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# Separate localization of sound recognizing and sound producing neural mechanisms in a grasshopper

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**Summary.** In the two acridid species *Chorthippus parallelus* and *Ch. montanus*, the sound template by which females recognize male song varies with temperature, as does the song itself. At relatively high temperatures the females respond best to simulated songs with high syllable frequencies, and at lower temperatures songs with lower syllable frequencies are preferred.

The temperature around the supraesophageal and metathoracic ganglia of female grasshoppers was monitored by implanted thermocouples, and either the head or the thorax was warmed selectively while the animal was free to move (within the limits of the wires). Then simulations of the conspecific song varying in syllable frequency corresponding to different song temperatures were presented, and the stridulatory responses of the animals were observed.

The results were as follows. 1. Song recognition (in particular, the position of the peak of the response curve) depended on the temperature of the head. 2. The rate of stridulatory hindleg movement was determined by the temperature of the thoracic ganglia.

This result provides strong evidence against the genetic coupling hypothesis.

## Introduction

In stridulating grasshoppers the frequency of hindleg movement is strongly dependent on the temperature (see, e.g., Walker 1975). The legs move more rapidly as the temperature rises, so that the number of up-and-down movements per unit time, and hence the syllable frequency of the sound signal, can change by more than a factor of four from 17 °C to 40 °C, the range within which grasshoppers sing.

The problem the receiver system encounters in identifying such variable signals is solved in diverse ways (D. and O. von Helversen 1981). One possibility is a temperature-dependent alteration of the receiver mechanism according to the temperature-dependent changes of the song pattern. This in fact has been demonstrated for some crickets (Walker 1957; Doherty 1985) and the acridid grasshoppers *Chorthippus montanus* and *Ch. parallelus* (von Helversen 1979, D. and O. von Helversen 1981).

The females of these two related species alter their innate sound template depending on their own body temperature in order to distinguish the conspecific song from that of the sister species. In an experiment the females were presented with a set of songs recorded at various temperatures; when the female themselves were at low temperatures they responded best to slower syllable frequencies, and when they were at higher temperatures they responded best to high syllable frequencies (D. and O. von Helversen 1981).

Given these two observations – a precise temperature dependence of both the singing movement and the sound template – we can formulate an approach to the underlying mechanism. By local temperature changes of single ganglia of the grasshopper's nervous system we tried to affect the two subsystems, song recognition and song production, in order to answer the following questions. Are the neuronal network that operates as a 'filter' for recognition of the conspecific song and the network that acts as a 'pattern generator' to drive the motoneurons to the singing muscles in the correct temporal sequence, localizable in the nervous system? Are pattern generator and pattern recognition coupled to one another, or are they independently functioning units?

## Materials and methods

*Animals.* The individuals of the two *Chorthippus* species, *Ch. parallelus* (Zetterstedt 1821) (syn. *Ch. longicornis* (Latreille 1804)) and *Ch. montanus* (Charpentier 1825) came from various wild populations in Southern Germany, in particular from Franken. The females were either caught as subadult larvae in the field or raised in the laboratory from collected eggs. They were kept virginal for all the experiments, since virgin females normally remain in a state of active readiness to mate from about one to two weeks after the imaginal molt until their death. In this state they respond to the songs of conspecific males, or to appropriate simulated songs, by making singing movements; only during the phases of egg-laying does this readiness to respond disappear for 12–24 hours.

*Construction of model songs.* A number of males of both species were first allowed to adapt for at least 15 min to various ambient temperatures (in a thermostat with no direct illumination). Then their calling songs were tape-recorded. The males were alone or with a female while singing, so that no rivalry songs were produced (the rivalry songs of both species have a higher syllable frequency than calling songs). The tape recordings were examined to determine the mean duration of the song units (a song unit or syllable is the sound produced by an upward and a stepwise downward movement of the hindlegs). No differences were found between these data and the curve published by D. and O. von Helversen (1981, Fig. 6; compare Fig. 6 of the present paper). The data are very closely approximated by the function $y = a + b/x$ ($y =$ duration of a song unit in ms, $x =$ temperature in °C). The best approximation was achieved by the values $a = -129$ and $b = 12435$ for *Ch. montanus* and $y' = \frac{1}{2}\, y$ for *Ch. parallelus*.

A computer was used to generate the simulated songs with these values, by amplitude-modulating approximately white noise with an envelope corresponding to that of a simplified natural song. Each simulated song comprised a series of about 15 identical units, corresponding to the syllables of the song. The simulations within a temperature series differed only in the duration of the pattern units; all the other pattern parameters were kept the same relative to unit duration, and the intensity as well as the modulated noise spectrum were kept constant. For both species a series of simulated songs was generated such that the production temperature would have ranged from 12 °C to 51 °C in steps of 3 °C. (We were actually able to record songs only between 16 °C and 40 °C. Hence the extra simulated songs, for 12 °C, 15 °C, 42 °C, 45 °C, 48 °C and 51 °C, presumably are sound patterns that do not occur in nature; however, they were of use in estimating the maximal response of the female at low or high temperatures.)

The simulated songs were stored on magnetic tape; in the experiment they were played back by a tape recorder (Uher-Report) by way of a loudspeaker (frequency range 5–20 kHz). Sets of four identical stimuli were presented, with the different types of stimuli following one another in irregular sequence. The time interval between consecutive presentations of stimulus sets was normally ca. 30 to 60 s and was adjusted according to the animals' behavior. The outcome of each test was recorded by direct observation of whether a response occurred or not.

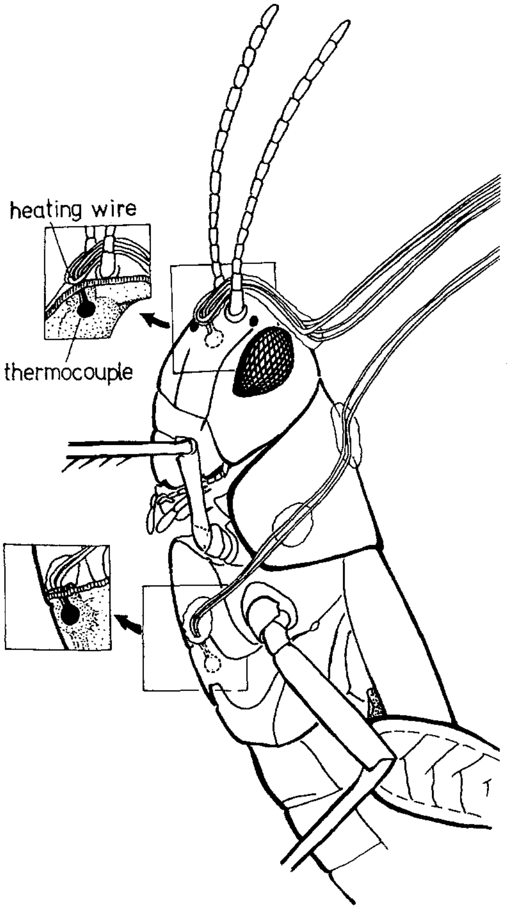

**Fig. 1.** Positions of the thermocouples. One NiCr-Ni thermocouple is inserted through a cuticular window above the median ocellus, to lie near the supraesophageal ganglion, and the second through a cuticular window in the mesosternum near the metathoracic ganglion. With the heating-wire placement shown, the top of the head is heated

*Temperature measurement and selective warming.* The devices for controlling and monitoring local body temperature were prepared and attached to the female grasshoppers as follows (Fig. 1). For temperature measurement, a NiCr and a Ni wire (each 50 μm in diameter) were welded together at a point to form a bead about 0.2 mm in diameter (a chromel/alumel thermocouple). The wires leading from the thermocouple were carefully insulated (with thinned nail polish). Holes were made in the cuticle of the animal's head and thorax, and two thermocouples were inserted, under microscopic control, so that one was in contact with the supraesophageal ganglion and the other with the metathoracic ganglion. The windows were sealed around the wires immediately with wax-colophonium, and droplets of the same material were used to fix the wires to the surface of the cuticle of head and thorax without exerting tension at the insertion site. The final point of attachment, so firm that the wires could not be pulled loose, was at the back of the head and on the pronotum, respectively. The positions of the thermocouples were checked after the death of the grasshopper.

A fine heating wire (Cu, 40 μm diameter) was also mounted on the grasshopper. In most experiments it was placed on the outside of the head, between the antennae (see Fig. 1), and fixed in position with wax-colophonium. As a control, in a smaller number of experiments the tip of the heating wire was passed through the window in the cuticle of the metathoracic

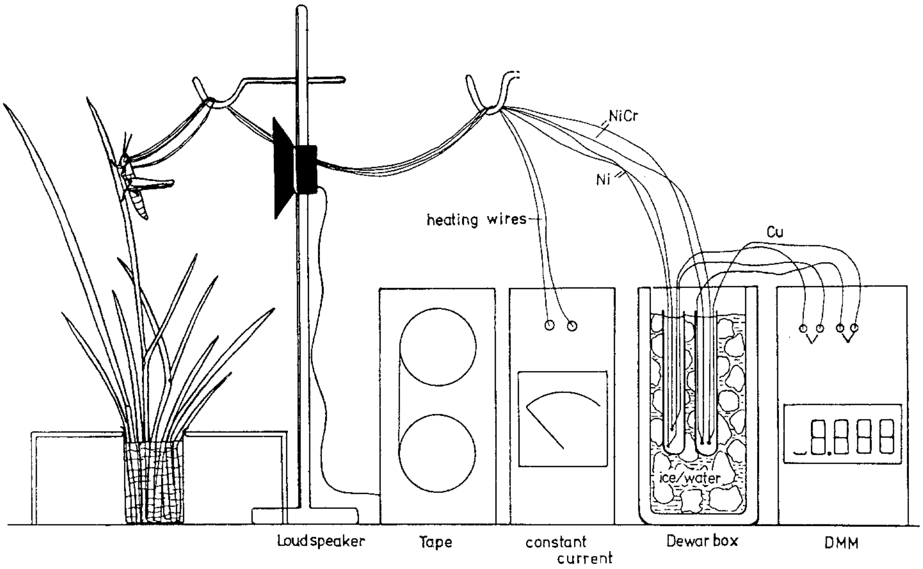

**Fig. 2.** Experimental setup. A constant-current source is used to warm the heating wires until the adjacent thermocouple has reached the desired temperature. The temperature of both thermocouples can be read off from a sensitive digital multimeter (*DMM*). For auditory stimulation, simulated songs varying in syllable frequency are played back by the tape deck

sternum together with the thermocouple, to warm the metathoracic ganglion selectively.

After this operation not all of the animals recovered sufficiently to exhibit the complete behavioral repertoire. Only undisturbed animals in a state of readiness to mate will respond to male song. For these experiments it was most important that the behavior be entirely unimpaired; therefore in some animals only one thermocouple was implanted, next to the heating wire. Because the temperature gradient that develops during heating depends not only on the amount of heating but also on the ambient temperature, the illumination, the substrate on which the animal is sitting, and so on, in these cases a control animal was positioned next to the one to be tested; the control animal had been prepared in the same way except that it had two implanted thermocouples, so that when it was warmed in just the same way as the test animal both temperature values could be monitored.

Although the grasshoppers were attached to the fine wires, about 40 cm long, they were otherwise free to move in their cage; in particular, they could find blades of grass to eat and deposit their eggs in the containers of sand provided. Some animals survived the operation for as long as five weeks.

During an experiment (Fig. 2) the first step was to pass current from a stabilized current source through the heating wire (usually 300–400 mA, with 2.5–3.5 V). The current was cautiously increased by hand until the desired temperature (usually not above 34 °C, and never over 38 °C) was reached at the ganglion next to the heater. For temperature measurement the thermocouple wires were connected to thicker NiCr and Ni wires by plugs of the same material. Each of the thicker wires was soldered to a flexible copper cable leading to a sensitive digital voltmeter. The soldered junctions were kept at 0 °C by immersion in a Dewar flask filled with a mixture of ice and water. The chromel/alumel thermocouples produced from 40 to 41 µV/°C; the voltages read off from the voltmeter were converted to temperatures by means of a calibration table.

*Recording of female stridulation.* Because the females of *Ch. parallelus* and *montanus* have very short wings, in general they stridulate silently. (However, some female *montanus* have wings long enough to produce a distinctly audible song; and occasionally in *Ch. parallelus*, especially in our laboratory colony, macropterous females appeared). The song of silently stridulating females was recorded by detecting the vibration induced by stridulation in the blade of grass on which the female was sitting, by means of an accelerometer (Brüel & Kjaer). The accelerometer output provides as clear a record of the song pattern frequency as do tape recordings.

## Results

### *Influence of selective warming on the recognition of song*

The first step was to determine how the responding animal's syllable-frequency preference depends on its overall temperature, without selective warming – that is, with the entire body of the grasshopper brought to a specific temperature by raising the temperature of the ambient air. The stimuli, each of which corresponded to a particular singing temperature, were presented in varying sequence, and the frequency of response to each was recorded. It turned out that female *Ch. montanus* quite clearly respond best to simulated songs that would be produced at the animal's own temperature (circular symbols in Fig. 5). By contrast, female *Ch. parallelus* at relatively high temperatures tended to prefer songs more rapid than those corresponding to their own temperature; when the animals were at 30–32 °C, they responded best to pattern frequencies that would have corresponded to the stridulation frequencies of a male at 36–40 °C (Fig. 3). The reason for this difference is not clear; perhaps a preference for a supernormal key stimulus in this case facilitates avoidance of the sister species *Ch. montanus.*

Then the curves obtained with overall warming were compared with the responses of animals bear-

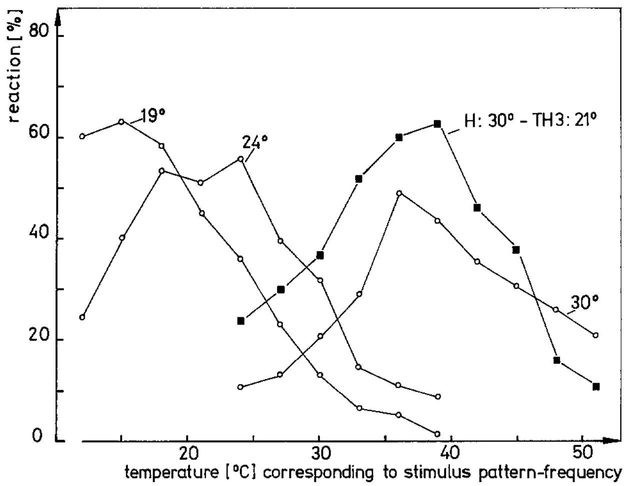

**Fig. 3.** Response curves of a female *Ch. parallelus* with and without selective warming of the CNS. Simulated songs differing only in syllable frequency were played back to the animal, to represent a male singing at different temperatures. The reaction is given as the percentage of stimuli that elicited a response. –o–: head and thorax at the same temperature; –■–: selective warming of the head to 30 °C, with thorax at approximately the ambient temperature (21 °C)

ing thermocouples and warmed locally, so that the temperatures of thorax and head differed. The animals behaved consistently in such a way as to indicate that their probability of responding is determined by the temperature of the head.

Three of the response curves shown in Fig. 3 represent the behavior of an individual *Ch. parallelus* in different ambient temperatures (19 °C, 24 °C and 30 °C; *n* per data point 104–132, 220–252, and 76–108), without selective warming. (The vertical position of these response curves depends on the animal's readiness to respond, which varies somewhat from day to day, but the position of the peak on the abscissa does not; cf., e.g., von Helversen 1972). Subsequently the head of the animal was warmed selectively until the thermocouple at the supraesophageal ganglion indicated a temperature of 30 °C. In this experiment the thoracic temperature reached 21 °C. The response curve of the animal under these conditions (*n* per data point 184–224) is also plotted in Fig. 3. It corresponds approximately to the curve obtained with the entire animal warmed to 30 °C. If the temperature of the thorax had determined the behavior, the response curve would have been expected to be shifted far to the left.

To enable simultaneous comparison of all the data on *Ch. parallelus*, the results were diagrammed as in Fig. 4. For each of the 10 individual experiments with selective warming of head or thorax, the peak of the response curve ('observed preference') was noted and set equal to zero. Then, on the basis of the responses of animals with head and thorax at the same temperature (without selective warming), two 'expected preferences' were determined – that is, the simulated song the animal would have been expected to prefer if its whole body had been at the temperature of the head or that of the thorax. The differences between the observed preference and the two expected preferences are shown in Fig. 4A for each of the 10 experiments. The preference expected on the basis

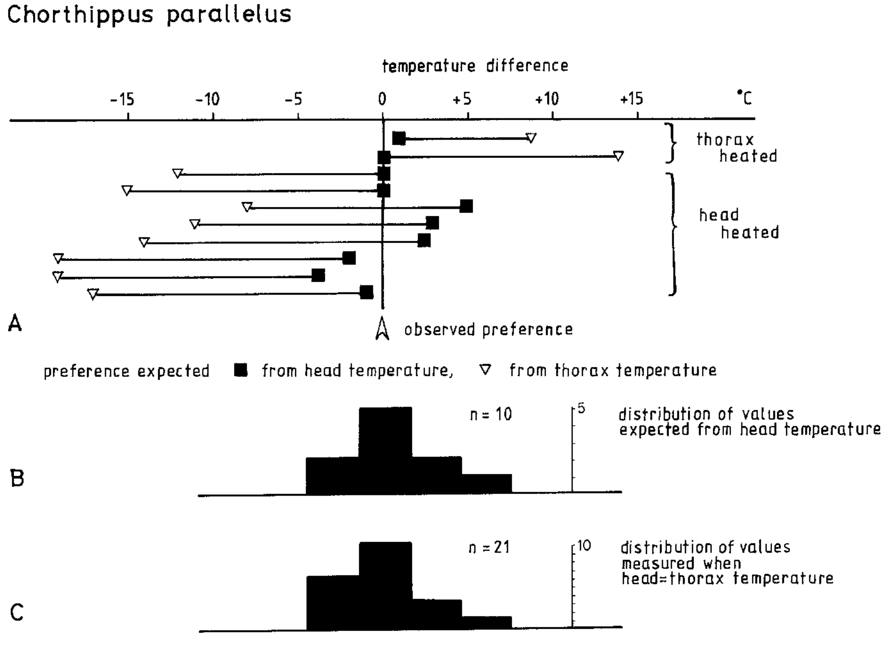

**Fig. 4A–C.** Position of the response peak observed during selective warming of the head or thorax of *Ch. parallelus* (10 experiments) as compared with the values expected if head and thorax were at the same temperature (see text). (**A**) The song-production temperature corresponding to the pattern frequency eliciting maximal response is set at zero on the scale (observed preference), and the expected values are plotted as the difference from this temperature, as follows: ■ expected value based on head temperature; ▽ expected value based on thoracic temperature. The distribution of differences between the values based on head temperature and the observed value (**B**) does not differ significantly from the scatter of the data without selective warming (**C**)

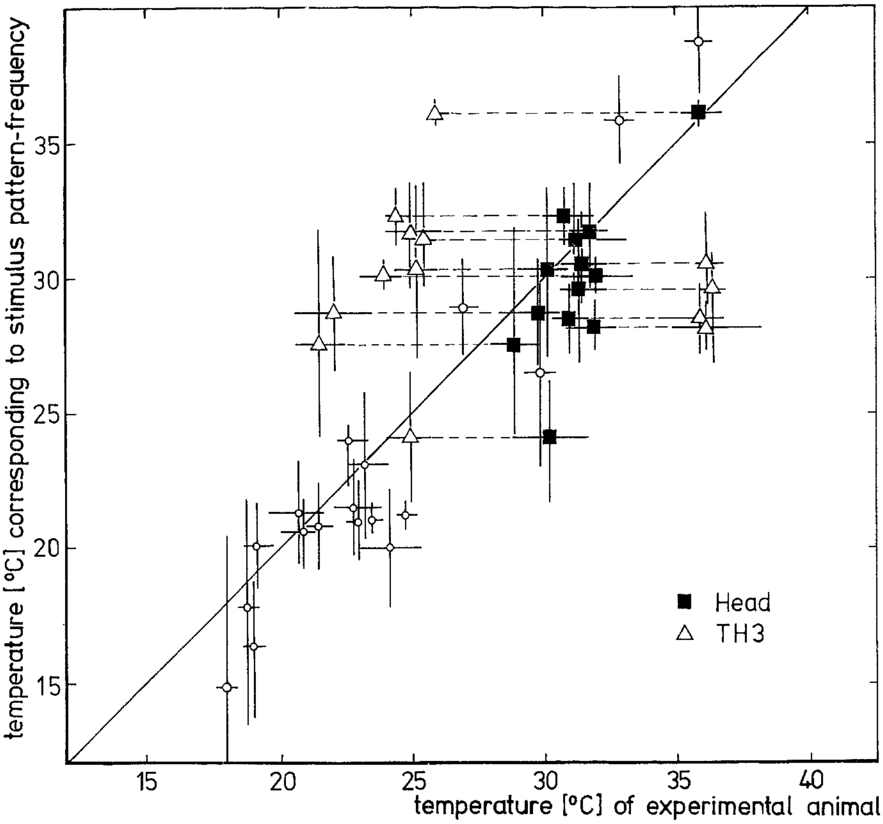

**Fig. 5.** Response peaks with and without selective warming of the CNS of female *Ch. montanus*. The abscissa gives the animal's temperature, either that of the whole body or those of head and thorax separately. Horizontal bars show the maximal range within which the temperature varied during the prolonged (several days) experiments. The ordinate indicates the simulated song that elicited the best response in each case, in terms of the temperature at which a male *Ch. montanus* would have produced this pattern frequency. Vertical bars give the width of the response curve at 90% of peak height. Data that belong together are joined by thin dashed lines. ■ temperature of the head, △ temperature of the metathorax, ○ experiments without selective warming

of the head temperature is always closer to the observed preference. The distribution of the differences between this expected value and the observed value (Fig. 4B) is not statistically different from the distribution of differences from the mean (i.e., the expected value) in the experiments without selective warming (Fig. 4C).

The experiments with female *Ch. montanus* (13 experiments with selective warming and 17 control experiments with head and thorax at the same temperature) gave the same result. The graph in Fig. 5 again clearly documents the decisive influence of head temperature on response behavior. In one experiment only, a female appeared to prefer the simulated sound corresponding to its thoracic temperature, but on the next day the same animal responded in the usual manner.

### *Influence of selective warming on the stridulation frequency*

Next we investigated the effect of overall and local temperature on the females' own stridulation frequency. In experiments without selective warming, the temperature dependence of the stridulation frequency of females of both species was found to agree entirely with that of the males, so that the mean curve for females lies within the scatter of that for males (shown in Fig. 6). The heads of a few females of both species were then selectively

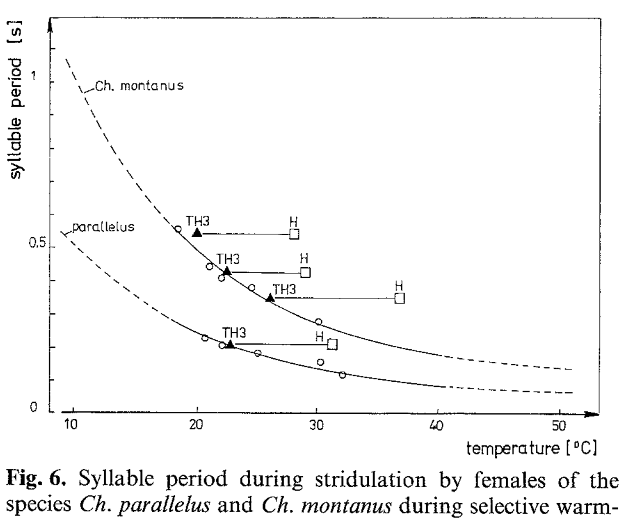

**Fig. 6.** Syllable period during stridulation by females of the species *Ch. parallelus* and *Ch. montanus* during selective warming of the CNS. The continuous curve is the mean curve for the pattern wavelengths produced by males of the two species at various temperatures (18–40 °C). Females sing with the same movement rhythm as males at these temperatures. (○: experiments without selective warming.) When the CNS is warmed selectively the syllable frequency is determined by the temperature of the thorax (▲) and not by that of the head (□)

warmed, and the result was just as clear as that in the preceding experiments, but reversed. That is, the temperature of the metathoracic ganglion was found to be the critical determinant of stridulation frequency.

## Discussion

The results can be summarized by the following simple statement: the recognition of songs by females of both these acridid species depends on the temperature of the head, whereas the stridulation velocity depends on the temperature of the thorax. As to functional separation of the different ganglia in each of these regions, the present data are not conclusive. That is, the thermocouples were next to the supraesophageal and the metathoracic ganglion; the temperatures of the ganglia adjacent to these, the subesophageal and mesothoracic ganglion, respectively, were not monitored but are unlikely to have differed greatly from those of the supraesophageal and metathoracic ganglia.

The most likely explanation of the different effects of head and thorax temperatures is that essential parts of the filter system for recognition of the conspecific song are located in the head ganglia (presumably the supraesophageal ganglion) and essential parts of the pattern generator for song production are in the thoracic ganglia (presumably the metathoracic ganglion). This explanation is consistent with all known electrophysiological findings, especially the absence of species-specific pattern recognition in the auditory system at the level of the thoracic ganglia (Kalmring, pers. comm.) and the presence of more complex auditory neurons in the supraesophageal ganglion of crickets (Boyan 1980; Schildberger 1984). Furthermore, ablation and transection experiments (see Ronacher et al. 1986) have shown that execution of the behavior 'response singing' requires at least one information loop involving the supraesophageal ganglion, whereas, on the other side, the thoracic ganglia are able to produce the song pattern even without connection to the supraesophageal ganglion (Kutsch and Otto 1972; Hedwig 1986).

However, an alternative explanation is not ruled out by the present experiments. That is, it is conceivable that the selectively warmed part of the body contains not elements of the recognition and pattern-generating systems themselves, but only thermosensors that send signals to other, unknown parts of the CNS. This interpretation is less likely for several reasons.

In the CNS of a poikilothermic animal the physiology of each individual nerve cell is temperature-dependent with different parameters showing different temperature effects (e.g. see Abrams and Pearson 1982). This situation presents evolution with the enormous task of developing compensatory interactions so that the function of individual neuronal networks remains as constant as possible over a wide range of temperatures – a problem that is not even understood in its basic aspects, and is often overlooked. Fischer and Florey (1981) demonstrated such a compensation of counteracting effects for the crayfish muscle. It is relatively easy to imagine that as such compensatory interactions evolved, networks were created that have different outputs at different temperatures. The provision of special temperature receptors would be an extra, unnecessary elaboration, especially since the selective warming of different parts of an insect's CNS is not likely to have been much of a factor in natural selection.

An additional interesting implication of these data is that, since *within an individual* the recognition and production of song can be completely uncoupled, the song generator (or part of it) cannot be used as a template for song recognition. This is of special interest, as in *Ch. parallelus* and *Ch. montanus* not only an analogous temperature dependence of sender and receiver exists, but also hybrid females were found to prefer hybrid songs, which in their syllable frequences are intermediate between the parental songs (von Helversen, unpubl.). These are the two main arguments brought forward in favour of the 'genetic coupling hypothesis' (Doherty and Hoy 1985). But our results show that even in these species the mutual adaptation of emitter and receiver must be based on their coevolution, and not on some form of physiological coupling (cf. von Helversen 1979).

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