Table 1. Enantiomeric resolution of racemates by TLC (development distance 13 cm, saturated chamber)

Racemate	Rf value (configuration)		Eluent[a]
Valine	0.54 (D)	0.62 (L)	A
Methionine	0.54 (D)	0.59 (L)	A
allo-Isoleucine	0.51 (D)	0.61 (L)	A
Norleucine	0.53 (D)	0.62 (L)	A
2-Aminobutyric acid	0.48	0.52	A
O-Benzylserine	0.54 (D)	0.65 (L)	A
3-Chloralanine	0.57	0.64	A
S-(2-Chlorobenzyl)-cysteine	0.45	0.58	A
S-(3-Thiabutyl)-cysteine	0.53	0.64	A
S-(2-Thiapropyl)-cysteine	0.53	0.64	A
cis-4-Hydroxyproline	0.41 (L)	0.59 (D)	A
Phenylglycine	0.57	0.67	A
3-Cyclopentylalanine	0.46	0.56	A
Homophenylalanine	0.49 (D)	0.58 (L)	A
4-Methoxyphenylalanine	0.52	0.64	A
4-Aminophenylalanine	0.33	0.47	A
4-Bromophenylalanine	0.44	0.58	A
4-Chlorophenylalanine	0.46	0.59	A
2-Fluorophenylalanine	0.55	0.61	A
4-Jodophenylalanine	0.45 (D)	0.61 (L)	A
4-Nitrophenylalanine	0.52	0.61	A
O-Benzyltyrosine	0.48 (D)	0.64 (L)	A
3-Fluorotyrosine	0.64	0.71	A
4-Methyltryptophan	0.50	0.58	A
5-Methyltryptophan	0.52	0.63	A
6-Methyltryptophan	0.52	0.64	A
7-Methyltryptophan	0.51	0.64	A
5-Bromotryptophan	0.46	0.58	A
5-Methoxytryptophan	0.55	0.66	A
2-(1-Methylcyclopropyl)-glycin	0.49	0.57	A
N-Methylphenylalanine	0.50 (D)	0.61 (L)	A
N-Formyl-tert.-leucine	0.48 (+)	0.61 (−)	A
3-Amino-3,5,5-trimethyl-butyrolactone·HCl	0.50	0.59	A
N-Glycylphenylalanine	0.51 (L)	0.57 (D)	B

[a] A: methanol/water/acetonitrile = 50/50/200 (vvv);
B: methanol/water/acetonitrile = 50/50/30 (vvv)

gand exchange [5]. The procedure for the preparation of the chiral plate was described in [6].

Using the plates thus prepared, we were able to perform TLC enantiomeric resolution for many racemates (Table 1): 2 µl of each of the racemic compounds were applied as a 1% solution to the TLC plates. After elution (30–90 min) and drying, the spots were visualized using 0.1% ninhydrin reagent. Respective antipodes could be determined at trace levels. In some cases $\geqq 0.25\%$ of the minor enantiomer could be detected.

The classes of resolved racemates include natural and non-natural amino acids, N-methylated amino acids, N-formyl amino acids and other derivatives of amino acids. Even dipeptides and a lactone derivative were resolved into enantiomers. This clearly indicates that a wide variety of enantiomeres can be resolved. To the best or our knowledge our method is unique in this aspect. Furthermore the analysis can be completed in less than 2 h.

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The Clockwork Cricket

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Singing field crickets produce very loud pure tones by rubbing their wings against each other. The frequency of the radiated sound is remarkably constant and almost unaffected by temperature. Our question is how such precise stability is achieved, when it is known that temperature changes the properties of nerves and muscles tremendously. This can be accounted for by the theory which describes the sound-producing system of the cricket as an analog of the clock escapement in a grandfather clock. New high-resolution measurements of the wing movements during singing provide fresh evidence for this theory: (1) the wing movements show oscillations corresponding to the individual teeth on the file, (2) the deletion of teeth produces high-speed slips until the next intact tooth is reached, and (3) loading the harp resonators in the wings reduces the closing speed by the same amount as it reduces the sound frequency. Thus the precision of the cricket's sound generator stems from the accurate regulation of the wing motion by a clockwork mechanism.

The loud sounds produced during the stridulation of crickets serve to attract females [1, 2]. In order to reach those far away, the sound must be as loud as practically possible [3–5]. One particularly efficient way to convert muscle work into sound energy is to drive a mechanical resonator with a high *Q*-factor and this is the method adopted by the male field cricket, *Gryllus campestris* [4]. Vibrations are set up in the wing during each closing stroke by scraping a plectrum in one wing across the file in the other. Part of the wing is made of a thin stiff sheet of cuticle – the harp – and this has a sharp resonance at 4.5 kHz, and this is used to radiate the sound energy efficiently [6]. Until now it has been unclear how the

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cricket manages to ensure that the energy is supplied to the wing so that the harp will vibrate at its resonant frequency, which is indeed the frequency of the calling song. The muscle force does not appear to be controlled to generate a smooth, constant impact rate of the teeth against the plectrum, which is a prerequisite for feeding energy into the resonator at a constant phase in each cycle. For example, the muscle force increases with each syllable in any chirp (Fig. 1a, [7, 8]), producing an increase in sound amplitude but no change in frequency. Again, the male sings at temperatures from 15 to 30 °C and this reduces the time course of muscle and neuronal events by up to 50% [8–10], but the sound frequency remains practically constant.

To explain these phenomena, we postulate that the stability of the closing motion is not caused by friction in the general sense, nor by sensory feedback controlling muscle excitation, but rather the cricket uses a mechanical oscillator and an escapement mechanism to enforce a constant impact rate of the plectrum across the teeth.

Such a system has a stricking analogy to a pendulum clock. A grandfather clock [11, 12] is composed of a source of mechanical energy (the weight), a mechanical oscillator (pendulum) and an escapement, whose role is to deliver small amounts of energy to the oscillator in the correct phase to compensate for the energy losses of the oscillator. Normally the escapement consists of a toothed scape-wheel (driven by the weight) and an anchor (coupled to the pendulum) whose pallets are capable of blocking or releasing just one tooth at a time. Just before its release, the scape-wheel tooth pushes on the pallet. By this means, little packages of energy are released, under the control of the pendulum movement, to support and maintain the pendulum oscillations.

The elements of the sound-generating system of the field cricket which correspond to those of the pendulum clock can be identified: the tension generated in the muscles following neuronal activation corresponds to the weight, the harp on the wing is the oscillator corresponding to the pendulum, while the plectrum and file provide the escapement mechanism (cf. [13]). The vibrations of the file are coupled to those of the harp [6] and so, as the harp moves upwards, the plectrum will come free from the file and be able to accelerate inwards to the next tooth, where the impact will transfer energy to the harp in the correct phase.

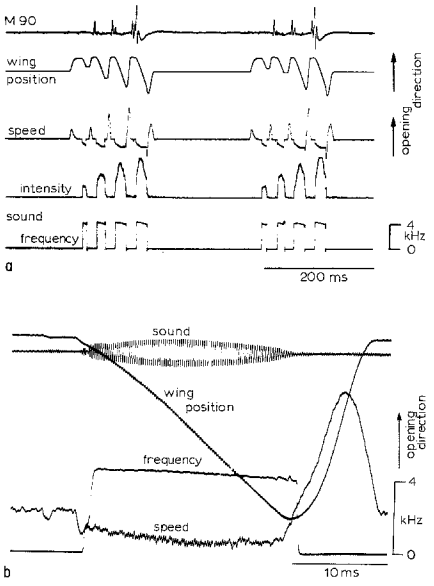

Fig. 1. An intact male field cricket *Gryllus campestris* producing calling song. The radiated sound was recorded with a microphone (Brüel & Kjaer 4138), 120 mm from the cricket and high-pass filtered (−3 dB point 2 kHz). The sound envelope was produced using a rectifier and low-pass filter. The sound frequency was measured using an instantaneous frequency meter. Wing position was measured with miniature-angle detectors and analysed using analogue computing [14–16]. The signal-to-noise ratio in the angle detector system was enhanced by using a stabilized RF oscillator (Tektronix FG 501) and improved power amplifiers for the external field coils. The bandwidth of the detector system was increased to 8 kHz. The speed signal is derived from the position trace by analogue differentiation and is low-pass filtered (−3 dB point 800 Hz). Electromyograms were made using 40 µm steel wire [7, 8]. a) Overview of two chirps, each of four syllables. Sound is generated in each closing stroke [8, 14] which is, at least in part, the result of the activity in muscle 90, the remotor. The muscle activity increases with each syllable in both chirps and this results in a longer closing stroke and higher sound intensity. Note, however, that the sound frequency remains constant. b) Detailed view of one closing stroke followed by an opening stroke. In the closing stroke, the wing position signal shows 'ripples' throughout sound production. The ripples correspond one-to-one with the sound oscillations. No ripples are seen in the opening stroke

If this theory holds it should lead to certain consequences which could be observed in the singing movements: during the closing stroke, because the plectrum jumps from tooth to tooth, stopping shortly at each one, the closing motion of the wings should not be smooth but should show ripples, corresponding to the individual teeth. This is indeed observed (Fig. 1b, 2a, 3). The closing motion is modulated by little peaks that are correlated one-for-one with the harp oscillations as monitored in the radiated sound wave. In the opening stroke, because no sound is radiated, the motion should be smooth, which is in fact the case (Fig. 1b).

In a grandfather clock, if one were to take out one or two teeth from the scape-wheel, the pallet cannot hold the wheel when the gap appears and so the scape-wheel slips forwards, being accelerated by the excess energy of the weight, moving on until the next intact tooth hits the pallet. Then the regulated movement resumes. Likewise when several teeth in the crickets' file are removed, we should expect an immediate increase in the closing speed, until the plectrum hits the next intact tooth. The results of this experiment are shown in Fig. 2. The scanning electron micrograph (Fig. 2b, kindly taken by K.-H. Schäffner) shows the file, from which teeth have been removed in two sections (Fig. 2c). As the recording (Fig. 2a) shows clearly, the closing speed of the plectrum across the file increases drastically as soon as it meets the first gap and runs free. The plectrum then catches back on and continues down the file until it reaches the second gap. There another speed increase is seen, and then the plectrum runs free, failing to catch any more teeth, until the end of the closing stroke. Between the gaps there are 48 teeth and in the mechanogram there are 45 or 46 oscillations (depending on the syllable) between the two speed increases. The electron micrograph shows 5 teeth missing in the first gap and the mechanogram shows a jump corresponding to 7 oscillations, so that each oscillation corresponds exactly with the jump across one tooth. Thus the results of this experiment conform to our hypothesis.

A second experimental manipulation possible with a clock is to alter the resonant frequency of the pendulum – most

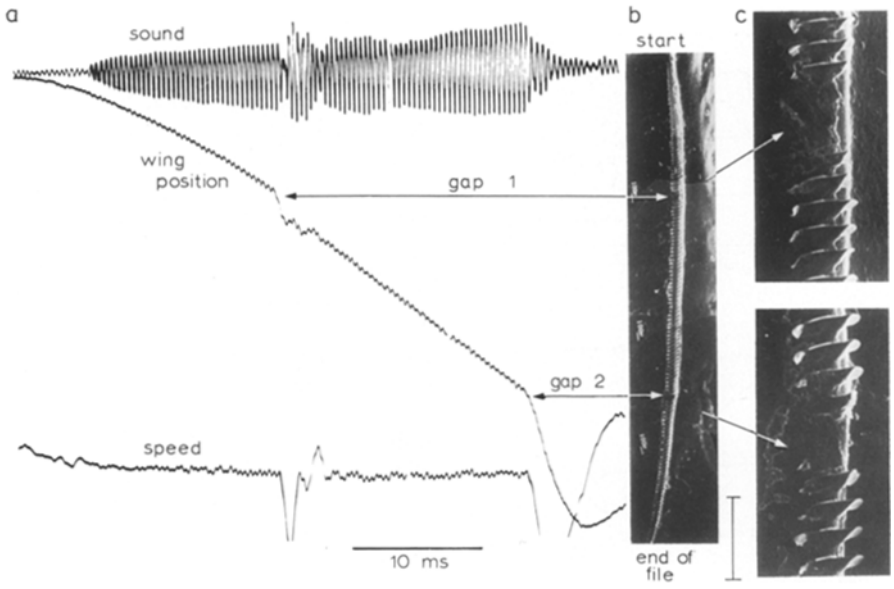

Fig. 2. a) Record from the same male as in Fig. 1 after removal of teeth at two points in the file with a dentist's drill. A single closing stroke is shown. Note the immediate increase in speed when either gap in the file is reached. After the first gap, the plectrum caught on the file again (with a certain amount of difficulty), and sound production resumed. However, after the second gap the plectrum was not recaptured and the sound died away. b) Scanning electron micrograph (courtesy of K.-H. Schäffner) of the operated file of the male whose wing movements are shown in a). Vertical bar corresponds to 1 mm. Note the correspondence of teeth and ripples in the position trace and of the gaps and increases in speed. c) Parts of the file at eightfold magnification

easily achieved by changing its length. Now the harp corresponds to the pendulum and Nocke [6] showed that the resonant frequency (f_r) of the harp is reduced by adding an extra mass (m_D) to the membrane in accordance with the equation

$$f_r = \frac{1}{2\pi} \frac{K}{m_H + m_D}$$

where K and m_H are the stiffness and mass of the harp, respectively. As with the clock analogy, reduction in the resonant frequency should result in slower, but still controlled motion. The wing movement and radiated sound measured in the closing stroke before and after loading both harps with wax are shown in Fig. 3. The first observation from this figure is that the wax does indeed reduce the frequency of the radiated sound (to 89% of its former value). Secondly, the speed of the plectrum across the file is reduced. This figure is arranged so that, at the beginning of the record, the two position traces are just touching and they gradually diverge, showing that the wing with the reduced resonant frequency travels slower (at 91% of its original speed).

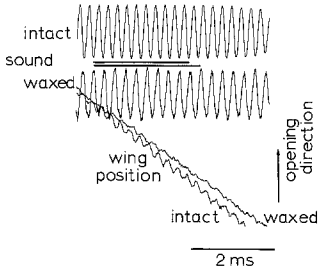

Fig. 3. Part of two closing strokes, one before and one after loading both harps of a male with wax. Both wing position traces represent movement over the same part of the file. The radiated sound from both closing strokes is also shown (with the gain of the 'waxed' trace increased by a factor of 5). The 'waxed' sound frequency is reduced: bars indicate the time for 10 cycles. When the harps are waxed, the speed of the closing stroke is reduced by the same proportion as the sound frequency

In each of the 3 crickets studied, a reduced sound frequency was associated with a proportional reduction in closing speed. Thus lengthening the duration of the cycle time of the harp vibrations lengthens the time between jumps of the plectrum from tooth to tooth.

If the pendulum is removed completely, then the clock will run much faster (at least with some escapements), and its speed will depend mainly on the driving force of the weight [12]. Likewise when both harps were removed, we observed that the closing speed was variable, reaching values up to 2.5 times faster than in the intact male.

Our experiments are in harmony with the hypothesis that the sound-producing motions of the wings in a singing field cricket are entirely regulated by the harp, a mechanical oscillator in conjunction with an escapement mechanism. As predicted by the theory, removing the teeth accelerates the wings, showing that muscle forces are not limiting the closing speed. This is also evident when the harps are totally removed. Again, lowering the harp resonance frequency was sufficient to induce a proportional reduction in closing speed; all other parameters remaining constant. The good temperature stability of a mechanical resonator explains why sound frequency is almost unaffected by temperature, although the muscle forces powering the sound generator are strongly temperature-dependent.

This mechanism, usual amongst crickets, may be compared with that of some bush crickets [13], where there is also a file and plectrum method of stridulation, but the sound recordings show that the vibration induced by any one impact decays before the next occurs. In these cases a continuous tone is not generated and the tooth impact rate does not correspond with the sound frequency, so no phase locking is necessary.

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Sexual Dimorphism of the Terminal Abdominal Ganglion of the Cricket

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Dorsal unpaired median (DUM) neurons, with their somata near the dorsal midline of each segmental ganglion, and bifurcating axons projecting symmetrically into left and right nerve roots of the ganglion, have been reported in several insect orders [1]. This paper briefly shows that in the adult cricket (*Gryllus bimaculatus*) the sexual dimorphism of the terminal abdominal ganglion (TAG) distinctly proves sex-specific differences not only in its external morphology, but also in the soma number, soma position, and projection of DUM neurons.

Comparison of the gross anatomy of the central nervous systems in both sexes shows considerable sex-specific differences only between their TAGs, each of which has eight pairs of nerve roots (R1–R8) and a median nerve. As shown in Fig. 1, the length of female TAG is significantly longer than that of male TAG, and the diameters of R2, R4 and R6 of female TAG are considerably larger than those of male TAG. In females, moreover, the nerve branches medially leaving the left and right R7s fuse in the midline to form a loop surrounding the posterior end of TAG without branching further. In males, they form another type of loop from which many axons emerge at two places centered around the midline towards the accessory glands (Fig. 2).

DUM neurons in the cricket are stained with neutral red [2], a dye which selectively stains monoamine-containing neurons [3]. Neutral red staining TAGs clearly demonstrate a sexually dimorphic character of DUM neurons in respect to the soma number and soma position, although they vary in different preparations: the total number of somata stained with 0.1% neutral red for 8–10 h at 4 °C is 20–30 in female TAG and 50–70 in male TAG. These somata together make up four clusters along the midline in both sexes. However, in females the gross organization of each cluster scarcely differs with different clusters, while in males it varies according to the position of the cluster within TAG: the closer the cluster is to the posterior end of TAG, the larger the soma number is in the cluster.

Of all the branches of eight nerve roots of TAG nickel chloride backfills reveal the following morphological characteristics of DUM neurons. Firstly DUM neurons of both sexes are stained with the dye only when it is backfilled selectively into the specific branch of R2, R4, R6, R7 or R8. Each of them shows the morphological characteristic that a single primary neurite arises from the soma located in the dorsal surface of TAG and runs ventrally

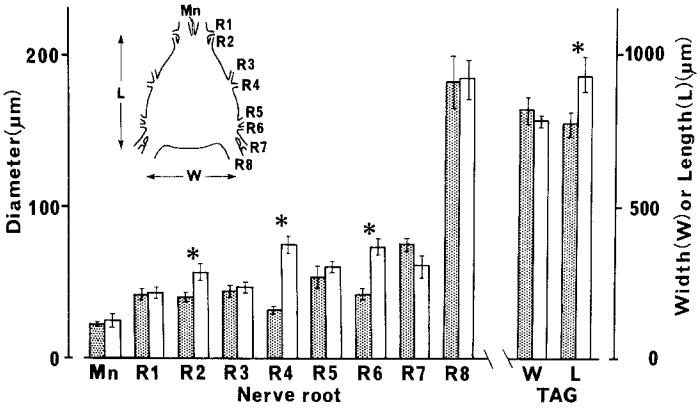

Fig. 1. Comparison of the diameters of nerve roots with the size of TAG between male and female. Each column with the standard deviation was obtained from ten animals: the white and the dotted columns represent the data from females and males, respectively. The diameter was measured just at the position where each nerve root starts to extend from TAG. It should be noted that the diameters of R2, R4 and R6 and the length of TAG marked with asterisks differ significantly between the two sexes. A probability level of 0.01 or less associated with the *t*-test was accepted as representing a significant difference. *Mn* median nerve, *R1–R8* first–eighth nerve root, *W* width, *L* length