5°C in *T. josei* (seven levels), and between 24°C and 34°C in *Ty. gastrica* (five levels). Error bars represent inter-individual standard deviation

The strong decrease in the syllable duration (SD) for the three species (*T. argentata*: 63%; *T. josei*: 58%; *Ty. gastrica*: 76%, values relative to first temperature, SD measured as the in-out interval, cf. Fig. 1) also agrees with the finding of Josephson and Young (1979) report for the temperature dependence of the pulse interval in *C. saundersii*. Both (syllable duration in the present study and pulse interval in *C. saundersii*) reflect the collapsing and restoring times of the timbal. These are likely to depend on the kinetics of both the timbal and tensor muscles but also on the elastic properties of the timbal membrane, since there are no antagonistic muscles involved. As temperature rises, the muscles contract and relax faster (Josephson and Young 1979) and hence the syllable duration decreases.

As observed with the syllable rate, the echeme rate (ER) increased steadily with temperature in *T. josei* and *Ty. gastrica*. This indicates that the cyclic activity of the neuronal oscillator generating the succession of echemes increases as temperature rises. On the other hand, in *T. argentata* a steady increase of the ER within the first four temperatures was followed by a significant decrease at 38°C (cf. Fig. 3a). This contrasted with the temperature dependence found for the syllable rate in this species, which continuously increased between 25.5°C and 38°C. Thus, these results strongly suggest that the neuronal pattern generators responsible for the echeme and the syllable rates are separate entities with different temperature kinetics, at least in *T. argentata*. In contrast, the temperature dependence of the vibratory communication signals in the spider *Cupiennius salei* pointed to the involvement of one single clock (Shimizu and Barth 1996).

The phrase rate (PR) was only analysed in *T. argentata* and *T. josei*, since in *Ty. gastrica* this element is very complex. This parameter presented the largest intra-individual variability of the three elements of the song analysed, which can reflect a higher susceptibility to the motivation of the animal. Indeed, if one animal is disturbed while singing, the phrase may abruptly stop or become abnormally prolonged, whereas the echemes and the syllables maintain their characteristic structure. Even so, in *T. josei* the PR increased from 3.3 to 8.7 phrases m^{-1}, suggesting that this parameter is also controlled by temperature-dependent structures within the central nervous system. Though to a smaller extent, this was also observed in *T. argentata*. The control of the phrase, much more behaviourally dependent, is likely to rely on a higher order oscillator, probably directly influenced by the brain.

The duration and interval of the phrases were also analysed but were not found to be temperature dependent, possibly due to the large intra-individual variability.

Implications for intraspecific communication

Finally, we investigated whether temperature could influence song parameters with potential behavioural significance and which consequences might arise for communication.

In different insects, communication relies on song analysis and the temporal parameters of a signal have been shown to be involved in intraspecific communication (e.g. Helversen and Helversen 1994; Schildberger 1994). In the same way, *Cicada barbara* males were recently described to be sensitive to fine manipulation of pause lengths between pulses (P.J. Fonseca and M. Allen Revez, unpublished observations). When long pauses were inserted in the conspecific song, males stopped responding to otherwise attractive stimuli. This indicated that manipulation of the temporal elements of the song can affect song discrimination in cicadas.

Here, we have demonstrated that the echeme rate, duration and interval are temperature dependent. Since these song parameters are potentially relevant for intraspecific communication, this temperature dependence might create a constraint for conspecific recognition. This problem can be solved in different ways. First, males could restrict their singing activity to a narrow range of body temperatures, thus minimising the song parameters' variability. This could be the case in *Tibicen chiricahua* and *Tib. duryi*, which regulate the thoracic temperature more precisely during singing than during other activities, keeping the temperature within ± 1°C (Hastings and Toolson 1991). Alternatively, if cicada males sing over a wide range of body temperatures, song recognition would still be possible if (1) the properties of the signal involved in conspecific recognition are not temperature dependent (Josephson and Young 1979; Doolan and Young 1989; Daws et al. 1997), or if recognition relies on the ratio between parameters that vary similarly with temperature (Skovmand and Pedersen 1983); (2) "temperature coupling" occurs (Gerhardt 1978; Doherty 1985; Pires and Hoy 1992); or (3) the female recognition system involves broad band filters that can cope with the variability of the song parameters.

The mechanisms by which conspecific recognition is achieved in the three species studied here is unclear, hence deserving further attention. In *T. argentata* some temporal parameters (e.g. SR, echeme duration) stabilise between 33.5°C and 38°C, thus being a reliable cue for recognition if males regulate their body temperature within these limits. On the other hand, some parameters of *Ty. gastrica* calling song vary similarly with temperature (e.g. SR and bilateral interval), making the ratio of those parameters temperature independent. In *T. josei* the inter-individual variability of the echeme parameters (rate, duration and interval) and the syllable rate is clearly reduced between 30°C and 36°C. Though in this range these parameters are still temperature dependent, a "temperature coupled" system would permit recognition to function accurately. Finally, some temporal parameters of these sympatric species do not overlap entirely (e.g. ER), and hence in principle broad band filters directed towards these parameters would be sufficient to maintain species isolation.

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