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Jaws that snap: control of mandible movements in the ant *Mystrium*

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Abstract

Ants of the genus *Mystrium* employ a peculiar snap-jaw mechanism in which the closed mandibles cross over to deliver a stunning blow to an adversary within about 0.5 ms. The mandible snapping is preceded by antennation and antennal withdrawal. The strike is initiated by contact of the adversary with mechanosensory hairs at the side of the mandible, and is powered by large yet slow closer muscles whose energy is stored by a catapult mechanism. Recording of closer muscle activity indicates that the mandibles are not triggered by any fast muscle. Instead, we suppose that activity differences between the left and right mandible muscles imbalance a pivot at the mandible tip and release the strike. The likelihood for the strike to occur can be modulated by an alarm pheromone. The presence of specialized sensilla and of a complex muscle receptor organ shows that the mandibles are also adapted to functions other than snapping and suggests that the force of the mandible can be finely adjusted for other tasks.

Keywords: Fast movements; Functional morphology; Insect mechanoreceptors; Motor control

1. Introduction

Ants belonging to the genus *Mystrium* employ a peculiar defence mechanism: they snap their jaws past each other to generate a powerful strike directed at their adversaries in the same way that we emit a sound by snapping our fingers (Moffett, 1986). A similar mandible snapping has also evolved in several termite genera (Deligne et al., 1981), presumably as defensive means against ants, the most significant predators of termites (Holldobler and Wilson, 1990).

Mystrium belong to the 'primitive' ant tribe Amblyoponini, a moisture-loving cryptic species that forage in soil, leaf litter or rotting logs. Little is known about the food preferences or hunting tactics of the Ablyoponini but they are supposed to be obligatory predators of other arthropods (Brown, 1960). They have been found feeding on beetle larvae and centipedes, but very little is known about the actual prey capture. The Amblyoponini, including the genus *Mystrium*, have very small or no eyes at all, so presumably they find their prey guided mainly by olfactory and mechanical cues. The long mandibles, clypeus and labrum of species of the Amblyoponini are toothed and appear well suited for grabbing and holding prey before it can be stung and subdued by the potent venom (Brown, 1960).

All species of *Mystrium* are equipped with long, specialized mandibles (Menozzi, 1929), and all perform the mandible snap. Like the other species of Amblyoponini, *Mystrium* have a very broad head and the base of the mandibles is set widely apart (Fig. 1(a,b)). Despite the similar head design of all Ambyoponini, *Mystrium* are the only ants to have evolved the snapping mandibles. This is in contrast to the so-called trap-jaws which have evolved repeatedly and independently in at least three ant subfamilies (reviewed by Holldobler and Wilson, 1990). We have recently investigated the function and control of trap-jaws in several ant genera (Gronenberg, 1995a, b, 1996b; Gronenberg and Ehmer, 1996), but very little is known about the mechanism of snapping mandibles in termites or ants. In the present report we describe the behavior, the morphology and mechanics,

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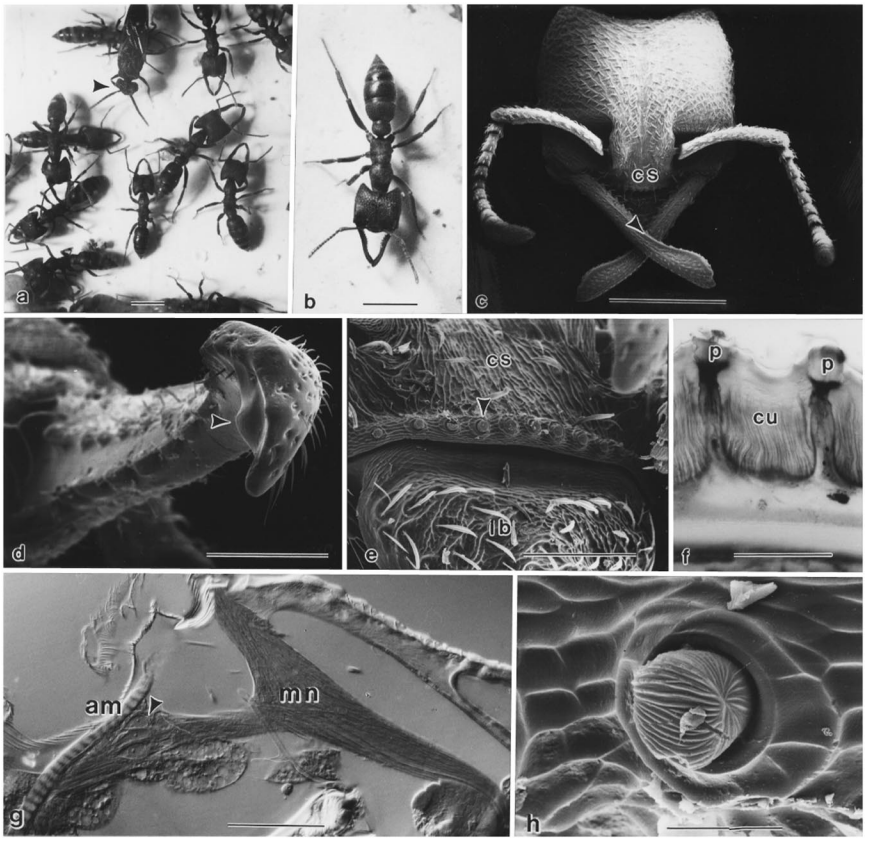

Fig. 1. Photographs (a, b), photomicrographs (f, g; osmium-stained material, Nomarski optics), and scanning electron micrographs (c–e, h) of *Mystrium* sp. (a, b) Ants in laboratory colony; note the different morphology of the male (arrowhead in (a)). (c) Head of worker (dorsal view) with spoon-shaped mandibles, arrowhead points at smooth mandible edge along which the mandibles slide, *cs* indicates the clypeus which is enlarged in (e). (d) Fronto-lateral view of tip and inner face of a mandible; note the hump (arrowhead) which is involved in the release of the mandible snap. (e) The frontal edge of the clypeus *cs* protrudes above the labrum *lb* and carries a row of putative mechanoreceptive sensilla (arrowhead). (f) The sensilla are embedded in the thick clypeal cuticle *cu* and terminate in plug-like structures *p* one of which is depicted in (h) together with its socket. (g) Chordotonal organ thought to measure mandibular tension; *mn* mandibular nerve, *am* accessory muscle fiber, arrowhead points at sensory cell bodies; scale bars: 2 mm in (a, b), 1 mm in (c), 200 μm in (d, e), 100 μm in (g), 50 μm in (f), 20 μm in (h).

and the control of the mandible snap in *Mystrium rogeri*. We demonstrate how small alterations in the general design of mandibles can lead to substantial functional changes and specializations, and yet allow the ant to perform all the subtle movements and force adjustments required for the delicate work involved in social interactions and brood care.

2. Material and methods

A colony of *M. rogeri* Forel was collected at Kirindi Forest, 48 km east/northeast of Morondava, Madagascar, under a baobab log on the ground, and kept in a moist plaster-of-Paris nest at 25°C and a 12 h light/dark cycle in the laboratory. The ants were fed freshly chopped crickets or flightless *Drosophila* daily. For comparison, we also studied a laboratory colony of *Amblyopone* sp., classified as '*sp. nr. reclinata*' by Stefan Cover in accordance with Brown (1960), and collected by Jurgen Liebig at Jog Falls, India (voucher specimens of the *Mystrium* and *Amblyopone* species were deposited in the ant collection of the Museum of Comparative Zoology of Harvard University, Cambridge, MA, USA).

2.1. Behavior

The behavior of unrestrained ants was observed in their nest and foraging arena and was videotaped with standard video equipment or using a high-frequency video camera and electronic strobe light (NAC HSV400) at 400 frames/s (strobe flash duration 20 μs). The defensive mandible snap was released by moving an object (e.g. forceps) into the nest entrance. To examine a possible involvement of pheromones in the defensive behavior, the freshly dissected mandibular, Dufour-, poison, and pygidial glands were crushed on the tips of hardwood applicators. Each applicator was then placed at the nest entrance, and after 30 s the number of snapping ants at the applicator were counted. As control we used blank applicators.

To analyse mandible movements in close-up videog-

raphy, ants had to be restrained. They were held with forceps at the thorax and the mandible snap was elicited by touching a mandible tip with a pin. To overcome the temporal limitation of the video system (2.5 ms), anaesthetized ants (enflurane vapors; Ethrane (Abbot)) were fixed at their heads and illuminated with a high-power red light-emitting diode from beneath. Through a microscope the magnified head was projected onto a ground-glass where the outlines of the mandibles were detected by two phototransistors arranged on the ground-glass so that the onset and the final position of the moving mandibles could be recorded (Gronenberg et al., 1993). In this way we were able to record the movement on a digital audio tape recorder (Biologic DTR 1800).

2.2. Morphology

The external morphology of the head and mandibles was examined by scanning electron microscopy of critical point-dried specimens using a Zeiss DSM 962 SEM. The internal organization of muscles, apodemes, nerves, and the central nervous system within the head was studied from aldehyde-fixed and then serial sectioned material (5–15 μm) stained with osmium-ethyl gallate or with methylene blue (Gronenberg, 1995a). In addition, sensory afferents originating from mandibular sensilla and sensilla on the clypeus were labelled retrogradely with Neurobiotin (Vectorstain). The tracer was applied to the stumps of the cut sensilla and after diffusion periods of 2–6 h, the heads were processed according to standard routines (paraformaldehyde fixation, reaction with lucifer yellow-coupled avidin (Vector), ethanol dehydration, plastic embedding (Fluka Durcupan), sectioning at 15 μm, epifluorescence micrography; Gronenberg and Peeters (1993)). Graphical reconstruction and microscopical measurements were made using a calibrated camera lucida attachment to the microscope.

2.3. Muscle recording

To record the electrical activity of mandibular muscles, ants were anaesthetized with enflurane and the head was attached with wax to a supporting rod. The cuticle of the head was pierced with sharpened tungsten needles and fine insulated copper wires were introduced into the respective muscle (two wires each for bipolar recording) and fixed in place with wax. A grounded reference electrode was placed in the thorax or gaster. Action potentials were amplified (DAM 50 differential amplifier, WPI) and digitally stored on magnetic tape (Biologic DTR 1800) together with the output of the phototransistors that recorded the mandible movement.

3. Results

3.1. Unrestricted behavior

The experimental colony consisted of a queen, a few alate females, about 70 workers and some brood (larvae and pupae). After about 2 months in the laboratory, the queen died and accordingly the production of new workers declined and later only males emerged from the pupae. During the first 2 months the ants appeared more aggressive and usually several workers were out in the arena to forage.

Mystrium workers generally walk around with closed mandibles (the tips of the two mandibles meet; Fig. 1(a,b)). This corresponds to the normal, relaxed position. During prey-catching the ants will open their mandibles before biting. Between the fully open (Fig. 2(a)) and the closed position (Fig. 2(b)) each mandible moves through an angle of about 38°. We found this normal mandible working range to be similar to that of the sister genus *Amblyopone*.

Unlike other ants, *Mystrium* are able to move their mandibles further to the inside by an approximate angle of an additional 40°, in such a way that the mandibles cross over (Fig. 1(c), Fig. 2(c)). This movement is very fast and cannot be resolved by the unaided eye or by normal videography. It is the main subject of the present study and will be referred to as the mandible snap. From the fully open to the crossed-over position each mandible covers an overall working angle of about 80°.

During the first 2 months (supposed to represent healthy colony conditions) workers vigorously defended their nest entrance and vicinity when they were disturbed by an ‘intruding’ object such as a pin or forceps. Unlike *Amblyopone*, *Mystrium* rarely bit the object (as they would do when confronted with prey) but instead used their mandible snap against the ‘intruder’. Since our pins and forceps would not yield to a mandible strike of the tiny animals, the ants were themselves propelled backwards by the power of their own mandible snap. This is shown in Fig. 3 where the snap is performed during the 2.5 ms following frame ‘0’. During the next 20 ms the ant performs a full looping, thus indicating how much power is released by the mandible strike. We have observed other cases where ants snapped themselves away for more than 10 cm, a large distance for such a small animal. However, under natural conditions small arthropods about the size of *Mystrium* are the probable intruders against which the mandible snap is employed. They would be stunned and catapulted out of the nest entrance by the snap.

When poking with forceps or a toothpick in the nest entrance area, only one to five ants that were touched directly by the object would snap their mandibles. When the object was contaminated with the secretions of one mandibular gland, the response was much stronger and

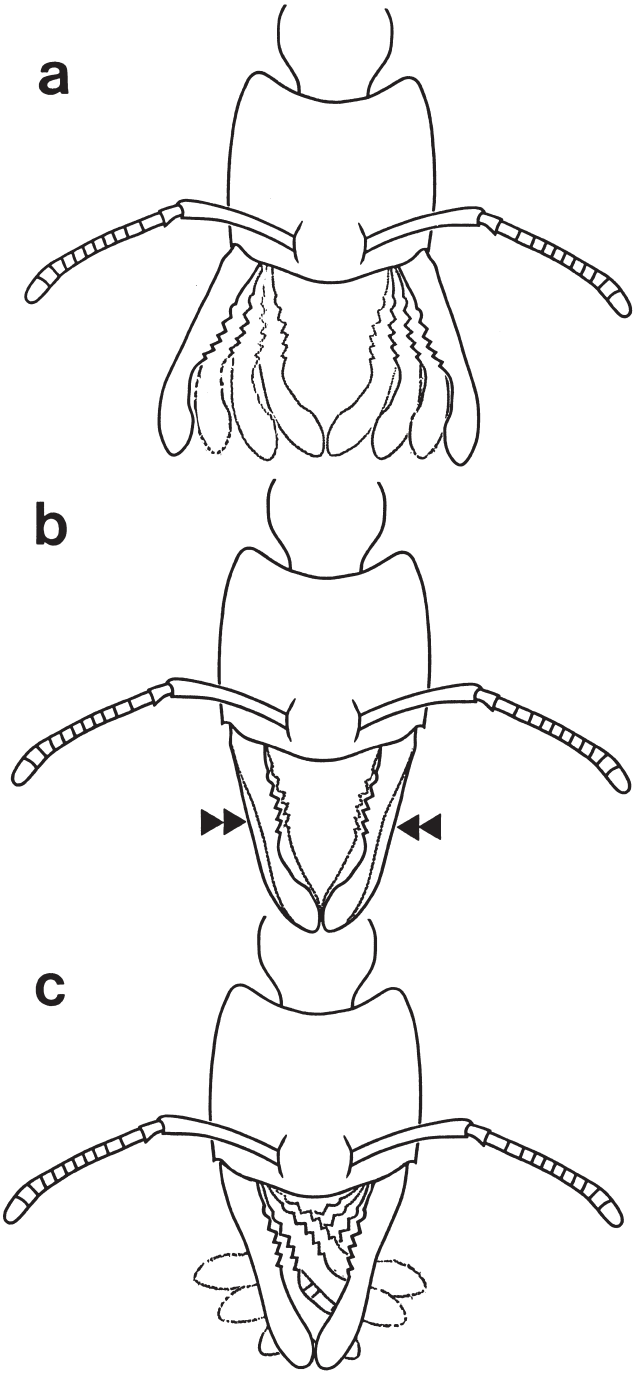

Fig. 2. Mandible movements in *Mystrium* (schematized): the 'normal' range (a) from fully open (solid) to closed (broken lines), the 'flexing' (b) before the snap (arrowheads indicate mandible bending), and the snapping movement (c) which results in crossed mandibles (broken lines).

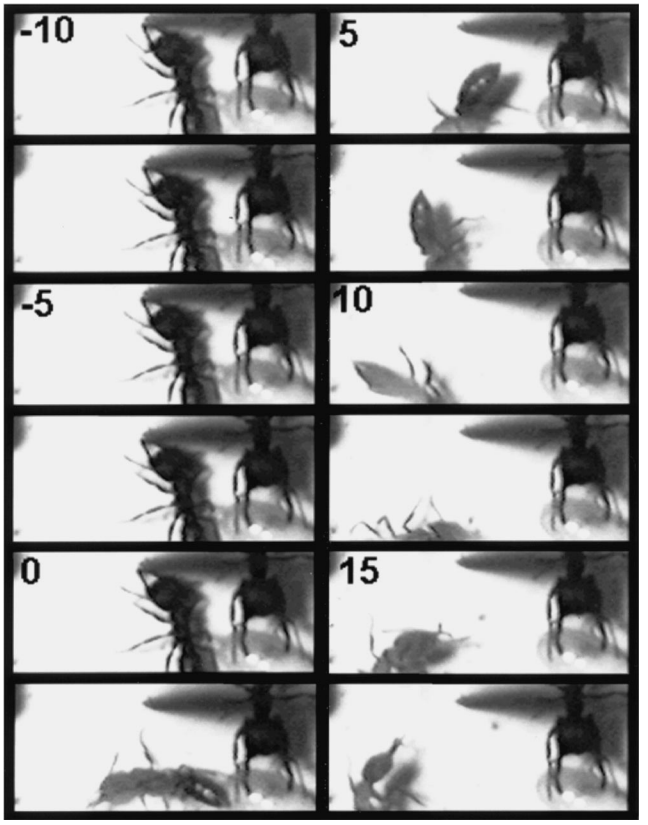

Fig. 3. High-frequency videographs showing a mandible snap response under unrestrained conditions. Each frame represents 2.5 ms, numbers indicate time before (− 10 etc.) or elapsed after the snap which occurs at time 0. The ant on the right carries a larva and does not move during the entire sequence while the left one presses her right mandible against a pin shown at top right of each frame and only slightly moves her antennae before the snap. The snap against the pin catapults the ant out of the frame in a looping.

five to eight ants would exhibit the snapping behavior (control: 2.37 ± 0.81; test: 6.62 ± 1.06; x ± SD; $n = 8$ trials). None of the other gland secretions tested elicited this escalated aggressive response. This indicates that the mandibular gland, but not the other exocrine glands located in the gaster, contains a component that acts as an alarm pheromone and modulates the aggressive mandible snap.

Some time after the queen had died, the behavior of the colony changed. When a foreign object was introduced into the nest entrance, the ants did not defend it any more. Instead, they would antennate the object and withdraw quickly into their nest chamber. Even the application of mandibular gland secretion evoked only a moderate defensive response and only one or two ants would strike against the object. Hence, the mandible snap is modulated not only by pheromones but also depends on the condition of the colony (tested individually, all the ants were able to perform the mandible snap when held by forceps and teased).

3.2. Temporal properties of the mandible snap

Under natural conditions (unrestrained animals), a novel object (or intruder) is always antennated before the mandible snap is employed. Before the snap is initiated, the respective object is touched with the lateral

side of the mandible tip (Fig. 3). If the antennae are still extended from previous antennation, they will always be withdrawn immediately before the mandible snap. The antennal retraction takes 41.8 ± 17.7 ms (n = 10; minimum duration of antennal movement 25 ms, maximum duration 80 ms) and starts 25–100 ms before the mandible strike. The mandible snap is released within 2.5–30 ms after the antennal withdrawal is completed, most often immediately (one frame = 2.5 ms) after retraction of the antennae (in Fig. 3 the end phase of antennal movement can be traced up to frame '0' after which the strike is released).

Under unrestrained conditions the mandible snap always occurred between two video frames (within less than 2.5 ms). The actual strike is preceded by a much longer phase during which the closed mandibles are slightly bent inwards (Fig. 2(b)), and which may take up to about 1 s (about 400 ms in the example of Fig. 4(a)).

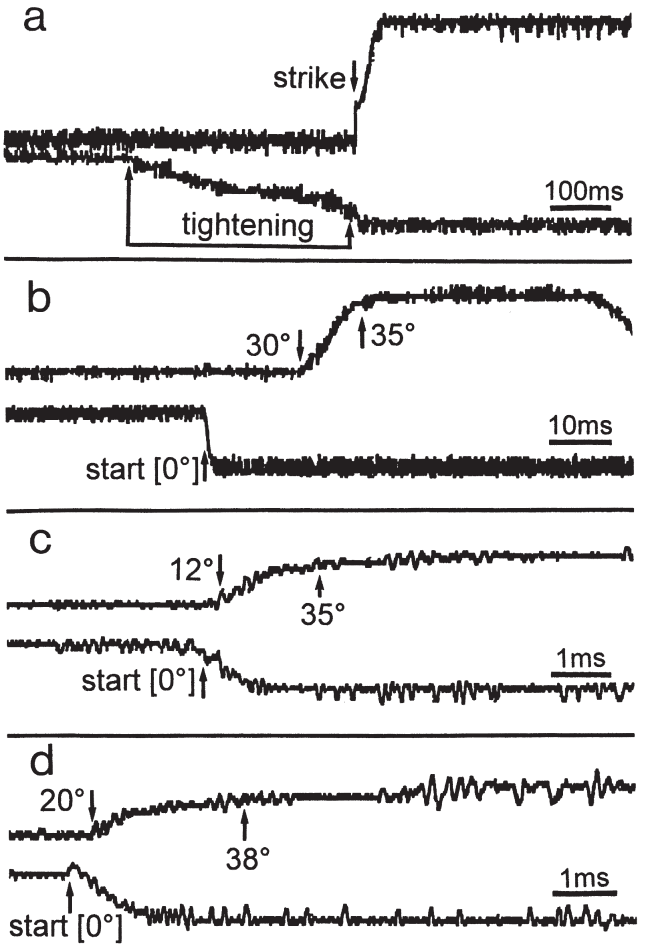

Fig. 4. Mandible snaps traced with two opto-electronic sensors (upper and lower traces); (a) flexing of the closed mandibles (tightening) before the spap is released (strike); (b) slow (mandibles not fully powered) and (c, d) faster snaps released at start, rapidly accelerated through 30°, 12° and 20°, respectively, and slowly decelerated to 35° or 38°, respectively. Note different time scales; jittery appearance of recording traces result from electric noise, not from mandible vibration.

During this time, the mandible closer muscles contract (see below). As the mandible tips already touch each other, the mandibles cannot move any further and the mechanical energy acting at their bases thus leads to deformation of the mandible. This deformation represents a form of energy storage. However, a much larger proportion of the mechanical energy set free by the muscle contraction is stored within the head (see 'Mandible muscles', Section 3.5).

Optoelectronic measurements of the mandible movement show that the strike may not always be of the same velocity (we show two examples for slow snaps in Fig. 4(a,b), and two fast ones in Fig. 4(c,d)). This probably results from differences in the cocking duration and in the amount of muscular power activated in advance of the snap. In addition, the two slow examples in Fig. 4(a,b) probably do not reflect the natural time course (these ants were fixed at their heads and had been repeatedly stimulated and teased). However, one important feature can clearly be seen in the slow examples (Fig. 4(b)) as well as in the fast ones (Fig. 4(c,d)): the mandibles are rapidly accelerated from the start through 30°, 12° and 20°, respectively, and slowly decelerated before they reach their final position at 35° or 38°, respectively. While the entire movement takes about 2 ms in Fig. 4(c and d), most of the time is consumed by the slower deceleration. Under normal conditions a potential intruder is hit at the peak mandible velocity after the mandible has travelled 10–20° within about 0.5 ms. The longer deceleration phase only occurs when no object has been hit and is thus irrelevant for the success of the strike.

In ants tethered at their thorax, the mandible snap can be reliably released when the lateral side of the mandible is stimulated mechanically. Contact of an object with the spoon-shaped mandible tip is most effective and, under natural conditions, generally appears to precede the mandible strike. However, in restricted animals we were able to release the snapping response by touching any point on the lateral side of the entire mandible including the mandible base. We therefore assume that the efficacy of mandibular neuronal input does not differ substantially between apical and distal mandible hairs, but a small object positioned (and releasing the strike) at the base of the mandible would receive a much less vigorous or no blow at all because of the deceleration and the limited range of the mandible tips (see Fig. 1(c), Fig. 2(c)).

We did not find any 'handedness' with respect to the mandibles: both jaws are functionally equivalent and either mandible can slide across the upper side of its counterpart. Closer inspection revealed that the mandible to which the touch stimulus is applied always will slide underneath (on the ventral side of) the unstimulated one. Hence, the mandible which strikes at the object or opponent is always the upper one. While we do not know

the behavioral significance of this peculiarity it certainly requires a side-specific motor control.

3.3. Morphology of the mandibles

The slender mandibles of *Mystrium* are longer than their heads and end in spoon-shaped tips. The inner face (the leading edge during biting movements) is covered with a double row of blunt teeth which apparently are not designed to penetrate prey (Fig. 1(d)). This contrasts with the mandibles of *Amblyopone*, the genus to which *Mystrium* appears to be related most closely. In the former genus, the mandibles form exquisite hunting weapons: they are slightly shorter than the head, are more pointed and carry apical teeth, and the inner rim of the mandibles is equipped with a row of sharp and inward pointing teeth that would penetrate the prey. These features also serve to discriminate the two genera taxonomically (Bolton, 1994). Unlike *Amblyopone*, the mandibles of *Mystrium* are equipped with a narrow and smooth dorsal (arrowhead in Fig. 1(c)) and ventral rim across which the mandibles slide when they cross over.

Like in other insects, the mandible is jointed to the head capsule by a hinge and rotates around a virtual dorso-ventral axis. Scanning electron microscopic inspection as well as manually moving the mandibles of live ants under the microscope shows that the fitting between the mandible joint and the head capsule is not very tight and allows slight rotaions around the mandible's long axis. This can explain the release of the mandible snap: within the spoon-shaped tip of the mandibles we found a peculiar hump (arrowhead in Fig. 1(d)). When the mandibles are closed, the two humps of the left and right mandible touch each other and seem to serve as a pivot. When the closed mandibles are cocked (set under tension), the two rounded humps will ride on each other in a kind of labile equilibrium. A slight rotation of one mandible around its long axis (clockwise with respect to the ant's right mandible) will disturb this balance and will let slide the other mandible across its counterpart. Hence, we think that the mandible snap is released by a slight inward rotation of the stimulated mandible's ventral side. However, due to the limited temporal and spatial resolution of our high-frequency video system we were not able to distinguish such minute movements supposed to occur only microseconds before the strike.

3.4. Morphology of sensilla

M. rogeri has minute eyes composed of only 10 ommatidia, and species such as *Mystrium camillae* are totally blind (Moffett, 1986). Accordingly, the sensilla covering the antennae, head and mouthparts are of special importance for prey-capture as well as defence. The lateral face of the mandible is covered with relatively short hairs (length ranging from 10 to 25 μm) thought to be involved in the activation of the mandible snap. In microscopic sections all these hairs appear to be innervated. The sensory afferents originating from these hairs (one per sensillum) project through the mandibular nerve into the central nervous system.

This is also true for the large blunt teeth at the inner face of the mandible (Fig. 1(d)) and at the mandible tip. These teeth are hump-like protrusions composed of harder cuticle (the more sclerotized cuticle appears darker in microscopic sections). The teeth are embedded deeply in the 'normal' (less sclerotized) cuticle which forms the mandible wall. Like other sensilla, the teeth are contacted by sensory dendrites. Unlike hair sensilla, the teeth do not seem to be sensitive to weak touch stimuli. Since the teeth are very firm and rigidly connected with the surrounding cuticle, we suppose that they measure strong strain within the cuticle such as would occur during biting and grasping of objects. We have found similar putative stress receptors in the mandibular teeth of other ants (Gronenberg and Tautz, 1994).

Another type of putative mechanoreceptive sensillum is shown in Fig. 1(e,f,i). A single row of these peculiar sensilla resides on the lip-like anterior rim ('apron') of the clypeus which covers the labrum and the mandible bases (Fig. 1(e)) in all Ambyoponini (Brown, 1960). Close-up electron micrographs (Fig. 1(i)) and microscopic sections (Fig. 1(f)) suggest that these sensilla function like bumpers in which a robust 'plug' is pressed into its socket. Unlike in the mandibular teeth, which are immobile, the insertion of the 'plugs' of the clypeal sensilla probably allows a certain degree of mobility. Touch stimuli directed to the 'plug' could thus excite the single dendrite which is attached to its inner face. In *Amblyopone* a touch stimulus directed at these clypeal sensilla reliably released a mandible strike (which, in these animals, does not involve any catapult mechanism or elastic energy storage). We speculate that in *Mystrium*, like in *Amblyopone*, these sensilla are involved in normal prey-catching behavior (with open mandibles) but not in the mandible snap, because the jaws are already closed before the snap and the clypeal sensilla are thus prevented from being touched by prey or an opponent.

3.5. Mandible muscles

As is the case in other ants, in *Mystrium* the mandibles are moved by a single large closer and a much smaller opener muscle. About three-quarters of the entire head volume is taken up by these muscles, both of which are composed of many tubular muscle fibers and attach to the mandible via apodemes (Fig. 5).

The opener apodeme attaches lateral with respect to the closer apodeme which inserts close to the inner face of the mandible base (Fig. 5). Contraction of the opener

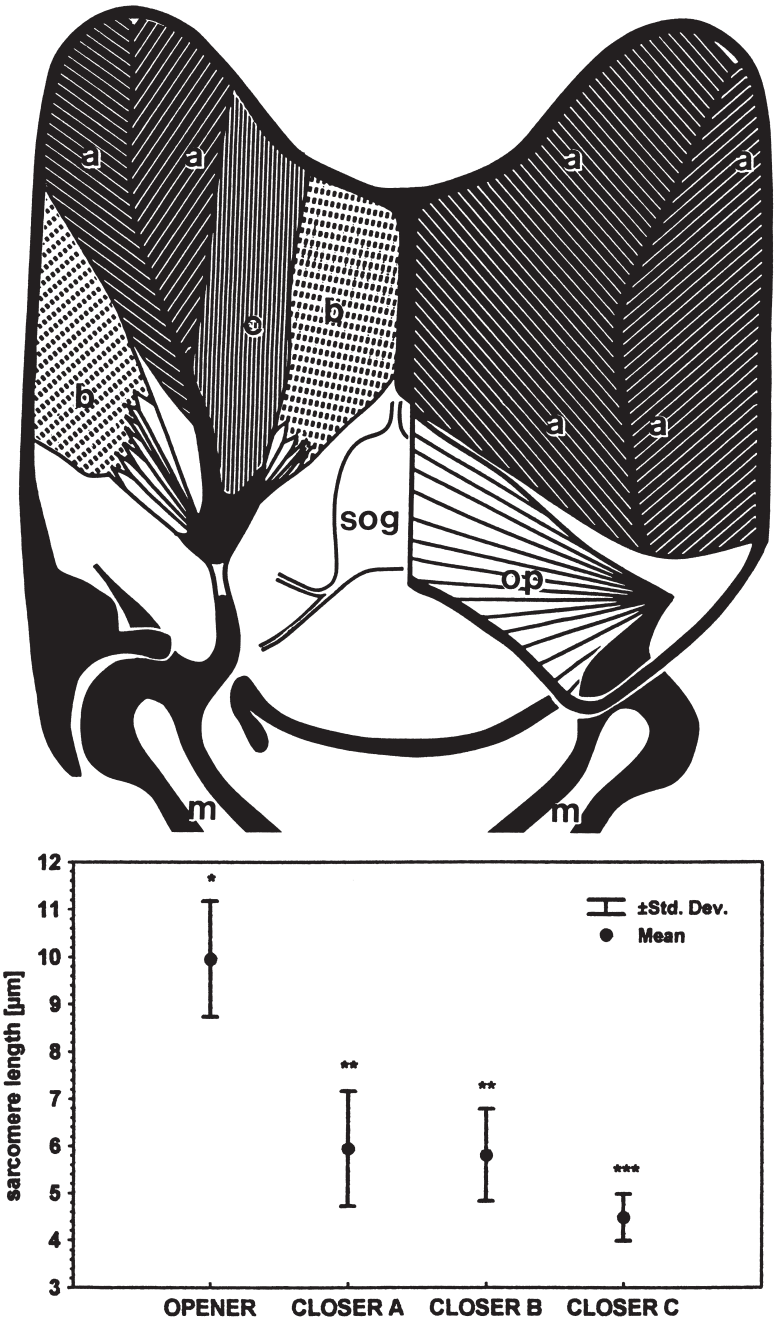

Fig. 5. Distribution of fiber types composing the mandibular muscles. Top drawing represents the head at two different dorso-ventral depths (left: central region at the level of the suboesophageal ganglion *sog*; right: about 150 μm from the ventral surface). Opener fibers *op* and closer fibers type (a) and (b) are supposedly slow, type (c) is supposedly faster; m, mandible. Bottom diagram shows the sarcomere lengths of the fiber types shown above; statistically significant differences indicated by different numbers of arrows above standard deviation (*t*-test, $P = 0.01$, $n = 25$ fibers, 2 animals).

muscle fibers, most of which originate from central and centro-frontal parts of the ventral head capsule (Fig. 5), will rotate the mandible outwards. While the opener muscle is much smaller than the closer muscle, it appears large when compared to opener muscles of other ants. Likewise, the opener apodemes are larger and more sclerotized (more tanned) than those of other ants (Gronenberg et al., 1997). This indicates that, unlike 'normal' ants, in *Mystrium* the opening movement requires more power because the friction between the two crossed-over mandibles has to be overcome before the mandibles will open completely. The sarcomere lengths of the muscle fibers composing the opener muscle indicate the same: the opener sarcomeres are almost twice as long as those of the closer muscle (9.95 ± 1.2 μm; diagram in Fig. 5), implying that they contract more

slowly but generate more power than do the shorter closer muscle sarcomeres. Moreover, the opener muscle fibers attach via thin cuticular threads to the main body of the opener apodeme (Fig. 1(h)), another indication of their slow shortening characteristics (Gronenberg et al., 1997).

The mandible closer apodeme is much larger and more complex than the opener apodeme. Its main part is sclerotized heavily and gives rise to less rigid (almost untanned) velum-like processes. These extend into the posterior head space almost up to the head capsule (Fig. 5) to collect the posterior, lateral, dorsal and ventral closer muscle fibers and funnel their force into the sturdy main apodeme. This connects to the mandible base by a thick untanned ligament which is probably more elastic than the main body of the apodeme and thus permits the positional changes resulting from mandible movements. It is also suited to store elastic energy prior to the snap. Like the opener fibers, part of the closer muscle fibers connect to the main apodeme via cuticular threads (fiber type 'b' in Fig. 5). These do not promote fast contractions of the attached muscle fibers, but make the best use of the available space and permit a more suitable angle of attack for the attached fibers (Gronenberg et al., 1997). Moreover, these cuticular fibers together with the entire apodeme probably serve to store mechanic energy by elastic deformation in advance of the mandible snap.

The mandible closer muscle is not only larger than the opener; it is also composed of different fiber types. The majority of closer muscle fibers (type 'a' in Fig. 5) directly attach to both sides of the velum-like processes of the apodeme (Fig. 1(g)). They are composed of sarcomeres significantly shorter than those of the opener muscle fibers (5.9 ± 1.2 μm; Fig. 5). These type 'a' muscle fibers always attack at a steep angle with respect to the main direction of pull and probably provide the main power involved in normal mandible action (grasping, holding, crunching) and are required for cocking the mandibles before the snap. We have mentioned already the thread-attached fiber type 'b'. They have essentially the same sarcomere length (5.8 ± 1 μm; statistically not significantly different) and similar angles of attack and, despite their different way of attachment, they seem to serve the same function as the type 'a' fibers. Type 'c' closer muscle fibers are different from type 'a' and 'b'. They are the longest fibers, directly attach to the main body of the apodeme, are arranged in parallel with respect to the axis of pull (Fig. 5) and have statistically significant shorter sarcomeres than the other fibers (4.5 ± 0.5 mm; $P = 0.01$). All these features indicate that they are faster muscle fibers. These fast muscle fibers traverse the head almost in the mid-horizontal plane and attach almost centrally to the apodeme. This indicates that they do not rotate the mandible around its long axis. We thus have no indication that the fast closer muscle fibers might have a triggering function for the mandible snap. They are probably involved in the generation of faster predatory jabbing movements during 'normal' mandibular actions.

For comparative reasons we have also measured sarcomere lengths of homologous muscle fibers in *Amblyopone*. In both genera we found the same fiber composition of the mandible closer muscle and the respective fiber groups occupied very similar locations within the head capsule. However, one difference was evident: all closer muscle fibers in *Amblyopone* have shorter sarcomeres than their counterparts in *Mystrium* ('slow' fibers 4.2 ± 0.7 μm, 'fast' fibers 3.2 ± 0.4 μm; 16 fibers from two animals measured from each type). Sarcomere lengths of 'slow' and 'fast' fibers are statistically significantly different from each other and from those of *Mystrium* ($P = 0.001$). Only the differences between 'slow' fibers of Amblyopone and 'fast' (type 'c') fibers of *Mystrium* were not statistically significant ($P = 0.18$).

3.6. Muscle activity

Our electromyograms were not suited to discriminate the different muscle fiber groups or to assign a muscle potential of a certain amplitude to a particular fiber type, but we could clearly discriminate opener and closer muscle activity. Fig. 6(a,b) shows an example of 'normal' mandible movements and associated muscle activity. We were able to discriminate up to six different units in the closer muscle by their amplitude (not regarding artifactual amplitude fluctuations due to muscle movements). This can be seen in both the opener and the closer trace of Fig. 6(a). Likewise, we found up to three different units in the opener muscle. Force and velocity of mandible movements are controlled by the frequency of muscle potentials, the number of active motor units and also by co-contraction of closer and opener units. This is demonstrated by Fig. 6(b): before the opening starts, the high-frequency closer discharge stops and only little closer activity is seen during the onset phase of the opening. With increasing opener muscle activity the closer also increases firing, and in the open position the mandible is held through the simultaneous activity of two closer and two opener units.

When more power is required for a movement (during the experiment, we pressed against the moving mandibles, thus increasing the load), the spike rate of a particular unit is increased and additional units are activated, suggesting that units may be specialized for tasks requiring little force (e.g. carrying of brood) or for powerful action (biting). Moreover, some muscle fibers were always activated in a particular mandible position (closer muscle in Fig. 6(a)), indicating that a range fractionation exists and that not all muscle fibers can be activated at any position.

Recording motor activity during a mandible snap was more difficult than during normal mandibular move-

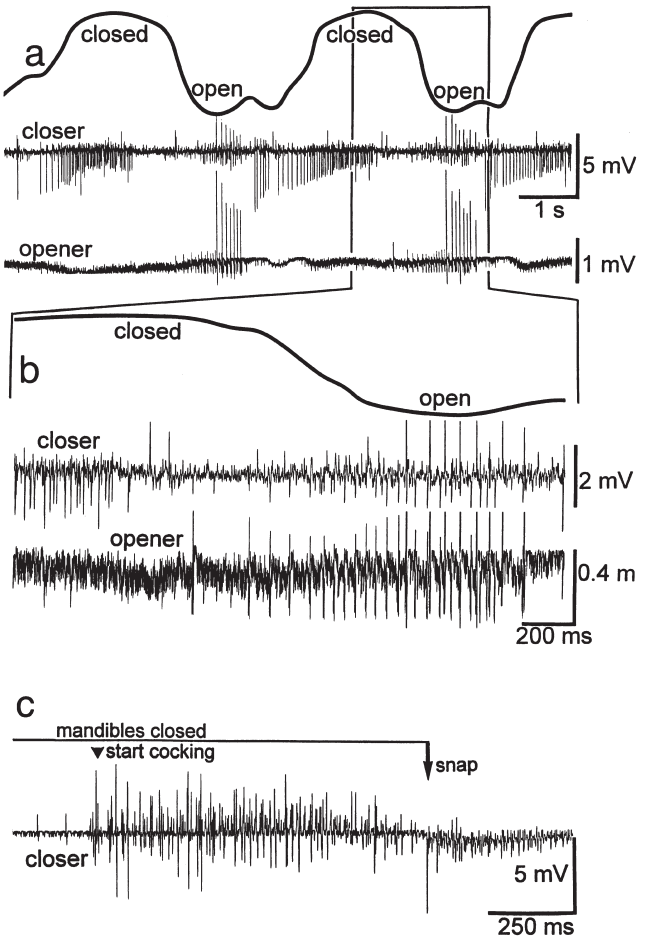

Fig. 6. Electrical recording of activity in the mandible closer (2. trace) and opener muscle (3. trace in (a) and (b)) during normal movements (a,b) and (c) before and during a mandible snap (arrow). Motor activity in (c) results in a slight inward bending (cocking) of the closed mandibles; top traces show the mandible position. Detail indicated in (a) shown at increased temporal resolution in (b).

ments because, with their head wired up and fixed to a support, the ants were not likely to perform their mandible snap. A typical record of the closer muscle activity is shown in Fig. 6(c). The mandibles are closed and only little activity is required to keep their tips together. The cocking movement, as indicated by a slight inward bending of both mandible shafts, is correlated with strong activity of three to four different closer muscle units which starts almost 1 s in advance of the snap. No unit shows particularly strong activity immediately before the release of the snap such as would be required for a triggering unit. While we cannot exclude the existence of such a specialized release motor unit (it might be located far away from the recording electrodes) it appears very unlikely because in none of the nine experiments in which we were able to provoke a snap while recording did we find any distinctive activity occurring immediately before the snap. We therefore conclude that the mandible snap is not released by the activation of a specialized fast motor unit or muscle, as is the case in trap-jaw ants (Gronenberg, 1995b, 1996a).

3.7. The neuronal substrate for the control of mandible movements

The behavior leading to the mandible snap is controlled by neurons in the suboesophageal ganglion (SOG). We will here describe a proprioceptive organ most likely involved in the control of mandibular force production, and the morphology of sensory input to and motor output from the SOG.

We have found a muscle receptor organ associated with the mandible and comparable (probably homologous) to a similar organ described for honeybees (Masuko, 1986). In principle, the organ consists of an elastic strand suspended between the frontal head capsule and the mandible closer apodeme (Fig. 7(a,b)) to which a group of about 15 sensory cells is attached whose axons join the mandibular nerve (Fig. 1(l), Fig. 7(c); the lump of sensory cells is referred to as the 'ganglionic mass' in the bee; Masuko, 1986). These sensory cells will be stretched upon closer muscle contraction. In addition, the receptor organ is attached to two parallel accessory muscle fibers (sarcomere length about 4.8 μm) which extend from the mandible base to the tentorium (Fig. 1(g), Fig. 7(b,c)). Mandible opening slackens these muscle fibers and accordingly decreases the tension acting on the sensory cells. We suppose that the two muscle fibers serve to set the working range of the sensory neurons. Some of the sensory cells are in particularly close contact with the muscle fibers suggesting that they may serve as muscle stretch receptors. Thus the actual activity of the sensory cells will depend on the position and the load of the mandibles as well as on the contraction state of the accessory muscle fibers. We found an almost identical muscle receptor organ in *Amblyopone*, and cursory inspections of other ant genera (*Myrmecia*, *Odontomachus*, *Harpegnathos*, *Atta*, *Camponotus*) suggest that such a muscle receptor organ is common among ants.

Mandible movements are controlled primarily by sensory input arising from mandibular sensilla. We have traced the sensory afferents originating from mechanosensitive hairs on the mandible and involved in the activation of the mandible snap (Fig. 7(d)). The mandibular nerve carries axons of diameters ranging from less than 0.5 μm up to 3 μm but no unusually thick fibers that would suggest a particularly fast mechanosensory reflex such as is found in trap-jaw ants (Gronenberg et al., 1993). Likewise, the terminals of individually stained neurons in the SOG do not suggest a particularly fast signal transmission between the sensory axons of mandibular hairs and mandibular motor neurons: we did not find many collaterals or even presynaptic 'blebs' in close contact with the primary motor neuron dendrites.

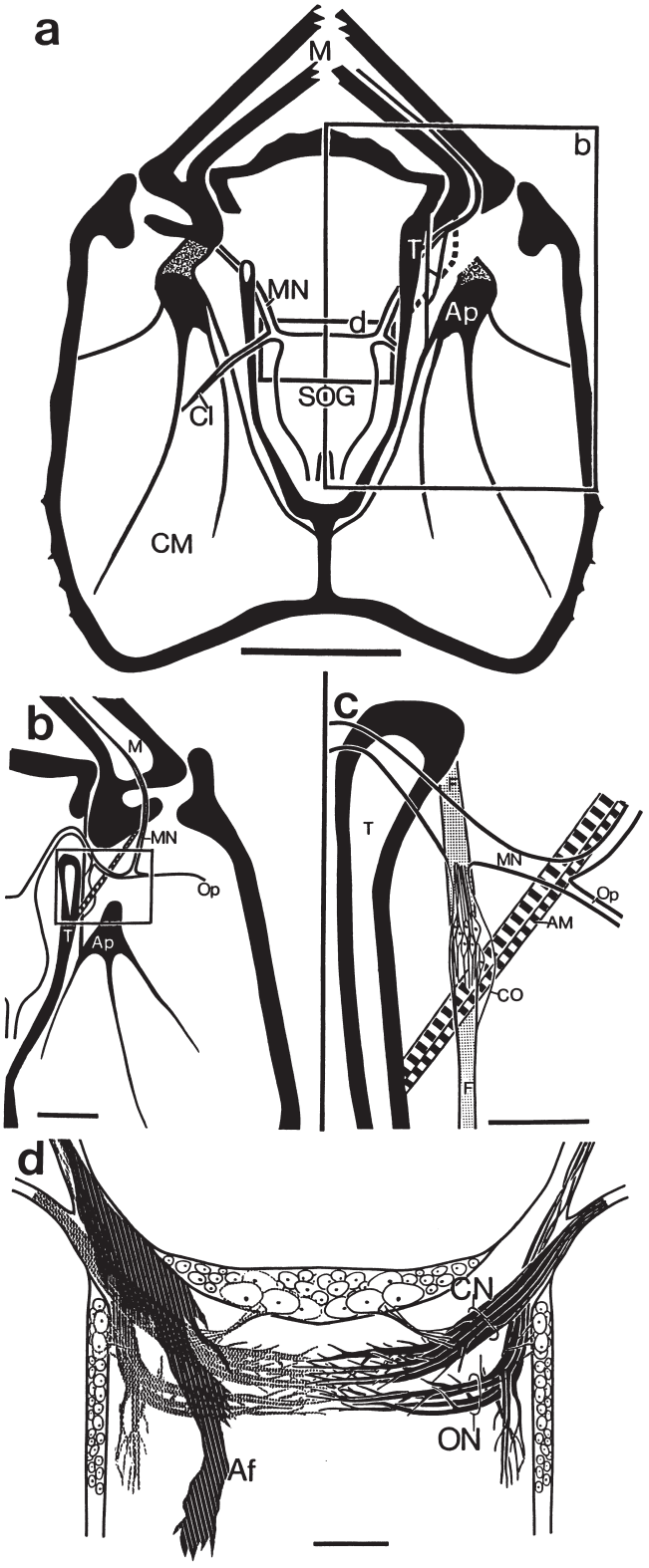

Fig. 7. The mandibular chordotonal organ (a–c) and mandibular neurons (d) in the suboesophageal ganglion SOG (dorsal views). (a) Position of the organ suspended within the head between the mandibles *M*, the tentorium *T*, and the mandible closer apodeme Ap; boxed areas (b) and (d) enlarged in (b) and (d), respectively. (c) Detailed view of the area boxed in (b); in (d) opener ON and closer CN motor neuron processes and cell bodies are shown solid on the right and cross-hatched on the left, where the projections of mandibular sensory afferents A_f are overlayed (hatched); accessory muscle fibers, AM; closer nerve, Cl; closer muscle, CM; chordotonal organ, CO; attachment fibers, F; mandibular nerve, MN; mandible opener nerve, Op; scale bars 500 μm in (a) 200 μm in (b) 100 μm in (c) 50 μm in (d).

Afferents originating from the putative touch receptors on the clypeus (Fig. 1(i)) project through the tritocerebrum and the SOG. They feature more terminals in the anterior and dorsal neuropil than do the mandibular hair afferents and many of their blebbed terminals are found in close vicinity of the mandibular motor neurons. Hence, it is likely that mandibular motor neurons receive monosynaptic input from the clypeus.

The mandible closer and opener muscle motor neurons supply their respective muscles through separate nerves (Fig. 7(d)). We found four opener motor neurons (axon diameter in the nerve about 2.5 μm) whose somata reside antero-laterally in the cell body rind (Fig. 7(d)). Their axons bifurcate and, before bending towards the midline (Fig. 1(k)), give rise to branches that descend posteriorly.

The mandible closer motor neurons leave the SOG through the closer nerve which carries about 10 profiles. Five of these form the largest profiles in the anterior SOG (diameter 2–3 μm in the nerve and up to 5 μm in the SOG) and are supplied by equally large cell bodies (diameters about 20μm) which reside in anterior parts of the cell body rind (Fig. 7(d)). They directly proceed towards the midline of the SOG without giving rise to (large) descending collaterals. Centrally, their finer dendritic branches partly overlap with their contralateral homologues (Fig. 7(d)). However, the overall organization of mandible closer motor neurons suggests that most of their rapidly tapering dendritic branches mainly integrate ipsilateral sensory input and do not receive particularly prominent direct input from sensory hairs on the mandible. We found essentially the same design of the mandibular sensory and motor system in *Amblyopone* and in *Mystrium*. Hence, the different defence mechanisms of the two genera which are based on distinct mandible shapes and modified muscle properties are controlled by similar neuronal networks.

4. Discussion

Little is known about the biology, and particularly about the prey capture behavior, of *Mystrium*. Our examination confirms the view of Moffett (1986), who suggests that the mandible snap is not involved in prey capture and only serves defensive purposes. However, we have not observed actual predation in our laboratory colony and thus cannot rule out that the mandible snap of

Mystrium may also occasionally be involved in predation.

4.1. Generation of the mandible snap

Our findings suggest that the mandible snap corresponds to the following scenario:

The snap is preceded by antennation of the respective object during which the ant gathers chemosensory and mechanical information about the object and its position. In preparation for the strike *Mystrium* arranges herself in such a way that the lateral side of her mandible tip touches the object. Mechanosensory information from mandible hair sensilla notifies the central nervous system about the correct position and probably initiates the next phase, the cocking of the mandibles. The large closer muscles contract against the closed mandibles which are thus bent and store some mechanical energy. However, most of the muscular energy is transformed and elastically stored within the apodeme and its cuticular threads, within the muscle fibers and probably also within the entire head capsule, as has been observed in comparably sized trap-jaw ants (Gronenberg et al., 1993).

Resulting from the unsymmetrical mandibular sensory input, some ventral closer muscle fibers of the touched mandible probably contract more strongly than their contralateral homologues. This is a speculation that could only be proved by an extensive series of muscle recordings which is beyond the aim of the present account. Still, this explanation best matches our findings. This slight ventral rotation causes the ventral edge of the stimulated mandible to bend slightly inwards. Since the 'humps' at the mandible tip serve as a pivot, this small movement unbalances the pivot and lets the contralateral mandible slide above the other one, powered and rapidly accelerated by the mechanical energy stored within the contracted closer muscles and also the mandible shaft. Immediately afterwards the unstimulated mandible hits the object and bounces it away. The stored energy thus is spent and the mandibles are decelerated during the second half of their trajectory and come to a hold before they could bump into the clypeus.

The defensive mandible strike of *Mystrium* is similar to that found in some termites. Soldiers of the genus *Termes* employ essentially the same mechanism in their symmetrical mandibles while some genera have even more specialized, unsymmetric mandibles (*Capritermes*, *Pericapritermes*, *Neocapritermes*, *Planicapritermes*; Krishna and Weesner, 1969). In *Homallotermes* this specialization has so far advanced so that only one mandible strikes while the other one is more elastic and serves as a spring (Deligne et al., 1981). The advantage of the snap-jaw design is that it can be employed within a narrow tunnel and does not need as much space as do the gaping mandibles of trap-jaw ants.

The basic design underlying the mandible snap in termites and *Mystrium* and the strike of trap-jaw ants employs a catapult mechanism (as do most insects that perform particularly fast movements; Bennet-Clark and Lucey, 1967; Heitler, 1974; Bennet-Clark, 1975; Christian, 1979; Alexander, 1995; Gronenberg, 1996a). However, unlike the snapping mandibles of *Mystrium*, the trap-jaw mechanism can be triggered precisely by specific sensilla and a specialized muscle in all the species examined (Gronenberg et al., 1993; Gronenberg, 1996b; Gronenberg and Ehmer, 1996). This suggests that the timing of the mandible snap is not crucial for its success which is a further indication of its defensive function: the blow is supposed to stun the adversary or to kick it off, and it seems not important to which particular body part or at which precise moment the blow is delivered.

The mandible snap can be explained in terms of a reflex-like sensory-motor interaction: touch information slowly activates the large closer muscles by direct (and probably also polysynaptic) sensory input onto the motor neurons, but additional input is required to start and modulate the reflex. Antennal sensory information precedes the cocking of the mandibles, and alarm pheromone from the mandibular gland increases the aggressiveness. The overall tendency to snap is also modulated by the condition of the colony: ants of the deteriorating queenless colony were much less likely to strike at an intruder even in the presence of alarm pheromone.

4.2. Sensory integration

For animals lacking visual feedback, mechanosensory input from the mouthparts is particularly important for foraging, prey capture or defence. The many hairs on the mandibles provide information about the position of objects close to or in contact with the mandibles. The sturdier putative touch receptors on the clypeus release the mandible closure in *Amblyopone* upon contact and may serve a similar function during prey capture in *Mystrium*. The sensory neurons associated with the large cuticular teeth on the mandible appear to represent a specialization for the assessment of large forces such as occur during biting. Similar sensory neurons have been described for the ant *Odontomachus* (Gronenberg and Tautz, 1994) and can probably be found in the mandibles of most ant workers.

It seems more difficult in terms of biological design to assess very small forces or movements of a particularly sturdy and large extremity such as the mandible. This task is accomplished by the muscle receptor organ. Mandibular muscle receptor organs very similar to those we describe here for ants have been found in the honeybee (Masuko, 1986), in beetles (Honomichl, 1976, 1978), and in stoneflies (Plecoptera; Liem et al., 1984). Different sensory cells of these organs assess the tension between mandible and mandible muscle as well as the contraction state of the accessory muscle fibers. We sup-

pose that the same is true for the mandible receptor organs of ants. In *Mystrium* (and other ants) the organ is quite elaborate (compared to the mandible receptor organs of other insects) suggesting that it supports the intricate movement control required for ant mandibles. While strong forces acting on the mandibles (large loads) can be assessed by a variety of mandibular receptors, we think that the muscle receptor organ is suited particularly well to the assessment and control of small forces and movements required for delicate actions involved in brood care, grooming or trophallaxis (food exchange between individuals). Only in bees is the mandible muscle receptor organ more complex (Masuko, 1986), presumably because bees do not perform powerful mandible movements but need a particularly fine force control when they care for their brood and build their straight, smooth and precisely oriented wax cells.

4.3. Motor control

The fiber composition of the mandible closer muscle in *Mystrium* is similar to that found in most other ants: a majority of slow fibers (attached directly or via cuticular threads) and a group of fibers with shorter sarcomeres supposedly involved in fast mandible movements (Gronenberg et al., 1997). In *Mystrium*, the sarcomere length between the two fiber types is not very different (4.5 and 6 μm, respectively), indicating that no strong specialization in fast and slow fibers exists. Likewise, our recordings suggest that the mandible snap is not released by the activation of specialized fast motor units. In *Mystrium* even the closer muscle fibers with short sarcomeres (type 'c' fibers) are not really fast when compared to those of other ants which have sarcomere lengths of about 3 μm (Gronenberg et al., 1997). The fastest muscles in ants may feature sarcomeres as short as 1.5 to 2.2 μm (Gronenberg and Ehmer, 1996).

This matches our behavioral findings: besides the specialized mandible snap, *Mystrium* do not seem to perform any particularly fast mandible movements. Interestingly, the highly significant statistical differences between homologous muscle fibers in *Mystrium* and *Amblyopone* nicely reflect the behavioral differences: unlike *Mystrium*, *Amblyopone* rapidly strike their mandibles together to catch prey. Our muscle recordings indicate that the control of mandible movement is much more complex than would be suggested by the existence of only two muscles, a closer and an opener. Except for the snap mechanism in *Mystrium*, these mandible movements are controlled similarly in other ants (considering the similar design of mandible muscles and motor neurons in other species) and provide an interesting system for the study of the fine control of movements and muscular power. Such an in-depth analysis would, however, require a better controlled apparatus for stimulation, force- and movement measurement, and the simultaneous recording from many muscle units. It would best be carried out on larger ants which are more accessible and about whose behavior and biology more is known. Regarding the snapping behavior, it would be interesting to examine analogous mechanisms and their control in termites.

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