

THE TYMBAL MECHANISM AND SONG PATTERNS OF THE BLADDER CICADA, *CYSTOSOMA SAUNDERSII*

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SUMMARY

1. In *Cystosoma saundersii* sound is generated by collapse of a pair of tymbals and radiated by a large, resonant, air-filled abdomen. Each tymbal comprises a flexible, biconvex membrane bearing seven long ribs. Tymbal collapse is caused by contraction of a large tymbal muscle, which acts on the tymbal plate. Each tymbal muscle is innervated by one motor neurone.

2. A single collapse of a tymbal produces two distinct pulses of sound, one when rib 1 buckles and one when ribs 2-4 buckle. A quieter sound is produced when the ribs click outwards.

3. A slowly contracting tensor muscle increases the convexity and stiffness of the tymbal, resulting in a reduction in the delay between the first and second sound pulse and in louder pulses.

4. *Protest songs* contain features of other songs. There is a delay between the spike in one tymbal motor neurone and its partner, and hence between sound produced by one tymbal and the other, of one-quarter of the interval between spikes in one motor neurone alone.

5. *Calling songs* are produced by males at dusk. Sound pulses have a smooth envelope and are very loud as a result of contraction of the tensor muscles and extension of the abdomen.

6. *Courtship songs* are triggered in a calling male by the presence of a female. Song is quite quiet, and broken into short chirps.

INTRODUCTION

The song of male cicadas is a fine example of a rhythmic motor output involving specialized cuticular, muscular and sensory structures. Pulses of sound are produced by the regular buckling of a pair of cuticular membranes, the tymbals. Pringle (1954) and Hagiwara (1955) showed that each tymbal is buckled inwards by the twitch contraction of a single, large muscle, and springs back to the resting position after the muscle relaxes. The form of the sound pulses is adjusted by accessory muscles,

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especially by the tonic contraction of the tensor muscle which inserts on the anterior rim of the tymbal. Subsequent work has shown that there is considerable variation between species in the details of this mechanism (e.g. Hagiwara & Ogura, 1960; Aidley, 1969; Reid, 1971; Young, 1972*b*). For example, the anatomy of the tymbal varies in the different species and the precise role of the tymbal in sound production is a rather neglected area of study (Popov, 1975).

Three types of song have been recognized. The *calling song* or free song (Pringle, 1954) is produced by undisturbed males and is the sound usually heard in the field. The *protest song* is the sound produced when the animal is disturbed, caught or injured, and is therefore the sound usually studied in the laboratory. In addition, the males of some species produce a distinct sound only in the presence of a female (Pringle, 1954) and this is termed the *courtship song*.

The Australian Bladder Cicada, *Cystosoma saundersii* (Fig. 1*a*), was chosen for this study because of its ready availability in season and its proven suitability for experimental work. Here we concentrate on the anatomy and mechanism of action of the tymbal, and on the neuromuscular activity underlying the different types of song, studied by means of electrodes implanted in freely singing animals. The calling song of *C. saundersii* has been described by Scott (1852) and Young (1972*a*). A preliminary physiological analysis was carried out by Young (1972*b*) and a detailed account of the acoustic behaviour is available from K. G. Hill and C. E. Hill (in preparation). This species has proved amenable to analysis of central neuronal mechanisms, and an intracellular study of activity in the tymbal motor neurones has been carried out by Simmons (1977).

MATERIAL AND METHODS

The Bladder Cicada, *Cystosoma saundersii* (Westw.) (Fig. 1), was collected in the neighbourhood of Port Macquarie on the central coast of New South Wales. Adults are found from November until mid-January and are most easily found at dusk when males produce their calling song. Observations in the field were made in collaboration with Drs K. G. and C. E. Hill in an area of quiet wasteland in the town, using a portable tape-recorder. Most laboratory experiments were performed on freshly collected animals in a room rented from 'Golden Sands Holiday Flats', Port Macquarie, but some were performed on animals air-freighted to Canberra.

Conventional anatomical and physiological methods were used throughout. To record myograms from intact males the animals were waxed securely to a holder fashioned from forceps and allowed to walk on a polystyrene ball that was covered with *Hydrangea* leaves and floated in a bowl of water (Fig. 1*b*). The tethered animals fed on the leaves. Electrodes were a pair of 50 μm silver wires, insulated to their tips, and were inserted into each muscle at least two hours prior to an experiment.

RESULTS

1. *Anatomy and innervation of sound-producing structures*

The sound-producing structures of cicadas are located mainly in the anterior of the abdomen and consist of a pair of tymbals, tymbal muscles, various accessory muscles and a tracheal air-sac (Myers, 1928; Pringle, 1954). For *C. saundersii* the principal

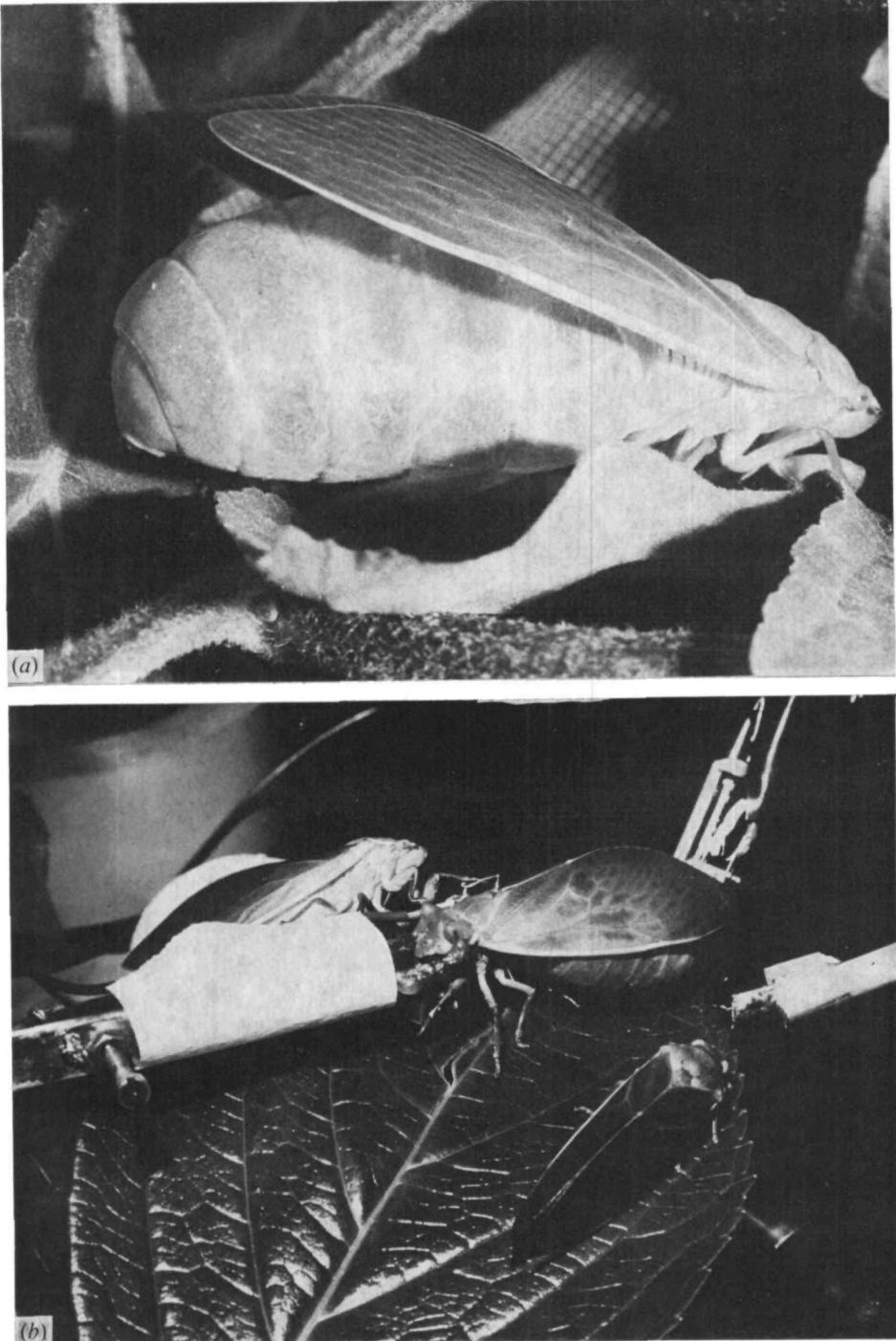


Fig. 1. (a) Male *Cystosoma saundersii* producing the calling song in the field. Note that the abdomen is distended so that it extends beyond the wings – at rest it is shorter than the wings – and that the wings are held clear of the body. Most males sing with the body inclined vertically. (b) Tethered male in the laboratory with wire electrodes implanted for recording myograms during singing. Two females, used to induce the male to change his song from calling to courtship, are also present.

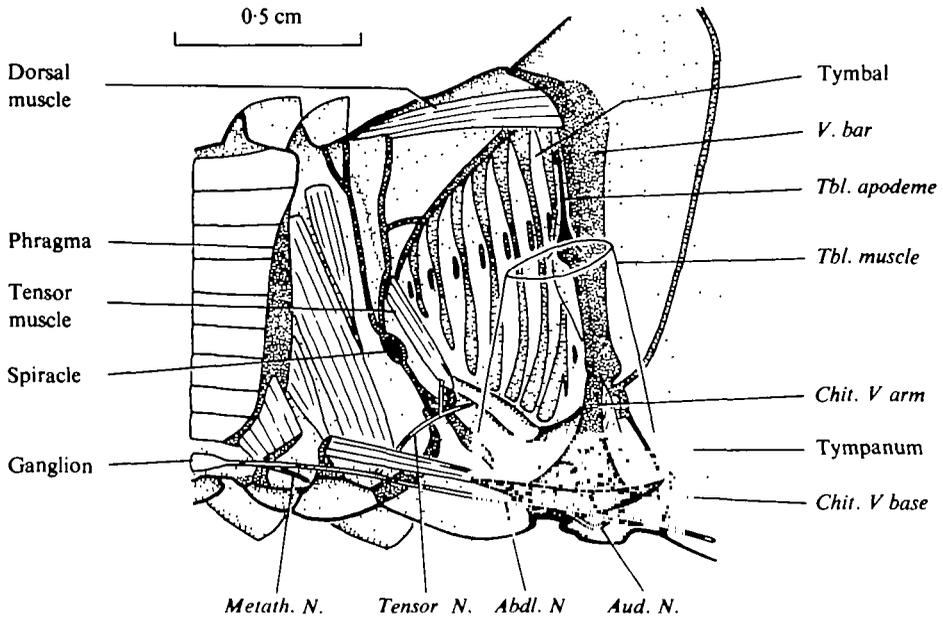


Fig. 2. Internal view of *Cystosoma saundersii* male to show the arrangement of the sound-producing structures. The drawing shows the right side of the cicada viewed from the midline after cutting the animal longitudinally in half. The large tracheal air-sac (not drawn), which originates from the spiracle shown here, occupies virtually all the internal space of the cicada posterior to the mesothoracic phragma. *Abdl. N.*, abdominal nerve; *Aud. N.*, auditory nerve; *Chit. V arm*, lateral arm of the chitinous V; *Chit. V base*, base of the chitinous V; *Metath. N.*, metathoracic nerves; *Tbl. apodeme*, tymbal apodeme; *Tbl. muscle*, tymbal muscle; *V. bar*, vertical bar.

components are shown in Fig. 2. The most conspicuous component is the large tracheal air-sac which originates from the spiracles on the posterior border of the metathorax (the spiracle labelled in Fig. 2). The expansions from the left and right spiracles fuse to form a single, large air-sac which occupies the metathorax and the entire abdomen except for a small dorsal space. The abdomen is thus almost filled with air and is greatly enlarged (Fig. 3).

In *C. saundersii* there is a conspicuous groove in the abdominal wall at the boundary of the 1st and 2nd segments (Fig. 3). The inner part of this groove constitutes an important sclerite, the vertical bar (Young & Hill, 1977), and the anterior surface forms the posterior part of the tymbal.

The tymbal is located on the side of the 1st abdominal segment. It comprises a thin, flexible membrane, biconvex in shape and with its posterior surface turned sharply inward (Figs. 3, 4*b*). The curved part of the tymbal bears a number of sclerotized ribs (Fig. 4*a*). There are usually seven long ribs, diminishing in size from posterior to anterior, but sometimes there is an incomplete eighth rib anteriorly. Interspersed between the long ribs are seven short ribs, arranged in a row and also diminishing in size from posterior to anterior. The long ribs are slightly raised above the membranous area but the short ribs are flush. Dorsally the long ribs fuse with the sclerotized tymbal rim but ventrally they stop short of the rim. The long ribs are assigned numbers according to the order in which they buckle (see below) so that the largest, most

posterior rib is rib 1 (Fig. 5) and the most anterior is rib 7, or sometimes rib 8. The inward-sloping, posterior face of the tymbal bears an irregularly shaped area of sclerotized cuticle which we term the tymbal plate (Figs. 4*b*, 5). The region of membrane between the tymbal plate and the vertical bar is especially flexible. The tymbal is bounded ventrally by the tymbal bar (Fig. 5), which projects forward from the 2nd abdominal segment (Young & Hill, 1977).

Dorsally on the tymbal plate there is a small indentation of less sclerotized cuticle and at the top of this is the insertion of the tymbal apodeme (Fig. 5). The tymbal apodeme is a slender shaft at this point of insertion but it expands internally to form a broad plate upon which the tymbal muscle inserts. Tymbal muscle fibres travel some way up within the shaft of the apodeme; the limit of their attachment is shown in Fig. 5 by a broken line. The shaft of the tymbal apodeme is shaded heavily in Figs. 2 and 5 for clarity of illustration but in the living insect it is quite transparent (unsclerotized) and flexible. The broad part of the tymbal apodeme is approximately kidney-shaped and this allows the distal part of the tymbal muscle to curve round the vertical bar. The tymbal apodeme touches the vertical bar in the resting position and at this point of contact there is a pronounced knob on the vertical bar and a corresponding dip in the broad part of the tymbal apodeme (Figs. 2, 4*b*, 5). The knob on the vertical bar is slightly hollowed on its anterior surface and there is a corresponding small bulge on the shaft of the tymbal apodeme, about level with the limit of muscle insertion (Fig. 5). This combination of raised and hollowed surfaces produces a precise fit between the tymbal apodeme and the knob on the vertical bar in the resting position. This arrangement strongly suggests the function of a 'return stop' to prevent the elasticity of the tymbal from causing overshoot during the relaxation of the tymbal muscle.

Each tymbal muscle has its origin ventrally, close to the midline on a specialized sclerite, the chitinous V (Myers, 1928) (Figs. 2, 5). The left and right tymbal muscles are apposed to one another in the midline so that the tymbal muscles slope outwards to the tymbals (Figs. 4*b*, 5). A large surface for muscle attachment is provided by the anterior and posterior extension of the base of the chitinous V. On each side the lateral arm of the chitinous V extends from the base to the vertical bar (Figs. 2, 5). The two arms form the compression members balancing tension developed by the tymbal muscles (Pringle, 1954).

The tensor muscle, situated at the anterior rim of the tymbal, is an important part of the sound-producing mechanism (Pringle, 1954; Hagiwara, 1955). It has its origin on a pronounced knob below the spiracle at the border of the metathorax and 1st abdominal segment (Fig. 2) and inserts above the spiracle upon a distinct oval area of cuticle immediately adjacent to the anterior rim of the tymbal (Fig. 5). This point of insertion is in line with the row of short ribs on the tymbal (Young, 1975). In *C. saundersii* the tensor muscle is comparatively slender and pale in colour. Two chordotonal organs (mechanoreceptors) are located near the tensor muscles (Young, 1975). The larger of the two, the tymbal chordotonal organ, inserts on the anterior rim of the tymbal and this point of insertion is discernible on the external surface (Fig. 5). Another muscle considered to have a possible role in sound production (Pringle, 1954) is the dorsal muscle (Fig. 2). This flat muscle extends longitudinally between the

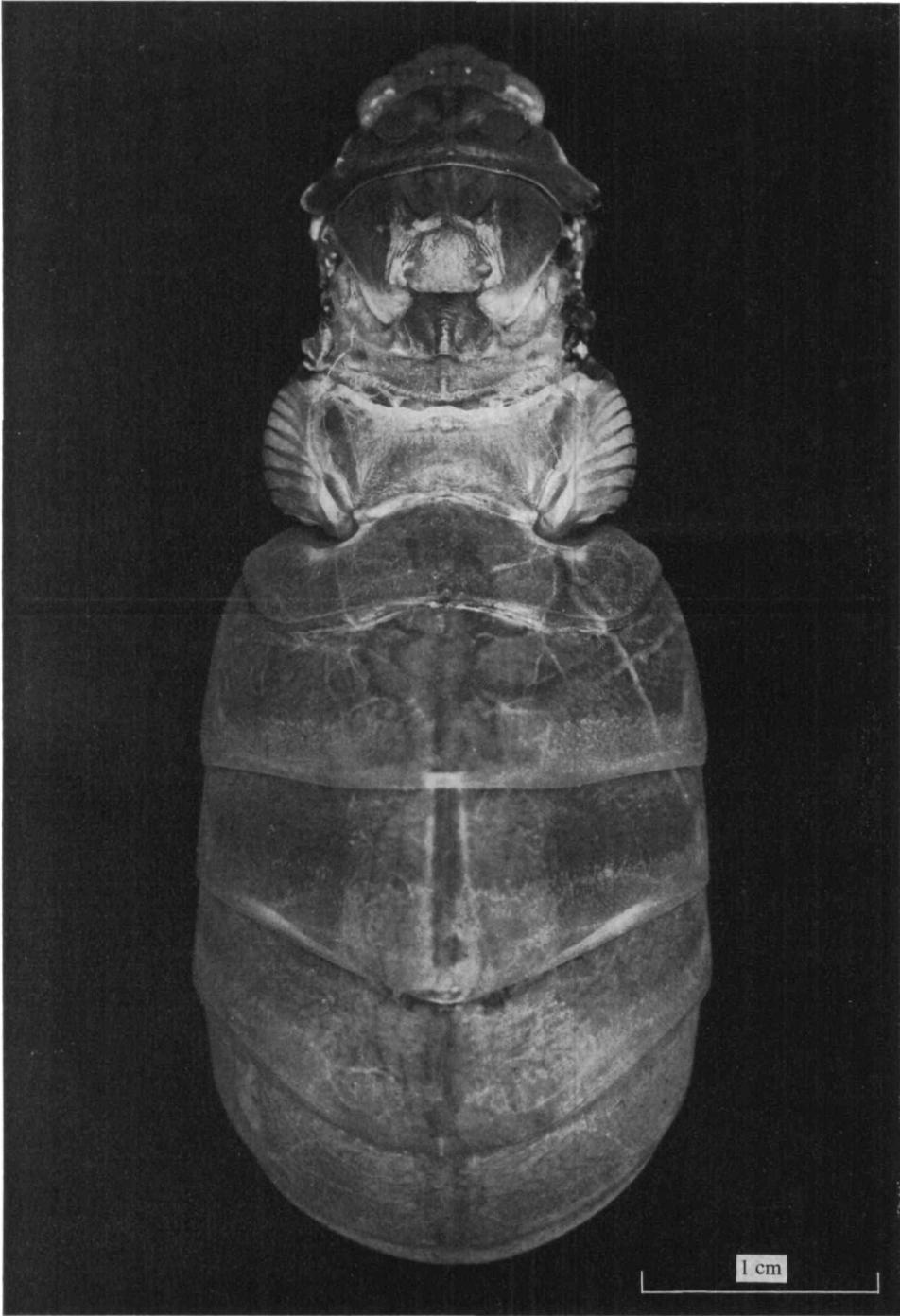


Fig. 3. Dorsal view of *Cystosoma saundersii* male with wings removed to show the ribbed tymbals and enlarged abdomen.

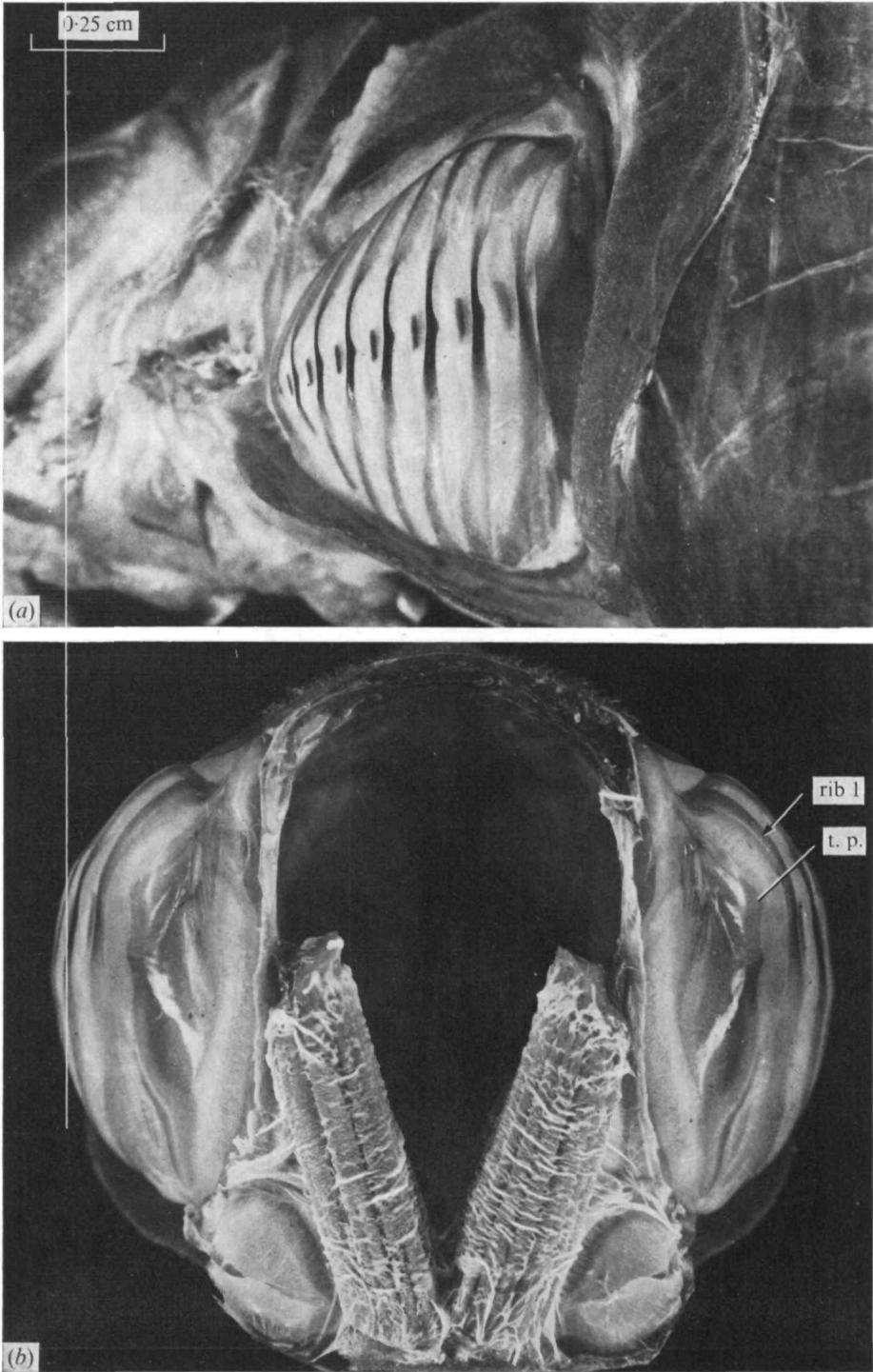


Fig. 4. (a) Lateral view of the left tymbal of *Cystosoma saundersii*. (b) Posterior view of *Cystosoma saundersii* cut open along the inner edge of the vertical bar. No internal structures have been removed: the tracheal air sac fills all the internal space except for a small dorsal region. The tracheal membrane is clearly visible enfolding other structures but has been peeled away from the top of the tymbal muscle to show the apodeme where it touches the knob on the vertical bar. The light patches on the posterior surface of the tymbals are due to the highly reflective surface of the tymbal plate. Magnification same as in (a). *t.p.*, Tymbal plate.

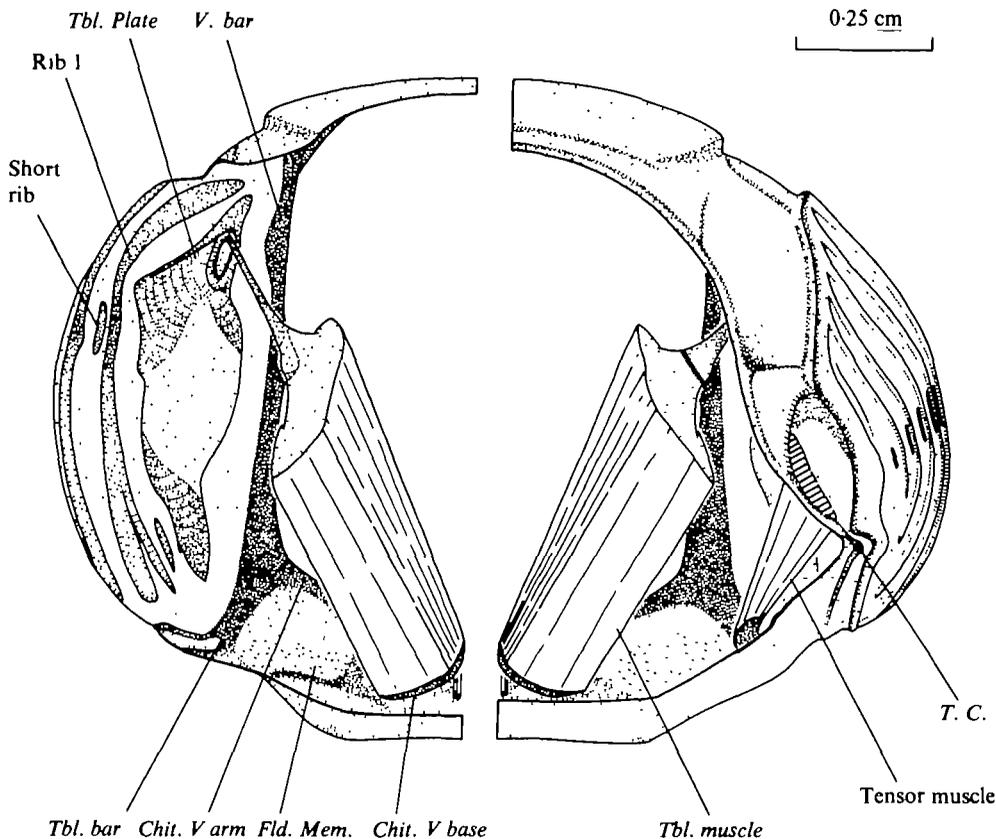


Fig. 5. Anterior view of the sound-producing structures of *Cystosoma saundersii*. The left drawing shows the cicada cut open at the transverse level of rib 2 on the tymbal. The right drawing shows the cicada cut open approximately along the anterior boundary of the 1st abdominal segment. The large tracheal air-sac (not drawn) occupies virtually all the internal space in this region. *Fld. Mem.*, Folded membrane; *T.C.O.*, insertion of tymbal chordotonal organ. Other abbreviations as in Fig. 2.

anterior and posterior margins of the 1st abdominal segment, passing across the dorsal rim of the tymbal.

All the muscles and sense organs involved in sound production are innervated from the thoracic-abdominal nervous system. In the species of cicadas studied by Pflugfelder (1937) and Pringle (1954), all the thoracic and abdominal ganglia are fused into a single mass, with at most a slight waist between prothoracic and mesothoracic regions. In *C. saundersii* the thoracic nervous system is not so condensed, the prothoracic ganglion being separated from the mesothoracic by short connectives and the mesothoracic ganglion being distinguishable from the metathoracic by a distinct waist and central hole (Fig. 6a). The metathoracic and abdominal ganglia are entirely fused together and this combined ganglion is located in the mesothorax (Fig. 2). The nerves concerned with sound production and hearing emanate from the posterior part of this metathoracic-abdominal ganglion (Fig. 6b).

The most medial of this posterior group of nerves is the abdominal nerve, which supplies all organs posterior to the 2nd abdominal segment. Next to it is the auditory

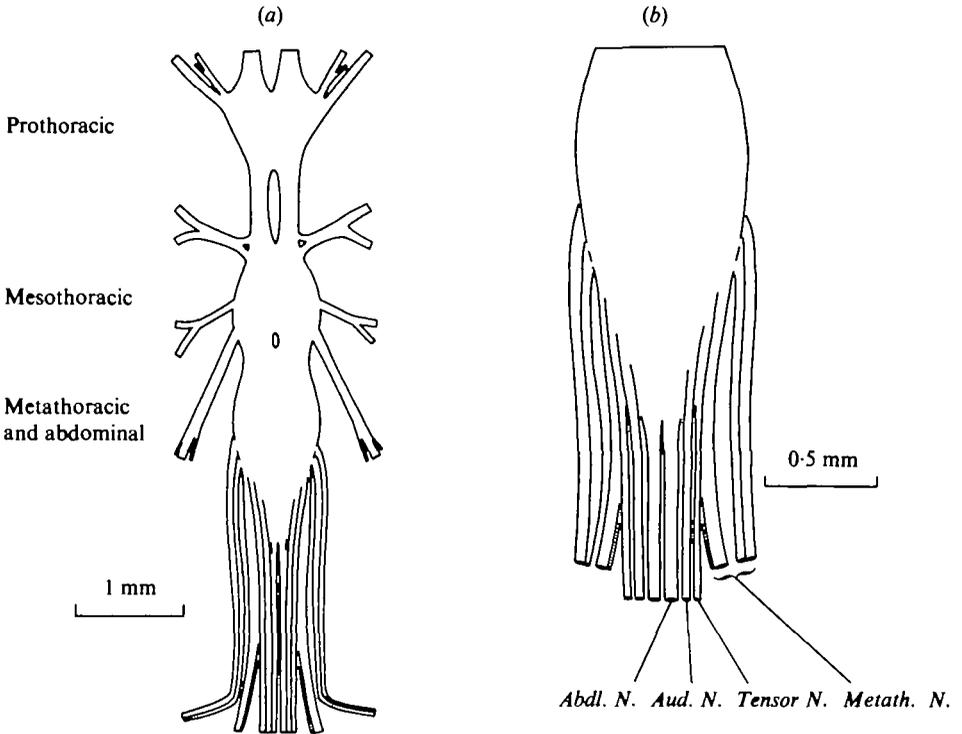


Fig. 6. The thoracic-abdominal ganglia of *Cystosoma saundersii*, dorsal view. (a) Shows the layout of the three thoracic and the fused abdominal ganglia. (b) Shows the nerves leaving the posterior part of the ganglion at the higher magnification. Abbreviations as in Fig. 2.

nerve, which leaves the ganglion about 0.4 mm anterior to the abdominal nerve. The auditory nerve is the most dorsal of these nerves in its origin on the ganglion (Fig. 6b). In *C. saundersii* the auditory nerve carries both the sensory fibres from the auditory organ and the motor innervation to the tymbal muscle (Young, 1972b), which consists of a single motor axon about 20 μm in diameter. In other cicadas the tymbal motor neurone axon is carried in a separate tymbal nerve which travels dorsally (Pringle, 1954). The auditory nerve travels with the abdominal nerve through the channel beneath the base of the chitinous V and then it turns peripherally along the edge of the tympanum to innervate the tymbal muscle from the peripheral side.

The tensor nerve leaves the ganglion slightly anteriorly and ventrally with respect to the auditory nerve (Fig. 6b). It travels closely with the auditory and abdominal nerves for a short distance and then turns laterally near the anterior margin of the 1st abdominal segment (Fig. 2). The majority of its target organs are in the region of the tensor muscle (Fig. 7), but one branch continues on round the ventral rim of the tymbal, travels up the vertical bar and around the tymbal apodeme to innervate the dorsal muscle. There are three axons which innervate the tensor muscle. About the same number of axons go to the spiracle muscle and to another small muscle below the spiracle. The motor axons to the accessory tensor muscle could not be found by any of the methods employed. A small sensory branch carries axons from a group of

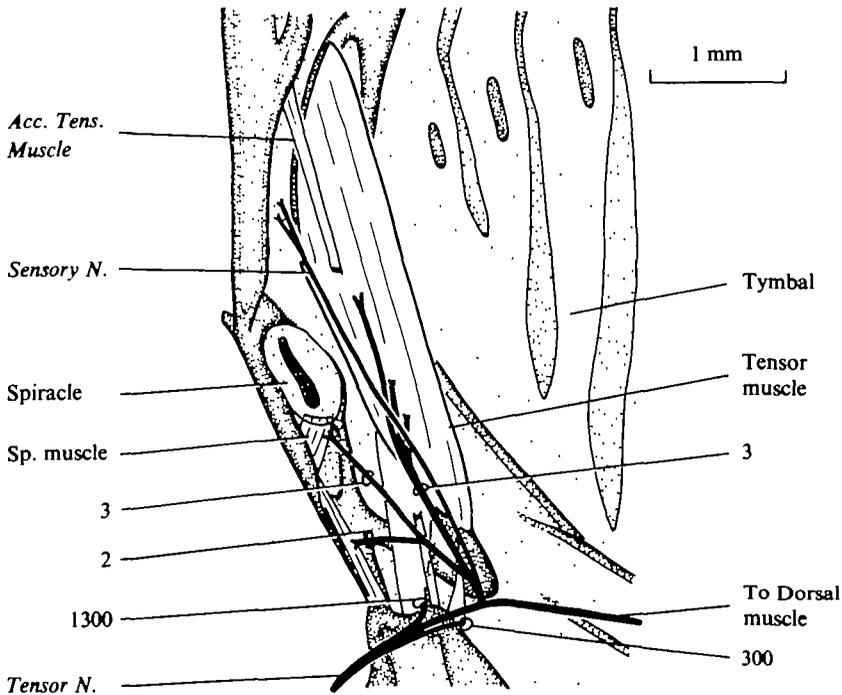


Fig. 7. Innervation of the tensor muscle region of *C. saundersii*. A medial view, enlarged from Fig. 2. This drawing is constructed from three methods of study: cobalt backfills, dissections and serial sections. The tensor nerve, which is drawn in solid black, branches to all the muscles in this region and to the dorsal muscle. It also innervates a group of hairs (the branch labelled *Sensory N.*) and two chordotonal organs. The numbers 1300 and 300 label the chordotonal organs and indicate the number of sensillae found by Young (1975). The smaller numbers label the motor branches of the nerve and indicate the number of axons seen in cobalt backfills. For clarity of illustration, the middle part of the small accessory tensor muscle (*Acc. Tens. Muscle*), described by Young (1975), is shown cut away. *Sp. muscle*, Spiracular muscle. Other abbreviations as in Fig. 2.

innervated hairs situated just anterior to the insertion of the tensor muscle. The tensor nerve also receives sensory branches from the tymbal and tensor chordotonal organs (Young, 1975).

2. The mechanism of tymbal action

In an intact animal the inward buckling of a tymbal is caused by a twitch contraction of its tymbal muscle. The delay between the spike and the buckling of the tymbal, as judged by the onset of a sound pulse, is 8–10 ms (Fig. 8*b*). The tymbal muscle develops maximum tension 12 ms after a spike invades it, as measured by a strain-gauge attached to its apodeme. This means that the tymbal begins to buckle and produce sound before the peak in the force that buckles it is attained. Following the inward buckling, the resting shape of a tymbal is usually regained by inherent elasticity. Sometimes a tymbal remains collapsed, particularly when high-frequency electrical shocks are applied to its muscle. Even in intact, freely singing males a tymbal occasionally remains collapsed for several seconds or even for some minutes.

The sound produced by one cycle of movement of a single tymbal can be seen from

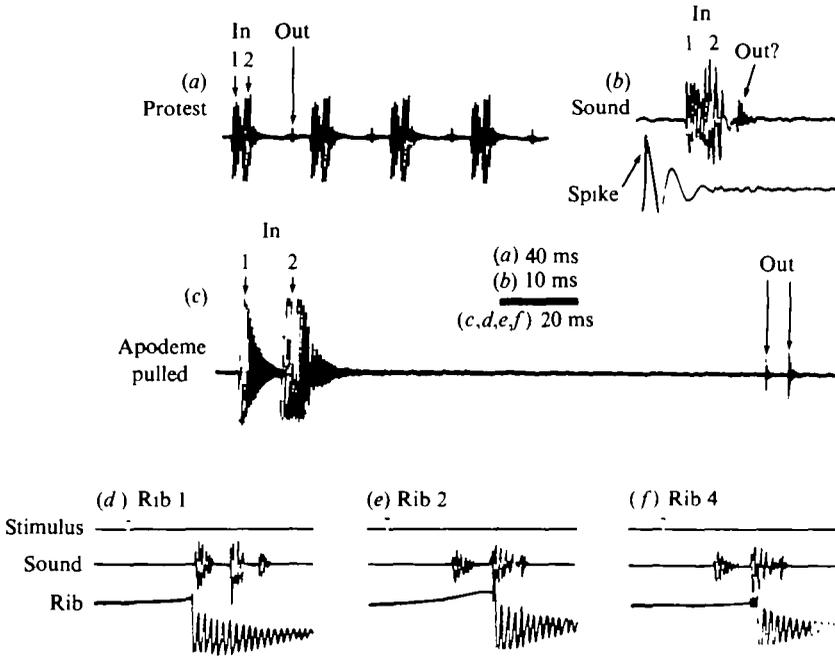


Fig. 8. Sounds produced by collapse of a single tymbal and the timing of movement of individual ribs during tymbal collapse. (a) Sounds produced by four sequential collapses of one tymbal during a protest song recorded in the field – the contralateral tymbal was destroyed. Three peaks in sound amplitude are distinguished following each collapse of the tymbal, two being produced as the tymbal collapses in and one as it clicks back out to its original shape. (b) A spike recorded from a tymbal muscle and the sound produced by collapse of the tymbal during a protest song of a tethered cicada. Three separate sound pulses can be distinguished, although it is impossible to be certain that the third is due to outward movement of the tymbal. (c) Sound produced by pulling sharply on the tymbal apodeme of a dissected cicada in which much of the abdomen had been removed. Three sound pulses can be distinguished clearly, with two separate peaks in the 'out' phase, which is unusual. The amplitude peaks are separated by longer intervals than in sound produced by contraction of a tymbal muscle. (d-f) Sound produced by stimulating a tymbal muscle electrically and movement of individual ribs registered by a strain-gauge. The tension registered by the strain-gauge increases gradually and then, when the rib buckles, the needle of the strain-gauge jumps free and vibrates at its natural frequency. (Rib 4 usually buckles slightly earlier than is shown in (f).)

a simultaneous myogram and sound record from an animal in which the contralateral tymbal has been destroyed (Fig. 8*b*). Three sound pulses are produced following each spike in the myogram. The peak in sound intensity is reached rapidly in each and is followed by an exponential decay. Usually the second pulse has a greater amplitude than the first and follows it by 5–7 ms. The third pulse is only sometimes observed; it follows the second by 15–20 ms and is always much smaller. In both intact and experimentally manipulated specimens, sound produced by the tymbal has a dominant frequency of about 850 Hz. Removal of the abdomen greatly reduces the intensity but does not significantly alter the form or carrier frequency of sound pulses.

We used three methods to investigate how the cycle of tymbal movement produces this characteristic group of sound pulses. First, in dissected animals, sound was produced by pulling on the tymbal apodeme with a pair of forceps. The pattern of sound produced in this way resembles that produced by an intact or dissected male during the protest song (Fig. 8*c*), but is much slower and the third sound pulse is sometimes

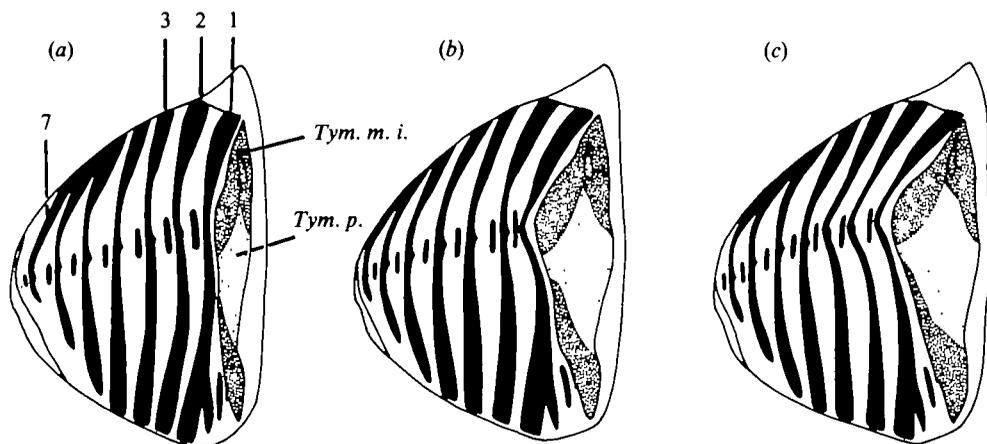


Fig. 9. The tymbal of *Cystosoma saundersii* in different stages of buckling. (a) The resting position; (b) with rib 1 buckled; (c) with ribs 1-3 buckled. The ribs are drawn in solid black and the tymbal plate is stippled. These drawings were made from dried specimens in which the tymbal had become fixed in the different positions but these reflect faithfully the appearance of the living cicada viewed with the stroboscope. Numbers refer to ribs; *Tym. m. i.*, Tymbal muscle insertion; *Tym. p.*, tymbal plate.

split into two. This method has the advantage that the investigator can distinguish with certainty between the inward and outward movement of the tymbal. It shows that the first two sound pulses are produced during the inward movement of the tymbal, and the third, much smaller pulse, during the outward movement.

Secondly, a tymbal muscle was stimulated at 20 Hz and the tymbal observed stroboscopically. Only three tymbal shapes were ever seen: the resting shape; a shape where only one rib had buckled inwards; and a shape where other ribs in addition to rib 1 had buckled inwards (Fig. 9). Initially, following contraction of the tymbal muscle, the tymbal plate swings forward and inwards, presenting a much broader profile to the observer (Fig. 9*b*). This movement of the tymbal plate brings about the buckling of rib 1, which is adjacent to it, thereby effecting the transition from shape 1 to shape 2. The transition to the third shape is effected by a smaller arc of movement of the tymbal plate as it continues to swing inward (Fig. 9*c*). In the third shape ribs 2 and 3 always buckled and other ribs sometimes buckled. The line of buckling coincides with the row of short ribs (Fig. 9).

In the third method, the time of buckling of an individual rib was determined at the same time as sound production was recorded. This was achieved by resting the needle of a strain-gauge against the rib. Care was necessary in performing this experiment because the time of the rib's buckling and the characteristics of the sound pulse were altered if too much pressure was applied by the needle to the rib. The needle was applied to the anterior sides of ribs to avoid interference from buckling of the next posterior rib. This method shows that the first sound pulse is coincident with the buckling of rib 1 (Fig. 8*d*). The remaining ribs buckle almost synchronously and their buckling coincides with the second sound pulse (Fig. 8*e, f*). All the ribs are restored to their original shapes almost simultaneously and this movement produces the third sound pulse.

3. *The action of the tensor muscle*

The form of the sound pulses produced by a single tymbal is modified by the tensor muscle, which has very different physiological properties from the tymbal muscle. A single shock applied to the tensor nerve produces little or no contraction of the tensor muscle. Tension develops gradually when a train of shocks is applied to its nerve and the muscle relaxes slowly when stimulation ceases. The rate at which it develops tension depends on the frequency of stimulation and is maximal at 40 Hz. In an intact animal, the tensor muscle, immediately following insertion of silver electrodes into the tensor muscle, was still developing tension 2 s after onset of a train of shocks applied to it at a frequency of 20 Hz.

Contraction of the tensor muscle increases the convexity of the tymbal. The modification that its contraction produces can be mimicked by pushing on its place of insertion in the direction of its contraction. With this procedure, the first two sound pulses occur closer together in time and often merge. Observations made with the strain-gauge and with the stroboscope show that rib 1 buckles sooner before the other ribs (Figs. 10*b-d*). The latency between excitation of the tymbal muscle and the production of sound is lengthened (Fig. 10*a*), indicating that the structural alteration to the tymbal increases its stiffness. Also the first two sound pulses increase in amplitude (i.e. loudness) when the tensor muscle contracts, which is readily understood in view of the tymbal's increased stiffness.

When other muscles in the region of the tymbal, including the dorsal muscles, were stimulated, no effect on sound production was found.

4. *The three song types*

(a) *Protest song*. Protest song contains several features common to all song types. It is readily elicited in the laboratory by electrical stimulation of the brain or abdominal connectives.

The pattern of sound produced when both tymbals are active differs from that when only one is active. This is shown simply by recording the protest song before and after the destruction of one tymbal (Figs. 11*a, b*). The sound recorded after the destruction of one tymbal clearly shows the pattern characteristic of a single tymbal: two loud pulses produced during the inward movement and the single quiet pulse of the outward movement. The sound recorded in the intact animal shows a pattern of three loud (inward) pulses and only one very quiet (outward) pulse, suggesting that there is a phase lag such that the second pulse of one tymbal overlaps the first pulse of the other. The third (outward) pulse of the leading tymbal will then be hidden by the second pulse of the trailing tymbal. The third pulse produced by the outward movement of the trailing tymbal hardly makes a significant contribution to the protest song and sometimes it cannot be detected. In some of the pulse groups in the sound record of the intact animal four loud pulses, rather than three, are just discernible (Fig. 11*a*). In these pulse groups the second pulse of the leading tymbal and the first pulse of the trailing tymbal are just separable due to the low frequency of tymbal muscle contraction and the high degree of damping of the sound pulses.

Measurements from the sound records show that such a phase lag between the tymbals will occur if the delay between the contraction of one tymbal muscle and the

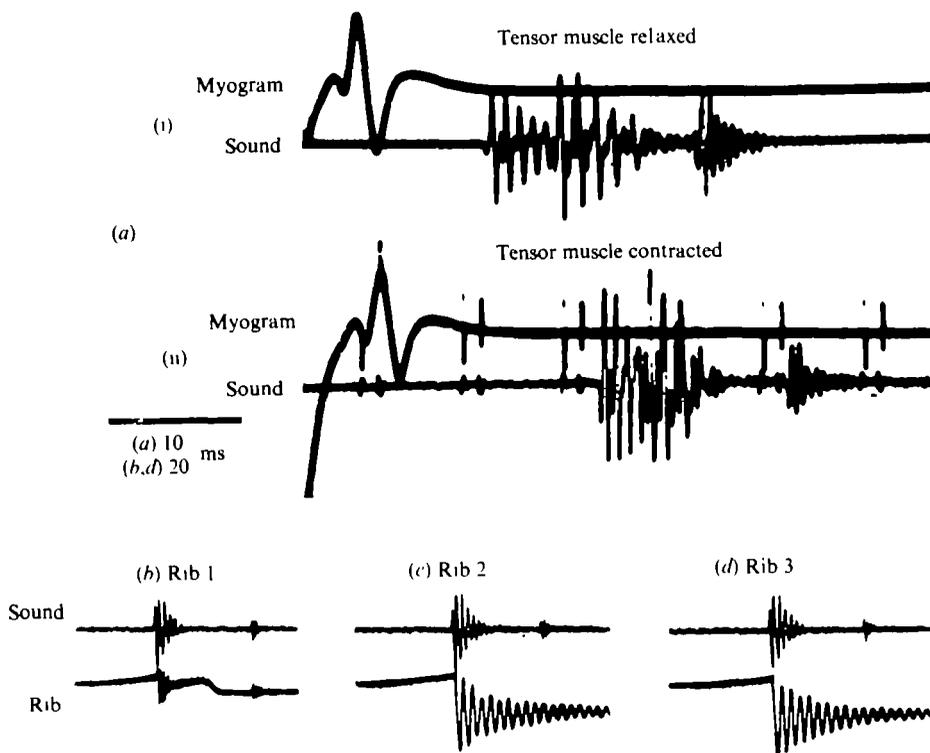


Fig. 10. Action of the tensor muscle on sound produced by tymbal collapse. (a) Comparison of sound produced by collapse of one tymbal: (i) when the tensor muscle is relaxed; and (ii) when shocks applied to it (stimulus artefacts in the myogram) have caused it to contract. The myograms are from the tymbal muscle, and the cicada is producing a protest song. (b-d) Sound produced by a single collapse of a tymbal and rib movement monitored by a strain-gauge when the tensor muscle is contracted. In (b) the strain-gauge did not jump clearly from the rib, and was hit as the rib clicked outwards.

other is equal to one-quarter of the contraction frequency of one muscle (= tymbal buckling frequency). Myograms from both tymbal muscles of a male, tethered as in Fig. 1(b), during protest song, reveal this patterning of activity in the tymbal muscles (Fig. 11c). We call the interspike interval for one motor neurone the *cycle period* and the shortest interval between a spike in one motor neurone and its contralateral partner the *intertymbal delay*. The intertymbal delay cannot be determined accurately from extracellular recordings of spikes in the tymbal muscles because it is affected by the positioning of the electrodes in the muscles, but intracellular recordings from the somata of the motor neurones show that the intertymbal delay is one-quarter of the cycle period (Simmons, 1977).

The cycle period is the same for both motor neurones and it varies at different times in one animal between 40 and 25 ms. For example, in one experiment in the field the tymbal buckling frequency of a male during a calling song was 40 Hz, but after a few minutes had dropped to 25 Hz, when a protest song was elicited. After the destruction of one tymbal, the buckling frequency of the other increased again. There is no tendency for one motor neurone to lead the other during song and after a pause the

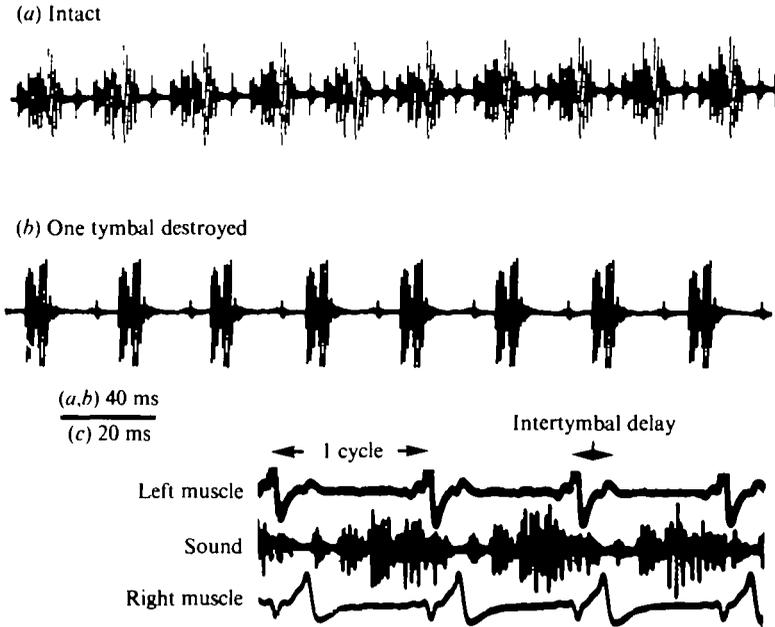


Fig. 11. Protest songs of male Bladder Cicadas. (a, b) Songs recorded in the field shortly after the calling chorus. In (a) both tymbals are intact; in (b) one tymbal was destroyed by making an incision in it and cutting through the tymbal muscle. After this operation the frequency of tymbal muscle contraction reduced from about 30 to 25 Hz. (c) Myograms recorded from the left and right tymbal muscles and the sound produced during a protest song of an intact cicada tethered as in Fig. 1(a).

motor neurones commonly change their order of spiking in a cycle. No more than one spike per contraction has been recorded from a tymbal motor neurone.

From variation in the degree to which sound pulses are separable during different protest songs we conclude that the extent of contraction of the tensor muscles varies in alarmed males. Often the tensor muscles do not contract sufficiently to affect the manner of tymbal buckling. Recordings that were made from tensor muscles during songs are impossible to interpret because they also reveal large spikes to other muscles. We have not managed to identify tensor motor neurone spikes in recordings from the tensor nerve, which carries many motor and sensory axons (Fig. 7).

(b) *Calling song*. Males of *C. saundersii* produce their calling song in a chorus that begins before sunset and continues for half an hour or longer, depending on the weather (K. G. Hill and C. E. Hill, in preparation). Before and during the early part of the calling song a male extends its abdomen and raises its wings from its body, thereby improving the effectiveness of the body as a sound radiator (Fig. 1a).

The sound envelope of pulses produced by a calling Bladder Cicada is very smooth compared with pulses produced during the protest song (Fig. 12). Nevertheless a careful comparison shows that the calling song is composed of groups of three loud pulses as in the protest song. Hence, this pattern may be attributed to the same phase relation between the two loud pulses produced during the inward buckling of left and right tymbals as in the protest song. Quiet pulses due to the outward movement of the tymbals are completely obscured by the loud inward pulses. The later pulses in

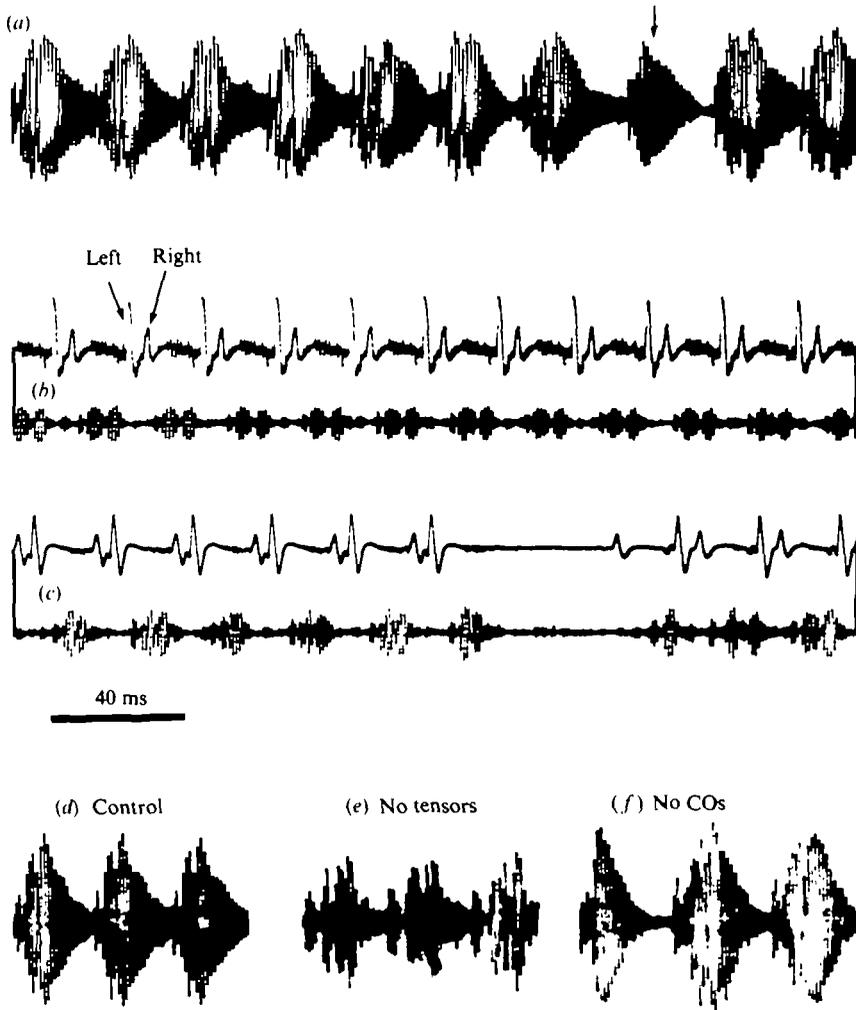


Fig. 12. Calling song of *C. saundersii*. (a) A song recorded in the field during a chorus. Note the smoothness of the sound envelope compared to that of protest (Fig. 11) or courtship (Fig. 13) songs. At the arrow one tymbal fails to collapse. (b, c) Myograms from the tymbal muscles and the sound produced by a tethered calling cicada. A pair of electrodes was inserted into the left tymbal muscle near its origin and picked up spikes from both muscles. In (b) the song is well under way and the electrodes were shaken enough for their movement to be registered. (c) A pause during the early part of the song. After the pause the right tymbal motor neurone recommences spiking before the left, and the order of the tymbal motor neurone spikes is reversed. (d) Calling song produced by a control animal in which a hole had been made in the cuticle. (e) Calling song produced by an animal in which both tensor muscles had been destroyed. Note the raggedness of the sound envelope compared with the rest of this figure. (f) Calling song produced by an animal in which both tymbal chordotonal organs had been destroyed. The sound envelope is smooth and the song was loud, but the tymbal buckling frequency is lower than in the calling songs of control animals.

these groups of three usually have a greater amplitude and longer decay time than the earlier ones. Occasionally only a pair of pulses is found, with the later one louder than the earlier. These pairs are attributable to the sound produced by only one tymbal and are especially common at the onset of song (see below).

One male in which wires were implanted to record myograms (Fig. 1*b*) sang in chorus with non-captive males at dusk. Although tethered, it extended its abdomen, raised its wings and walked vigorously on its polystyrene ball during the early part of its calling song. Myograms from this male show cycles essentially identical to those recorded during protest songs, with a number of additional features (Fig. 12*b, c*). The calling song of this male began with some short chirps, which increased in cycle number as song progressed. Cycle periods became shorter as song continued: at 7.40 p.m. they were 24 ms long and by 7.45 p.m. they had contracted to 22 ms. The intertymbal delay shortened in proportion to the cycle period. Accompanying the increased cycle frequency, sound production became louder and the recording electrodes were shaken enough for vibrations of the animal to be registered (Fig. 12*b*).

In five cases the onset of calling song was clearly recorded in the field. Some of these examples show an increase in cycle frequency and all show a steady increase in loudness of the song. In four of these cases, only one tymbal was operating for the first few cycles of the song, then both the tymbals operated, and then only one again. This alternation continued for some seconds until a steady rhythm was established. The fifth case was similar but began with both tymbals operating. In all five cases the song began in the form of short chirps, which gradually merged into continuous song.

Recordings from the wired-up animal show that short, irregularly occurring pauses occur throughout a calling song. Sometimes only one spike by one motor neurone is omitted, but up to ten cycle periods of silence by both are common. The left and right motor neurones commonly swap the lead following a pause (Fig. 12*c*). Pause lengths are roughly, but seldom exactly, equal to a multiple of the partner delay and this, as discussed later, indicates that sensory feedback affects cycle frequency. In an uninterrupted sequence of song about 1 in 200 cycles is slightly shorter or longer than usual and, most often, unusual lengths of cycle occur in both motor neurones together.

The contraction of the tensor muscle is a major factor in determining the envelope shape and amplitude of sound pulses in the calling song. This can be seen from comparison of normal calling songs with those of males in which the tensor muscles have been ablated. This ablation was achieved by making a cut round the insertion area of the tensor muscles (Fig. 5). This operation was carried out during the day and the animals put out on a shrub at least 2 h before the normal chorus time. Such operated animals extend their abdomens, raise their wings, and call in chorus with intact males. But they produce sound pulses which are highly damped compared to normal so that three sharply distinguished sound pulses are evident in each group, as in protest song (Fig. 12*e*). Judged subjectively their songs are much quieter than those of intact males. Also the waveform of the sound pulses is rather ragged compared to normal calling song, which indicates a higher harmonic content in the song. Control males, with holes cut in the thorax wall near to the insertion of the tensor muscle, produce calling songs which are apparently normal, showing that minor punctures to the air-sac are not deleterious to the quality of the song (Fig. 12*d*). As with the protest

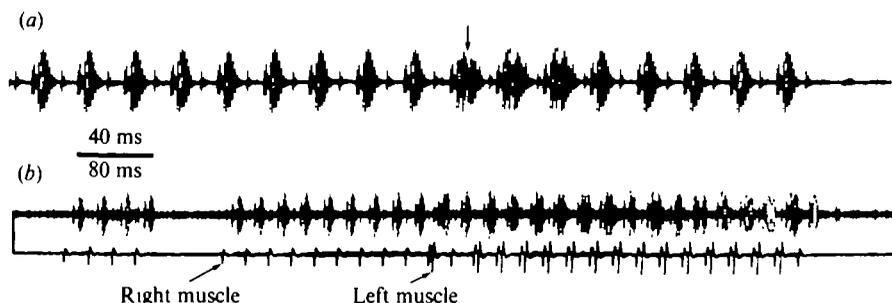


Fig. 13. Chirps during courtship songs of *C. saundersii*. (a) Courtship song recorded in the field. Most cycles involve the collapse of only one tymbal, but for three cycles, the first of which is arrowed, both tymbals collapse. This figure shows the second half of the chirp. (b) Courtship song and myogram from the tymbal muscles of a male tethered as in Fig. 1(a).

song, recordings from the tensor muscles during calling were incomprehensible, so we know nothing of the patterning of spikes in tensor motor neurones.

The function of the tymbal chordotonal organs was investigated by ablating the insertion of these organs in a manner similar to the tensor muscle operation (Fig. 5). The calling songs of males operated on in this way usually have a lower cycle frequency than intact males, and the waveform is rather ragged as in tensor-ablated animals (Fig. 12*f*). However, the sound envelope is quite normal.

(c) *Courtship song*. Courtship song is triggered in a calling male of *C. saundersii* after a female has landed nearby. The abdomen is drawn in, sound intensity decreases and the song becomes broken into short chirps. Chirp lengths vary from 0.3 to 1 s and the silent periods between chirps are about 1–2 s long during a brisk courtship sequence but often lengthen to 1 or 2 min. Usually a chirp begins with only one tymbal operating and the contralateral one may or may not join in toward the end of the chirp (Fig. 13).

Fig. 13*b* shows myograms and sound recordings from the male which produced calling song while wired-up. When females were placed near to him, as in Fig. 1(*b*), he produced the courtship song. These records show clearly that only one tymbal muscle is operating at the beginning of a chirp and sometimes the contralateral muscle does not join in at all. In both field and laboratory situations the sound pulses resemble those of animals in which the tensor muscles have been ablated, in respect of both sound envelope shape and rather ragged waveform (cf. Figs. 12*e*, 13*a*). Hence it is likely that the tensor muscle is relaxed during courtship.

DISCUSSION

1. *Tymbal mechanism and radiation of sound in C. saundersii*

Two important consequences of the tymbal mechanism may be distinguished. First, the tymbal converts a single muscle twitch into a cuticular movement that occurs in three stages (two inward and one outward) so that two loud sound pulses and one quieter sound pulse result from each contraction of a tymbal muscle. Secondly, the tymbal has the property of vibrating rapidly (about 850 Hz) when suddenly displaced. In the terminology of Michelsen & Nocke (1974) the tymbal acts as a frequency

multiplier. That the property of vibrating at about 850 Hz is intrinsic to the tymbal is shown by the fact that the tymbal vibrates at this frequency even when the abdomen is cut off (Fig. 8c). Therefore the tymbal is tuned to the frequency of the species' song independently of the abdomen. The basis of this tuning is not identified, but the ribbed construction of the tymbal must affect its stiffness and hence its natural frequency of vibration. Most other species of cicada have much stiffer cuticle in their tymbals and higher carrier frequencies for their songs than *C. saundersii*. Also a comparison of different species shows that there is a correlation between the arrangement of the ribs and the harmonic content of the calling song (Young, 1972b).

Michelsen & Nocke (1974) state that the tymbals of cicadas act as radiators of sound as well as frequency multipliers, but they do not discuss the function of the air sacs. From their analysis, one would anticipate that in *C. saundersii* the large, air-filled abdomen would be better suited than the relatively small tymbals to radiate sound at the low frequency of 850 Hz. Three lines of evidence can be presented to support the view that the abdomen acts as a resonant sound radiator. First, if the abdomen is cut off during a protest song, the volume of sound produced decreases by 8–10 dB relative to the initial sound field (Young, 1972b). Secondly, when the abdomen is stimulated with sound frequencies near 800 Hz, the sound pressure inside the abdomen is augmented by up to 11 dB (Young & Hill, 1977). Third, Fletcher & Hill (1978) have shown that when the dimensions of the tracheal air-sac are incorporated in standard acoustic equations, the result is close to that required for resonance at 850 Hz. Therefore we conclude that the function of the abdomen is to radiate sound, and the function of the tymbals is to drive the abdominal resonator. Pringle (1954) provided experimental evidence that the abdominal air-sacs resonate at the frequency of the species' song, but he did not explicitly discuss whether the sound of a cicada singing is radiated by the abdomen or by the tymbals.

From the mechanisms of the production and radiation of sound described here, a possible function of the phase lag between the two tymbals in optimising acoustic communication can be suggested. The volume of sound emitted by a Bladder Cicada will be greatest when the abdomen is resonating. Since the abdomen is a damped mechanical system, appreciable time is required to reach and decay from resonance. This means that the sound produced by collapse of the second tymbal in a cycle will add with the sound produced by the first tymbal, creating a louder and longer sound pulse than if both tymbals were to collapse in synchrony or half a cycle out of phase. Thus the sound pulses in a calling song (see Fig. 12) are quite loud and distinct, which we assume to be advantageous for efficient intraspecific communication. The auditory organs of both males and females are sharply tuned to the frequency of the species' song (Young & Hill, 1977), but nothing is known about responsiveness of cicadas or their neurones to sound pulses of different lengths and repetition frequencies.

Elsner (1974) has found a similar phase lag between movements of the sound-producing structures on either side of the body in Gomphocerine grasshoppers. The phase lag results in a partial obscurity of pauses between syllables and between chirps. Unlike the situation in cicadas, where sound is radiated by a single tuned resonant structure, there is considerable variation in the frequency of sound in a grasshopper song. Elsner suggests that frequency discrimination may be an important aspect of acoustic communication in grasshoppers, and that the phase lag between the

two sides can accentuate the range of frequencies produced. Thus, a phase lag in the movements of sound-producing structures on either side of the body appears to have evolved in two separate groups of insects for different reasons.

General comments on tymbal mechanisms of cicadas

The pattern of long and short ribs anteriorly and a tymbal plate posteriorly seems to be general among cicadas. This pattern can be seen clearly from the drawings and text of Pringle (1954) as well as from published photographs of tymbals (Hagiwara, Uchiyama & Watanabe, 1954; Reid, 1971; Young, 1972; Popov, 1975).

Pringle (1954) showed that a hand-manipulated tymbal produces a noise during both the inward and outward movement and that each tymbal muscle contraction elicits two sound pulses close together. He concluded that the first of these two pulses is produced by the inward movement of the tymbal and the second by the outward movement of the tymbal. He was followed in this interpretation by other authors who found species with two sound pulses per muscle contraction (Aidley, 1969; Young, 1972*b*). In *C. saundersii* this interpretation is shown to be mistaken. Although the outward movement of the tymbal does produce a noise during hand-manipulation, this does not contribute significantly to any of the natural song patterns and the two pulses are due to the two-stage buckling during the inward movement. Thus, it is possible that the two-stage mechanism may be applicable to those other species with two pulses which have hitherto been ascribed to the inward and outward tymbal movements. For instance the two pulses shown in Fig. 8 of Pringle (1954) closely resemble the two pulses produced by the inward movement of *C. saundersii* under similar conditions (Fig. 8). At all events, we may conclude that there is no published example in which it has been clearly established that both the inward and outward movements of the tymbal normally produce sound pulses.

It may be proposed that all cicada tymbals work in the same way, namely with sound pulses being associated with the buckling of individual ribs during the inward movement of the tymbal. The movement starts with displacement of the posteriorly placed tymbal plate, and then the ribs buckle in orderly succession from posterior to anterior. Differences in the structure of tymbals between species gives rise to differences in the pattern of sound pulses they produce. The situation in *Cystosoma* contrasts with that in *Abricta* and *Magiccicada*. In the latter two genera a separate sound pulse is associated with the buckling of each of about ten ribs (Moore & Sawyer, 1966; Reid, 1971; Young, 1972*b*). The long ribs of these cicadas are heavily sclerotized, raised well above the membranous area of the tymbal and placed rather close together (illustrated in Reid, 1971 – where the photograph of the tymbal in Fig. 1*c*) has evidently been mounted upside-down; and Young, 1972*b*). In *Cystosoma* the ribs are not so heavily sclerotized, lie more flush with the membranous area of the tymbal, and are not very close together. Tymbal collapse occurs in two distinct stages, each emitting a sound pulse: rib 1 buckles, followed by the almost synchronous buckling of a variable number of other ribs. In some other genera the sound pulses occur in groups of 3 to 5 and the tymbals of these species are constructed in a similar way to those of *Abricta* and *Magiccicada*, but with fewer ribs (Popov, 1975; D. Young, unpublished observations). One species at least, *Arunta perulata*, produces only one sound pulse per muscle contraction (Young, 1972*b*).

Action of the tensor muscle

Our observations on the action of the tensor muscle confirm and extend Pringle's (1954) ideas about its role in modifying the sound pulses produced by tymbals. Contraction of the tensor muscle increases the force that must be applied to the tymbal plate to cause collapse of the tymbal and also alters the geometry of the tymbal. These changes result in an increased delay between nerve impulse and sound pulse, an increase in amplitude of vibration, and a decrease in the interval between the first and second sound pulses during the inward movement of the tymbal. Pringle (1954) and Aidley (1969) found a similar relationship between pulse interval and amplitude in paired pulses. They both interpreted this relationship in terms of pulses produced during the inward and outward movement of the tymbal but, as suggested above, the two-stage buckling mechanism may be applicable to the species they studied. In *C. saundersii* animals in which the tensor muscle was ablated produced sound pulses with a higher harmonic content. In this species, therefore, contraction of the tensor muscle may be required to maintain the stiffness of the tymbal in order to keep it sharply tuned to the carrier frequency of the calling song.

Neural control of songs in C. saundersii

Intracellular recordings from tymbal motor neurones of *C. saundersii* show that the neuronal oscillator that drives singing is continuously active in quiescent animals (Simmons, 1977). It produces smooth waves of depolarization which are in antiphase in the left and right tymbal motor neurones and spikes occur at most upon every second depolarization. Therefore the neuronal oscillator has a frequency twice that of the tymbal buckling frequency and the shortest delay between spikes in the left and right motor neurones is equal to one-quarter of the cycle period (Figs. 11*c*, 12*b*, *c*). This ensures constancy in the relation between cycle period and intertymbal delay and, for restricted times, constancy in cycle frequency (= pulse repetition frequency). The neuronal mechanism underlying song also ensures, in a way that is not yet understood, that no more than one spike can occur in either tymbal motor neurone during a cycle – even strong depolarization of a tymbal motor neurone does not cause bursts of spikes. This is important as tetanic contraction of the tymbal muscle causes the tymbal to stick in the collapsed position and this requires time to reverse.

That sensory feedback affects the song-driving neuronal oscillator is suggested by two sets of observations. The first set are those of calling songs of animals which had their tymbal chordotonal organs destroyed (Fig. 12). Secondly, pauses in calling songs do not have lengths that are exact multiples of the period of the intertymbal delay although cycle period is very constant during periods of uninterrupted spiking (see Fig. 12). This means that the neuronal oscillator frequency is very constant while the tymbal motor neurones are spiking but alters when the tymbals do not collapse due to the tymbal motor neurones not spiking. The simplest explanation for this is that proprioceptive feedback that monitors tymbal collapse affects the frequency of the neuronal oscillator. An alternative and more complex explanation is that a pause occurs when the central nervous system anticipates a change in oscillator frequency.

Our results show that the transition from one type of song to another can be understood in terms of quite simple adjustments to the neuronal song generator and to the

■und-producing structures. Protest songs may be regarded as the 'raw' form of singing, with no refinements to increase specific communicative value. Whether protest songs do have communicative value is questionable, and cries from protesting cicadas do not appear to deter predatory birds. Production of a calling song is achieved by: increasing the frequency of the song-driving neuronal oscillator; contraction of the tensor muscles to alter the envelope shape of sound pulses; and extension of the abdomen and raising of the wings from the body to improve sound radiation. In the change from calling to courtship the song is reduced to short chirps; one motor neurone often spikes without the other; the cycle frequency decreases slightly; and sound pulses become much quieter probably because the tensor muscles relax and the abdomen is drawn in.

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