# The Mouthparts of Ants

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# THE MOUTHPARTS OF ANTS -

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## General introduction

Beginning from unsegmented body appendages of annelids (parapodiums), further evolution created segmented limbs that gave the name to arthropods. These articulated extremities were endowed with the great potential to modify to sensory organs (antennae), locomotory organs (legs for walking, jumping, or swimming), copulatory apparatuses, and a variety of differently adapted mouthparts. Among insects, the mouthparts comprise a pair of mandibles and maxillae laterally, and a labium forming the lower lip. In addition, the unpaired labrum in front and the median hypopharynx behind the mouth are considered as mouthparts as well, although they are not derived from extremities. Primitive mouthparts converted to biting, chewing, licking, sucking, and piercing organs, often being used as tools also for tasks other than feeding. Possessing such a capacity of diversity, mouthparts contributed notably to the enormous success of insects, counted in numbers of species.

One of the most wide-spread groups of insects is constituted by ants. Besides the human being, ants are the predominant terrestrial organisms. According to calculations based on a conservative estimation that 1% of all living insects on earth belong to ants, the entire population of ants consists of approximately $10^{16}$ individuals, corresponding to a biomass as large as that of all humans (Hölldobler and Wilson, 1994). As social insects, ant colonies accommodate a caste of only one or few reproductively active individuals and another caste of infertile worker ants. In consequence of their social life, worker ants of different species share several of their daily jobs, such as caring for the tender brood, grooming the dependent queen, or guarding and defending the nest. But many other tasks that workers do are species-specific, in compliance with the ecological niche they are adapted to. Workers catch elusive prey, or cut tough leaves and use the plant material as substrate for their underground fungus gardens. They harvest seeds and hoard them in their food store within the nest, or collect nectar or honeydew and care for their aphid colonies. They gather all the dead animals of their size they can find by chance, or move forward in fan-shaped formations and systematically prey on almost everything they can get. They live as nomads, or construct their nests between leaves that are interwoven with silk. For almost any of these various tasks, that either reflect the species-specific lifestyle or are common among ants corresponding to their social life, ants use their mouthparts. The mouthparts are to ants what the bill is to birds, or what hands are to humans – the most important tools to master a diversity of tasks.

Since the mouthparts are crucial for the life of ants, they should be highly adapted to fulfil the requirements according to the specific ecological niche as well as to the peculiarity of a social life. Differences between species are thought to reflect particular adaptations. By using interspecific comparisons, the present study tries to point out distinct and shared characteristics of ant mouthparts and their physiological performance, including the whole system that controls their movements (joints, apodemes, muscles, motor neurons). For that purpose, the current research projects focus on biomechanics, functional morphology, behavioral physiology, and neurobiology. The respective differences between the various ant species are then correlated with species-specific lifestyle parameters such as feeding habits. This allows to derive common principles of how a movement apparatus and the accessory structures are optimized in nature to manage a variety of both specialized and standard tasks. Furthermore, it explains how specific adaptations enable a species to establish in an ecological niche during evolution. Because of their versatility within the same systematic group, ants are very well suited to address such questions, using a comparative approach.

# A. THE MANDIBLES

## 1. Review: Mandible movements in ants

## Introduction

For the majority of insects, the mouthparts are very important tools for almost any task. This is particularly true for the mandibles of ants. Ants employ their mandibles for fast or powerful actions like prey-catching, fighting, digging, leaf-cutting, and also for delicate tasks such as grooming, brood care, carrying nestmates, transporting liquids, and communication (Hölldobler and Wilson, 1990). Accordingly, ant mandibles have to perform many different kinds of movements in terms of velocity, force output and precision. While some tasks are common among ant species (e.g. brood care), others represent specific adaptations. Predators often have long jaws equipped with piercing teeth and sharp edges, whereas herbivorous ants have more compact mandibles suited for the special task of processing plant material (Gronenberg et al., 1997). In many ant species, however, the mandibles resemble a general type found in many other insect groups: they are sturdy, shovel-like, unsegmented limbs. But mandible specialization is not only based on shape but also depends on the speed of movement and on the force the jaws can generate. Catching elusive prey obviously requires different movement characteristics and tactics than cracking seeds.

Force and velocity of a limb movement depend on the muscles and the accessory structures that control the particular limb. In ants, the mandible design is simple. It conforms to the common mandible organization of other hymenopterans (Snodgrass, 1935, 1956): the mandibles are connected to the head capsule by a hinge joint, movable only in a single plane (inwards/outwards), operated by only a single closer and opener muscle on each side of the head. Even though opener-closer muscle co-contraction may occur, the mandible closer muscle is much larger and the key to the versatility of mandible functions (Gronenberg et al., 1998a). All the fast, forceful, or delicate mandible movements are generated by the mandible closer muscle.

Because of its relative simplicity and its great behavioral relevance, this movement system is very well suited for studying adaptive muscle morphology, biomechanics, and motor control. This review chapter gives a concise overview of ant mandible movements and some of the underlying mechanisms considering an evolutionary context by comparing species adapted to different lifestyles.

## Muscle fiber types of the mandible closer muscle

In ants, as in most other animals, the mandible closer muscle is much larger than the opener muscle. In large species or in large individuals of polymorphic species (Hölldobler and Wilson, 1990), the mandible closer occupies up to two-thirds of the entire head capsule volume (see chapter B.4., Fig. 4.3e). The proportion of the mandible closer muscle volume in relation to the head capsule volume decreases with decreasing size of the ant. In small species or small individuals, the mandible closer muscle only fills about 25% of the head capsule (see chapter B.4., Fig. 4.3e). The reason for this allometric correlation is that other organs and structures within the head take up relatively more space when the head capsule volume decreases (e.g. antennal, labial, and maxillary muscles; cf. chapter B.4., Fig. 4.3; Paul and Roces, 1999). Most notably, the brain is relatively larger in small ants (Jaffe and Perez, 1989). However, the mandible closer is the largest muscle in any ant worker, even in very small species or individuals.

In almost all ants, the mandible closer muscle is composed of two morphologically distinct types of muscle fibers (Gronenberg et al., 1997; Paul and Gronenberg, 1999): fibers with short sarcomeres (sarcomere length 2-3µm) and fibers with long sarcomeres (5-6µm). As a general rule, in long sarcomeres, more myosin-actin cross-bridges act in parallel (Huxley, 1974; Tregear and Marston, 1979; Cooke, 1997). Therefore fibers with long sarcomeres generate larger forces. In muscle fibers with short sarcomeres many units simultaneously shorten in series, resulting in a high contraction velocity (Jahromi and Atwood, 1969, 1971; Lang et al., 1977). This correlation between sarcomere length and contractile properties of a muscle fiber has been shown in many different studies (O´Connor et al., 1982; Stephens et al., 1984; Günzel et al., 1993; Taylor, 2000). Hence, muscle fibers with short sarcomeres are fast contracting fibers, whereas long sarcomeres indicate slow but forceful muscle fibers. Ultrastructural observations and considerations support this classification of muscle fiber types in ants (proportion and density of myofilaments, mitochondria, sarcoplasmatic reticulum, and thickness of z-discs; van Leeuwen, 1991; Gronenberg et al., 1997). However, absolute values of sarcomere lengths and other structural properties do not always accurately predict physiological performance of muscle fibers in arthropods (Costello and Govind, 1983; Silverman et al., 1987; Günzel et al., 1993). A causal relationship between the characteristics of the mandible movement and the electrical activity of particular muscle fiber types has been established (see chapter A.3.). In conclusion, the mandible closer muscle of ants is composed of two different sets of fibers: slow but forceful muscle fibers (sarcomere length 5-6 µm) and probably less forceful but fast

ones (sarcomere length 2-3 μm). In some arthropod muscles, including the ant mandible closer, muscle fibers may connect to thin thread-like processes of the apodeme (the functional analogue of the vertebrate tendon) rather than attaching to its main body through the entire cross-sectional area. These fibers are referred to as 'filament-attached' fibers to differentiate them from the more common type of 'directly attached' muscle fibers. Fast contracting fibers of the ant mandible closer always attach directly to the apodeme, whereas slow muscle fibers are either directly or filament-attached fibers.

The muscle fiber types of the ant mandible closer (fast directly attached, slow directly and filament-attached fibers) are arranged in bundles of like fibers (Gronenberg et al., 1997; Paul and Gronenberg, 1999). In all ants studied, the mandible closer muscle is thus composed of sub-units that each comprises only a single muscle fiber type and occupies characteristic positions within the head capsule (Fig. 1.1). While the relative and absolute size of each muscle fiber group differs across species and in some cases between individuals of different body size, the relative position of the different muscle fiber bundles is very similar in different species (Fig. 1.1). These typical positions of the muscle fiber types possess functional significance (see chapter A.2.).

In contrast to the mandible closer of ants, other insect muscles are either homogeneous or comprise several morphologically, histochemically, and physiologically different muscle fibers or a continuum of fiber types (Hoyle, 1974, 1978; Rathmayer and Maier, 1987; Müller et al., 1992). Other muscles in ants do not consist of distinct muscle fiber bundles as well. Antennal muscles contain fibers of different properties arranged concentrically (fiber diameter and sarcomere length increase from the center to the periphery of the muscle; Gronenberg and Ehmer, 1995; Ehmer and Gronenberg, 1996). The smaller mandible opener, labial, or maxillary muscles may comprise only a single muscle fiber type.

## Muscle fiber composition reflects adaptive differences among species

The fiber composition of the mandible closer muscle is species-specific (Gronenberg et al., 1997). The drawings in Figure 1.1 illustrate the variation in fiber composition among species. Most ants have both fiber types (fast and slow) and both types of fiber attachment (direct and via filaments) in their closer muscle. In the small *Leptothorax sordidulus*, no fast fibers were found. In *Atta sexdens* and small individuals of some *Camponotus* species, all slow muscle fibers are filament-attached. In the ponerine genera *Odontomachus* and *Anochetus* (Gronenberg and Ehmer, 1996), almost all fibers are of the directly attached type

with long sarcomeres. The drawings in Figure 1.1 represent sections in the mid-frontal plain, and roughly reflect the distribution of the muscle fiber types. However, since the distribution is not homogeneous throughout the entire head, these drawings are not a quantitative rendering of the fiber composition.

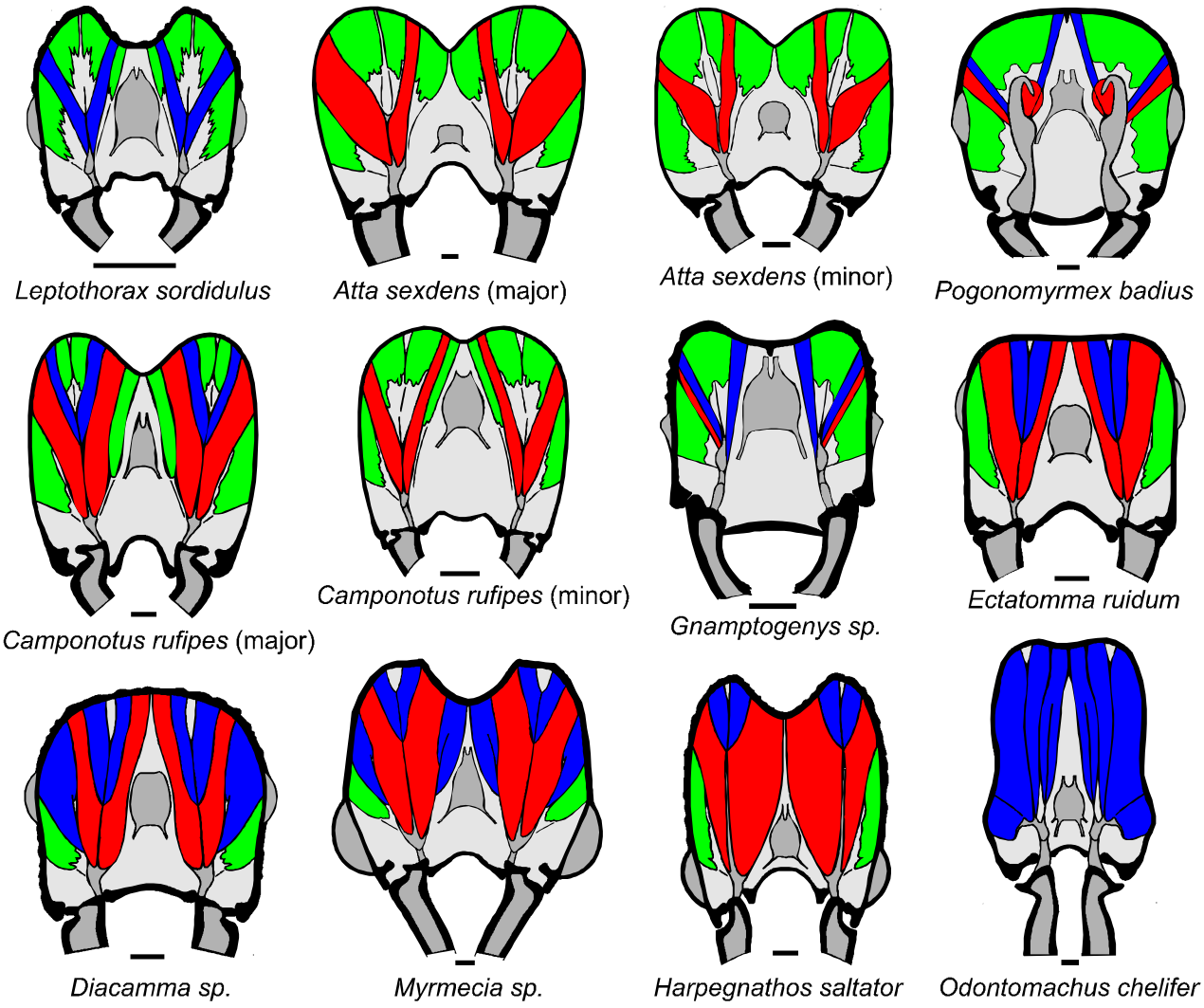



Fig. 1.1: Schematic drawings of mandible closer muscle fiber groups of selected ant species (modified from Paul, 1996). Each drawing represents the fiber composition of several mid-frontal sections at the level of the suboesophageal ganglion, but does not reflect the amount of fibers located more frontally or more caudally in the head. Arrangement of ant species reflects the decreasing amount of filament-attached muscle fibers. Solid black: cuticle; dark grey: mandibles, eyes, closer apodemes, suboesophageal ganglion; green: filament-attached slow muscle fibers (long sarcomeres); black lines indicate filaments; red: directly attached fast fibers (short sarcomeres); blue: directly attached slow fibers (long sarcomeres); scale bar: 250 µm.

Figure 1.2 shows the actual volume ratios of the muscle fiber types in different ant species. The species are arranged according to their proportion of fast fibers. *Camponotus rufipes* features an average ratio of fast to slow muscle fibers (Fig. 1.2). Accordingly, its morphology is suited to illustrate the 'generalized' ant mandible muscle design (Paul and Gronenberg, 1999). *Odontomachus*, *Pogonomyrmex*, and *Atta* have only few fast muscle fibers, whereas in *Myrmecia* and *Harpegnathos*, the majority of mandible closer muscle fibers are fast ones (Fig. 1.2). The proportion of fast fibers correlates positively with mandible closing velocity (Fig. 1.3). The fastest closing movements were measured in species that feature a high proportion of fast fibers (e.g. mandible closing velocity of *Harpegnathos saltator*: 1.25°/ms; relative proportion of fast fibers: 69.9%; Fig. 1.3; Gronenberg et al., 1997). *Myrmecia* and *Harpegnathos* are predators. *Myrmecia* is known as a predator with particular rapid mandible closing movements (Gray, 1971a, b).

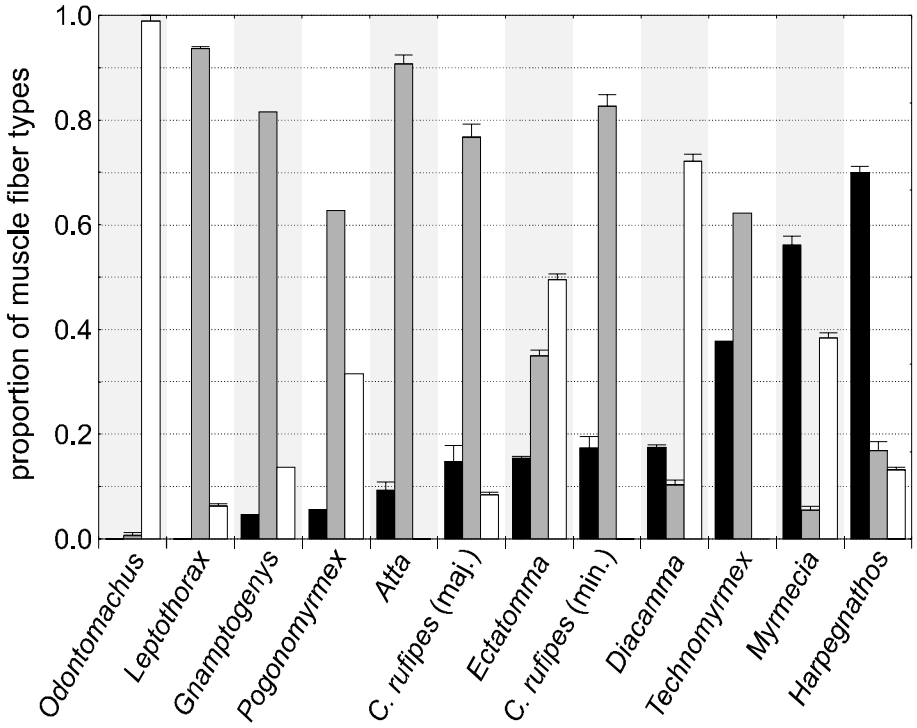



Fig. 1.2: Ratio of the three mandible closer muscle fiber types in different ant species (modified from Paul, 1996). Black bars: directly attached fast muscle fibers (short sarcomeres); open bars: directly attached slow fibers (long sarcomeres); grey bars: filament-attached slow fibers (long sarcomeres). Mean ± standard deviation (3 animals); bars without standard deviation (1 or 2 animals); *Atta*: 2 majors and 2 minors; *Camponotus rufipes*: 3 majors and 2 minors. The organization of ant species on the abscissa reflects the increasing proportion of fast fibers.

*Harpegnathos* species display jumping behavior and are able to catch flying prey in mid air (Ali et al., 1992; Baroni Urbani et al., 1994; Tautz et al., 1994). Hence, the mandible closer muscle of species that depend on fast mandible actions such as predatory ants consists of many fast muscle fibers.

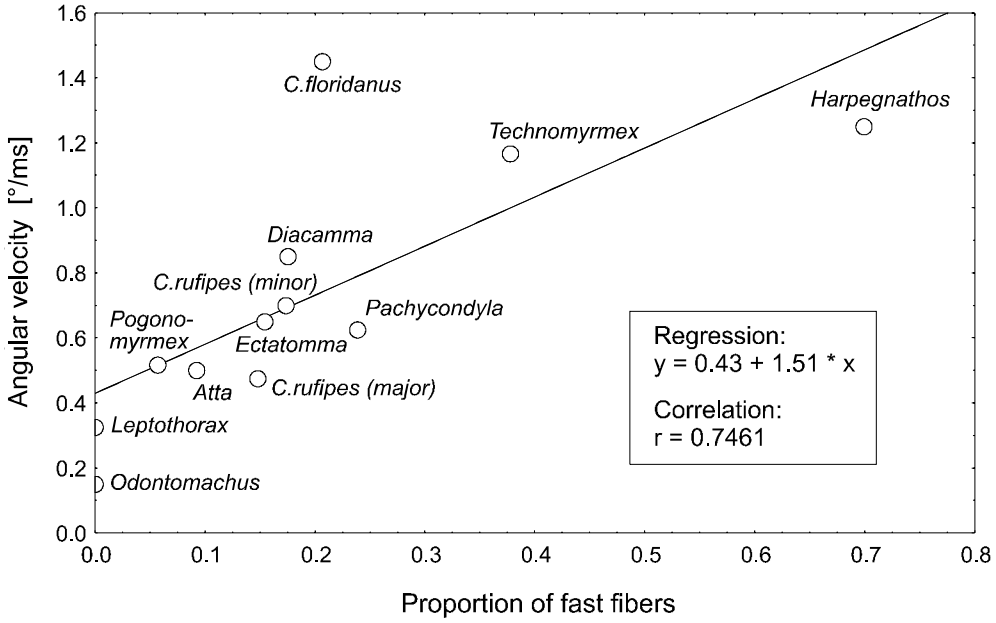



Fig. 1.3: Correlation between the angular velocity of mandible closing movement (ordinate) and the proportion of fast fibers in the mandible closer muscle (abscissa) of different ant species. Angular velocities were measured using a high frequency video system. For detailed interpretation see Gronenberg et al., 1997 (e.g. the data point for *C. floridanus*).

Ants of the genera *Odontomachus*, *Pogonomyrmex*, and *Atta* have many slow but forceful muscle fibers, whereas the mandible closer of the predatory ants *Myrmecia* and *Harpegnathos* contains only few of such particularly forceful muscle fibers (Fig. 1.2). *Pogonomyrmex* and *Atta* are herbivorous ants. As a harvesting ant, *Pogonomyrmex* needs very forceful mandible closing movements to crack dry seeds (Hölldobler and Wilson, 1990). The leaf-cutting ant *Atta* relies on a powerful mandible closer muscle to cut through tough leaves (Hölldobler and Wilson, 1990). In *Atta sexdens*, the mandible closer has a very high metabolic rate approaching that of insect flight muscle, and cutting leaves consumes almost 30 times as much energy as inactivity does (Roces and Lighton, 1995). Although *Odontomachus* is a predator (Hölldobler and Wilson, 1990), its mandible closer features slow muscle characteristics (e.g. it exclusively consists of slow muscle fibers; Fig. 1.2). This finding in *Odontomachus* and other 'trap-jaw' ants may seem puzzling, but we will see the reason for this combination of fast movement and slow muscle characteristics later.

To conclude, lifestyle variables such as feeding habits determine the species-specific volume ratios of mandible closer muscle fibers. Predators like *Harpegnathos saltator* depend on very fast mandible strikes to catch their prey, corresponding to a high proportion of fast fibers (Fig. 1.2). Herbivorous ants like *Atta sexdens* need a forceful mandible closer muscle for processing plant material, corresponding to a high proportion of slow but forceful muscle fibers (Fig. 1.2).

## Trap-jaw mechanism yields maximum velocity

Some ant genera (the so-called trap-jaw ants) feature a particular catapult mechanism to overcome the temporal limitations inherent to muscular contraction (Alexander, 1988). Such spring-loaded systems are widely employed by insects (e.g. the jumps of fleas, Bennet-Clark and Lucey, 1967; springtails, Christian, 1979; click beetles, Evans, 1973; flea beetles, Furth et al., 1983; locusts, Bennet-Clark, 1975). Trap-jaw mechanisms have evolved convergently in the ponerine ant tribe Odontomachini and in two other unrelated ant tribes, the formicine Myrmoteratini and the myrmicine Dacetini. These ants possess large mandibles that can be closed extremely rapidly to trap prey between them (Wheeler, 1900; Wilson, 1962; Dejean and Bashingwa, 1985; Dejean, 1986; Moffett, 1986; Carlin and Gladstein, 1989; Hölldobler and Wilson, 1990). In some ant genera this mechanism also serves a defensive function (Jaffe and Marcuse, 1983; Carlin and Gladstein, 1989). When the mandible strike is used against a large solid object, the ant will bounce off that object (retrosalience; Wheeler, 1900, 1922).

Mandible closing of trap-jaw ants is known as one of the fastest movements in the animal kingdom. Photoelectric scanning has revealed that these trap-jaws can be closed in less than 0.5 ms (Gronenberg, 1995a). In all trap-jaw ants, however, the mandible closer muscle features slow muscle characteristics. It is solely composed of muscle fibers with particular long sarcomeres (up to 11.4 µm, Tab. 1.1). Fast muscle fibers with short sarcomeres are completely absent within the mandible closer (e.g. *Odontomachus* in Fig. 1.1, 1.2). The slow muscle fibers directly attach to the apodeme at the optimum angle for maximum force output (approximately 45°, see chapter A.2). In some trap-jaw ants (*Strumigenys*), the majority of mandible closer muscle fibers are filament-attached (Gronenberg, 1996; cf. chapter A.2). Hence, the mandible closer of trap-jaw ants is specialized to generate large forces.

The high velocity of trap-jaw closing is based on a specific catch mechanism that keeps the extended mandibles open during contraction of the powerful mandible closer

muscle. This catch mechanism allows the potential energy the mandible closer muscle produces to be stored within cuticular elements, the head capsule, apodemes, and the closer muscle itself (Gronenberg, 1995a; Gronenberg et al., 1998a). The mandible strike is released in a reflexlike action when particular trigger hairs are touched. During a strike a relatively small and highly specialized trigger muscle unlocks the catch, instantaneously releasing the stored energy to accelerate the mandible.

| | Ponerinae | | Myrmicinae | | | Formicinae |
|---|---|---|---|---|---|---|
| | *Odontomachus sp.* | *Anochetus sp.* | *Acanthognathus sp.* | *Daceton armigerum* | *Strumigenys sp.* | *Myrmoteras sp.* |
| catch mechanism formed by | mandible joint | mandible joint | accessory mandibular processes | labrum | labrum | ? |
| Sarcomere length [µm] of mandible closer muscle | 9.8 | 11.4 | 8.4 | 7.2 | 4.9 | ? |
| trigger muscle derived from, sarcomere length [µm] | mandible closer, 2.7 | mandible closer, 2.9 | mandible closer, 3.0 | labral adductor, 1.8 | labral adductor, 1.8 | ? |
| number of trigger hairs and sensory neurons | 4 large | 2 large, 2 slightly smaller | 2 large | 2 large | 2 large, 2 small | none (visual) |
| number of trigger motor neurons | 4 | 4 | 4 | 2 | 2 | ? |
| References | 1, 2, 6 | 1, 4 | 5 | 3 | 3 | 7 |

Table 1.1: Major features underlying and determining the trap-jaw mechanisms (modified from Gronenberg, 1996). [1] Brown (1978); [2] Gronenberg (1995a, b); [3] Gronenberg (1996); [4] Gronenberg and Ehmer (1996); [5] Gronenberg et al. (1998a); [6] Just and Gronenberg (1999); [7] Moffett (1986).

The catch mechanism differs among species. In ponerine species, it is formed by specific adaptations of the mandible joint, whereas in myrmicine species, the mandibles are locked in the open position by the labrum, which functions as a latch, or by opposing accessory mandibular processes (Tab. 1.1). The trigger hairs can be located on the mandible surface or on the labrum, respectively. The trigger muscle of ponerine species is probably evolutionary derived from the fast muscle fibers of the mandible closer muscle (Tab. 1.1). In ponerine ants, upon contraction of the trigger muscle, the hump at the ventral tip of the mandible base slightly moves which serves to unlock the mandible catch (Tab. 1.1). In *Acanthognathus*, mandibles are unlocked by contraction of fast mandible closer muscle fibers that rapidly rotate the closer apodeme and the mandible around its long axis (Tab. 1.1). In the other two myrmicine genera, the labral adductor muscle serves as trigger muscle as the mandibles are blocked by the labrum (Tab. 1.1). Comparison of trap-jaw mechanisms among species reveals a remarkable example of convergent evolution.

Ants of the genus *Mystrium* employ a peculiar defensive 'snap-jaw' mechanism in which the closed mandibles cross over to deliver a stunning blow to an adversary

(Gronenberg et al., 1998b). The strike is initiated by contact of the adversary with mechanosensory hairs at the side of the mandible, and is powered by slow closer muscles whose energy is stored by a spring mechanism. Recording of closer muscle activity indicates that the mandibles are not triggered by any fast muscle. Instead, it is supposed that activity differences between the left and right mandible muscles imbalance a pivot at the mandible tip and release the strike (Gronenberg et al., 1998b).

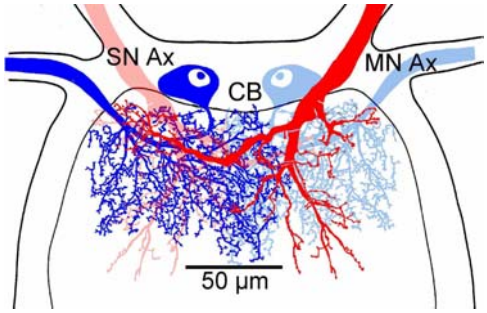



Fig. 1.4: Schematic drawing (by W. Gronenberg) of the sub-oesophageal ganglion of *Odontomachus* showing the overlap of a giant mandibular sensory neuron's axon terminal SN Ax (red) and a trigger motor neuron's dendritic tree MN Ax (blue). Light colors indicate the respective contralateral neuron to reveal the bilateral overlap. CB: motor neuron cell body. Two similar sets of neurons exist on either side, only one of which is shown here. Sensory and motor neurons are connected by highly efficient chemical synapses, motor neurons are mutually coupled by electrical synapses.

Previous work on mandible motor control in ants focused on the specialized trap-jaw ants. Eight mandible closer motor neurons have been found in *Odontomachus* (Just and Gronenberg, 1999). The trap-jaw reflex takes 4 to 10 ms and is one of the fastest reflexes yet described for any animal (Gronenberg, 1995a). In *Odontomachus*, it is controlled by a system composed of two giant sensory neurons (trigger hairs) and two giant motor neurons (trigger muscle) on either side (Fig. 1.4; Gronenberg, 1995b). The giant neurons are most likely monosynaptically coupled (Fig. 1.4). The large axon diameter and the synaptic coupling result in high conduction velocity which underlies the very fast mandible reflex (Gronenberg et al., 1993; Gronenberg, 1995b). The trigger motor neurons are dye-coupled and receive input from both sides of the body without delay, which ensures the synchronous release of both mandibles (Just and Gronenberg, 1999). Reflex activity is modulated by antennal and other sensory input probably converging onto the large dendritic trees of the trigger motor neurons (Fig. 1.4; Gronenberg, 1995b).

In order to generate fast mandible closing movements ants have adapted in two different ways: In predatory ants such as *Harpegnathos* or *Myrmecia*, the mandible closer muscle is mainly composed of particular long muscle fibers with short sarcomeres directly attached to the apodeme at small angles, combining characteristics that result in high mandible closing velocities (cf. chapter A.2). Alternatively, trap-jaw ants developed highly specialized catapult mechanisms which rely on slow mandible closer muscles to produce large forces. Their mandibles close even faster.

# A. THE MANDIBLES

## 2. Optimizing force and velocity: Mandible muscle fiber attachments in ants

## Introduction

To many insects, and to ants in particular, mandibles are important tools. Ants use these shovel- or tong-like mouthparts for almost any task, including prey-catching, fighting, digging, leaf-cutting, and wood-scraping, as well as for subtle tasks such as grooming, brood care, carrying nestmates or liquids, and communication (Hölldobler and Wilson, 1990). Accordingly, ant mandibles have to perform many different kinds of movements in terms of velocity, power output and precision (Gronenberg *et al.* 1997). Even though opener-closer muscle co-contraction may occur, the mandible closer muscle is the key to the versatility of mandible movements (Gronenberg *et al.* 1998b). The mandible closer muscle is much larger than the opener muscle and occupies up to two-thirds of the entire head volume. It is the largest muscle in any ant worker and is always composed of several motor units that may be activated individually, sequentially or synchronously to generate a variety of different types of movement (Just and Gronenberg, 1999; see chapter A.3.).

In almost all ants, the mandible closer muscle is composed of two distinct types of muscle fibers: fibers with long sarcomeres (5-9µm in length) and fibers with short sarcomeres (2-3µm). According to their morphological, ultrastructural and biochemical properties, these fibers contract either slowly or relatively rapidly, respectively (Gronenberg and Ehmer, 1995; Gronenberg *et al.* 1997; see chapter A.1.). Hence, the closer muscle is composed of two different sets of fibers: powerful slow fibers and less forceful fast ones (Gronenberg *et al.* 1997). Below I will refer to the closer muscle fibers with long sarcomeres as slow fibers and to the fibers with short sarcomeres as fast fibers. The fiber distribution and the ratio of fast to slow fibers is species-specific and (besides the overall size of the muscle) determines the maximum contraction velocity and force of the mandibles (Gronenberg *et al.* 1997; see chapter A.1.).

In addition to the physiological properties of the muscle fibers, speed and force of a whole movement system depend strongly on the musculo-skeletal design such as joint characteristics and on the geometrical arrangement of the muscle fibers (Full et al., 1991; Full and Ahn, 1995). The muscle fiber's angle of attachment with respect to the muscle's overall direction of pull is a particularly important determinant of the force that a single fiber contributes. In arthropods, muscle fibers attach to the exoskeleton (e.g. the thorax or head capsule) directly *via* a deeply serrated area of adhesion between the muscle fiber and the cuticle (Neville, 1975; Gronenberg *et al.,* 1997). The opposite end of the muscle fiber connects to a specialized region of the moving body part (e.g. leg or mandible) referred to as the apodeme, which is the functional analogue of the vertebrate tendon (Pringle, 1972). In

some arthropod muscles, including the ant mandible closer, muscle fibers may connect to thread-like processes of the apodeme rather than attaching to its main body through the entire cross-sectional area (Fig. 2.1; Janet, 1907; Gronenberg *et al.* 1997). In principle, all these muscle fibers attach to an individual secondary apodeme branch and I will refer to them as 'filament-attached' fibers to differentiate them from the more common type of 'directly attached' muscle fibers.

The presence of such filament-attached fibers has been described previously in ants (Janet, 1907a, b) and other insects (Snodgrass, 1935), but their functional significance has never been addressed. In the present study, I examine the occurrence and distribution of such fibers among different species of ants and I then discuss their possible functional advantages. I also compare the geometry of mandible closer apodemes across various ant taxa and examine whether different muscle fiber types (fast or slow, directly attached or filament-attached) differ with respect to their attachment angles at the apodeme.

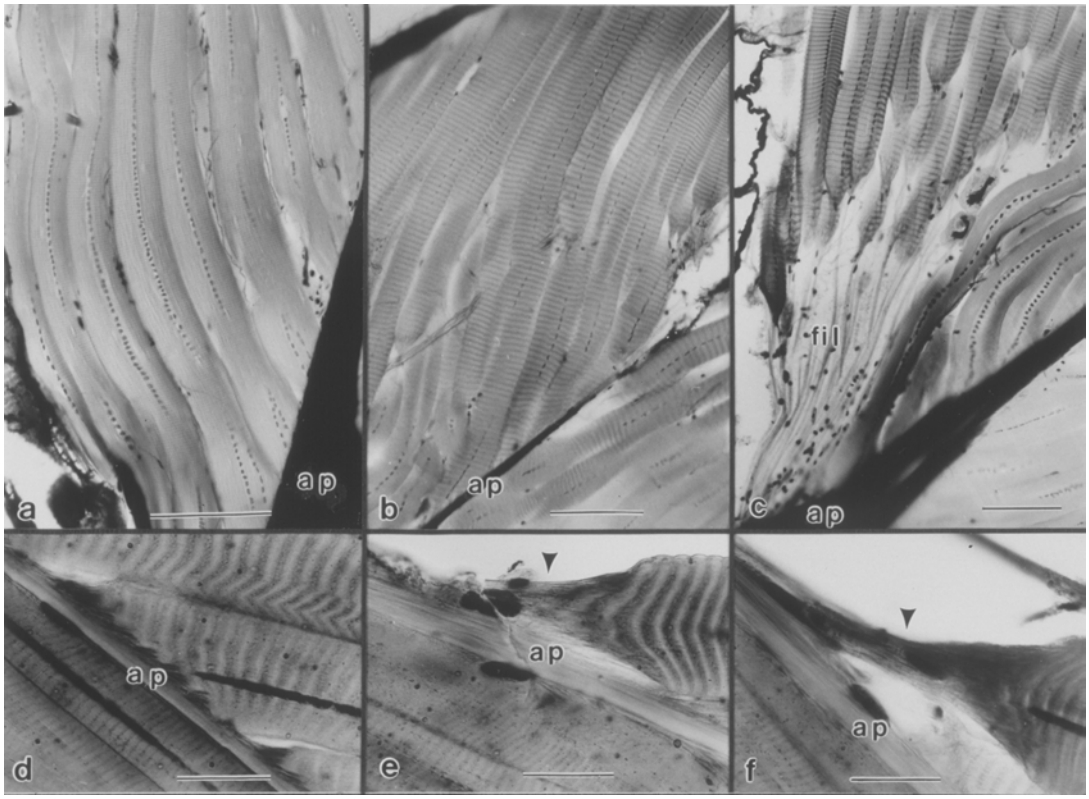



Fig. 2.1: Photomicrographs of mandible closer muscle fibers. (a) Fast fibers, (b) slow directly attached fibers, (c) filament-attached fibers in *Camponotus rufipes*; (d) directly attached fiber, (e) intermediate attachment and (f) filament-attached fiber in *Myrmecia* sp.. ap, apodeme; fil, filament; arrowheads indicate short filaments in e and f. Scale bars, 100µm (a-c), 20µm (d-f).

This leads to two simple models which explain why, in general, fast or forceful mandible movements require longer or broader ant heads, respectively, why fast or slow muscle fibers should attach at different angles at the apodeme, and how filament-attached fibers can help to optimize the use of the available head capsule volume and the total power output.

## Materials and methods

Measurements were made on the following species of ants: *Myrmecia* sp. (Clark), Myrmeciinae, preserved material only; *Diacamma* sp., *Ectatomma ruidum* (Brown), *Gnamptogenys* sp. (Brown), *Harpegnathos saltator*, *Odontomachus bauri*, *O. chelifer* (Brown), Ponerinae; *Acanthognathus rudis* (Brown and Kempf), *Atta sexdens* (Borgmeier), *Leptothorax sordidulus* (Bernhard), *Pogonomyrmex badius* (Cole), Myrmicinae; *Technomyrmex* sp. (Wheeler), Dolichoderinae; *Camponotus rufipes* (Yasumatsu and Brown), Formicinae. The ants were kept in plaster-of-Paris nests under a 12h : 12h L : D cycle at 25°C and 50% relative humidity. They were fed chopped cockroaches, crickets or wingless *Drosophila*, and honey-water (30%) or fresh leaves of various kinds (*Atta sexdens*). To examine the morphology of mandible muscles and apodemes within the head, ants were decapitated, and the head capsule was opened under fixative (buffered 4% formaldehyde or 2.5% glutaraldehyde). The heads were then stained either with Methylene Blue or with osmium/ethyl gallate according to Gronenberg (1995) or silver-impregnated (Gronenberg *et al.* 1997), dehydrated, embedded in Fluka Durcupan and horizontally or vertically sectioned at 10-15µm. Specimens were drawn from microscopic images using a *camera-lucida* attachment to the microscope (Zeiss Axiophot). The acute angle between the longitudinal axis of the apodeme and the muscle fiber (here referred to as the attachment angle of a muscle fiber) was determined from those drawings. Muscle cross-sectional area was traced from digitized microscopic video images (Gronenberg *et al.* 1997) and muscle volumes were calculated using the section thickness. I analyzed three animals per species with a few exceptions: two minor and three major workers each in *Atta sexdens* and *Camponotus rufipes*, and only a single specimen each in *Pogonomyrmex badius*, *Technomyrmex* sp., and *Gnamptogenys* sp..

## Results

The volume of the mandible closer muscle is correlated with the body size of the ant worker, taking up approximately two-thirds of the head volume (cf. chapter B.4., Fig. 4.3e). This is not the case in most male ants, which have reduced mandibles, and in very small ants.

In both these groups, the brain occupies a larger portion of the head than it does in 'normal' workers. Accordingly, the overall volume of the closer muscle varies greatly among species (Table 2.1): the smallest muscle among the species sampled was found in *Leptothorax sordidulus* (total volume = 0.01mm³ per hemisphere) and the largest one in a major *Atta sexdens* (4mm³ per hemisphere). As a result of polymorphism, even within a single colony the size of the closer muscle was found to differ by more than a factor of 30 between soldiers and small workers of *Camponotus rufipes* (Table 2.1). In *Atta sexdens*, the maximum intra-specific difference will probably be greater than indicated in Table 2.1 as I did not examine minims, the smallest worker subcaste (Wilson, 1985). The number of closer muscle fibers also depends on the size of the ants. The muscle may be composed of as little as 80 muscle fibers (*Leptothorax sordidulus*) or more than 1000 fibers (*Myrmecia* sp., *Atta sexdens* major workers). The closer muscle fibers originate from all over the posterior two-thirds of the head capsule and are oriented towards the apodeme in an antero-frontal direction. Before discussing the different fiber types and their distribution within the muscle, I will first describe the mandible closer apodeme in more detail.

| Species | N | Muscle volume ($10^6$ µm³) | Directly attached (%) | Filament-attached (%) |
|---|---|---|---|---|
| *Odontomachus chelifer* | 2 | | 99.3 ± 0.5 | 0.7 ± 0.5 |
| *Myrmecia* sp. | 2 | 1710 ± 46 | 94.6 ± 1.3 | 5.4 ± 0.7 |
| *Diacamma* sp. | 3 | 355 ± 3 | 89.7 ± 0.9 | 10.3 ± 1.0 |
| *Harpegnathos saltator* | 3 | 1291 ± 159 | 83.2 ± 0.9 | 16.8 ± 1.8 |
| *Ectatomma ruidum* | 3 | 282 ± 11 | 65.0 ± 0.7 | 35.0 ± 1.0 |
| *Technomyrmex* sp. | 1 | | 37.8 | 62.2 |
| *Pogonomyrmex badius* | 1 | | 37.3 | 62.7 |
| *Camponotus rufipes* (soldier) | 3 | 954 - 3244 | 23.3 ± 1.8 | 76.7 ± 2.6 |
| *Gnamptogenys* sp. | 1 | | 18.4 | 81.6 |
| *Camponotus rufipes* (worker) | 2 | 98 - 157 | 17.3 ± 1.1 | 82.7 ± 2.2 |
| *Atta sexdens* (major) | 3 | 1091 - 3889 | 10.3 ± 1.8 | 89.7 ± 1.8 |
| *Atta sexdens* (minor) | 2 | 182 - 796 | 8.1 ± 0.7 | 91.9 ± 0.7 |
| *Leptothorax sordidulus* | 3 | 10.5 ± 0.2 | 6.3 ± 0.2 | 93.7 ± 0.4 |

Table 2.1: Absolute volume of mandible closer muscles and their relative compositions with respect to attachment type in different ant species. Values are means ± S.D.. Measurements were made on one side of the heads only. Size differences between individuals of *C. rufipes* and *A. sexdens* were too large to calculate a meaningful mean value; therefore, a range is given for these species.

**Composition of the apodeme**

In all species, the mandible closer apodeme follows a basic plan that may be modified to a greater or lesser extent (Fig. 2.2). A broad, unsclerotized, flexible ligament connects the inner flank of the mandible base to the apodeme base. This main body of the apodeme is a massive sclerotized structure which funnels the forces of all the closer muscle fibers into the mandible. The apodeme base gives rise to apodeme collaterals. Typically, three branches project from the apodeme base posteriorly into the closer muscle: a central principal branch and two accessory branches, a median and a lateral one. This pattern can be seen best in *Myrmecia* sp., the most primitive ant in my study (Fig. 2.2) where the principal branch is the thickest apodeme process. Like the apodeme base, it is often partly sclerotized and it projects in the direction in which the largest forces are to be expected. The sturdy nature and usual orientation of the principal apodeme branch can be seen clearly in *Harpegnathos saltator* (Fig. 2.2). This principal branch receives the majority of muscle fibers. In cross section, it is not circular as might be suggested by the dorsal views in Fig. 2.2. Rather, the principal branch extends in a dorso-ventral direction and is ribbon or velum-like, depending on the size of the ant and the number of muscle fibers that attach to it. Fig. 2.3F reveals that in the vertical plane the principal apodeme branch is slightly S-shaped and tilted with respect to the dorso-ventral axis. The two accessory apodeme branches vary in length between species but are always shorter than the principal one. They are unsclerotized, narrower in the dorso-ventral axis (less velum-like) and connect to fewer muscle fibers.

Among different species, the most variation from this basic apodeme design is in the accessory branches (Fig. 2.2). In the genera *Diacamma*, *Ectatomma* and *Harpegnathos*, the inner accessory branch is much shortened. In the former two genera, the principal branch is slightly bent centrally, compared with other genera, and it carries an additional side branch (Fig. 2.2). In *Ectatomma ruidum*, muscle fibers attach only to the tip of this additional branch while in *Diacamma* sp. muscle fibers attach all along its length.

The most significant alteration to the basic design is the introduction of apodeme filaments. Bundles of such filaments, each of which connects exclusively to a single muscle fiber, may replace the accessory branches entirely although the basic organization can still be discerned. In the genera *Ectatomma* and *Harpegnathos*, filaments replace the lateral apodeme branch, and in *Atta*, *Camponotus* and *Leptothorax* both accessory branches are replaced by apodeme filaments (Fig. 2.2). In the latter three genera, even the principal apodeme branch is shortened and its tip fans out into filaments. The tendency to substitute longer apodeme branches for filaments is most pronounced in *Atta sexdens* where the

filaments are longest and approximately 90% of the muscle fibers are filament-attached (Table 2.1). Filament length varies across species as well as within individual muscles. Besides *Atta*, other myrmicine genera and the formicine *Camponotus* (Fig. 2.1) feature long-apodeme filaments, while many ponerines have short filaments. In some species, most notably *Myrmecia* sp., all permutations from directly attached to filament-attached fibers exist (Fig. 2.1D-F). As for filament length, the proportion of filament-attached fibers is smaller in ponerines than in the other subfamilies (Table 2.1). Likewise, smaller species (*Leptothorax sordidulus*, *Gnamptogenys* sp., *Technomyrmex* sp.) and smaller individuals (small workers of *Camponotus rufipes*) appear to have relatively more filament-attached muscle fibers (Table 2.1), while *Myrmecia* sp. and the large ponerines (*Diacamma* sp., *Harpegnathos saltator*) feature relatively small proportions of these fibers.

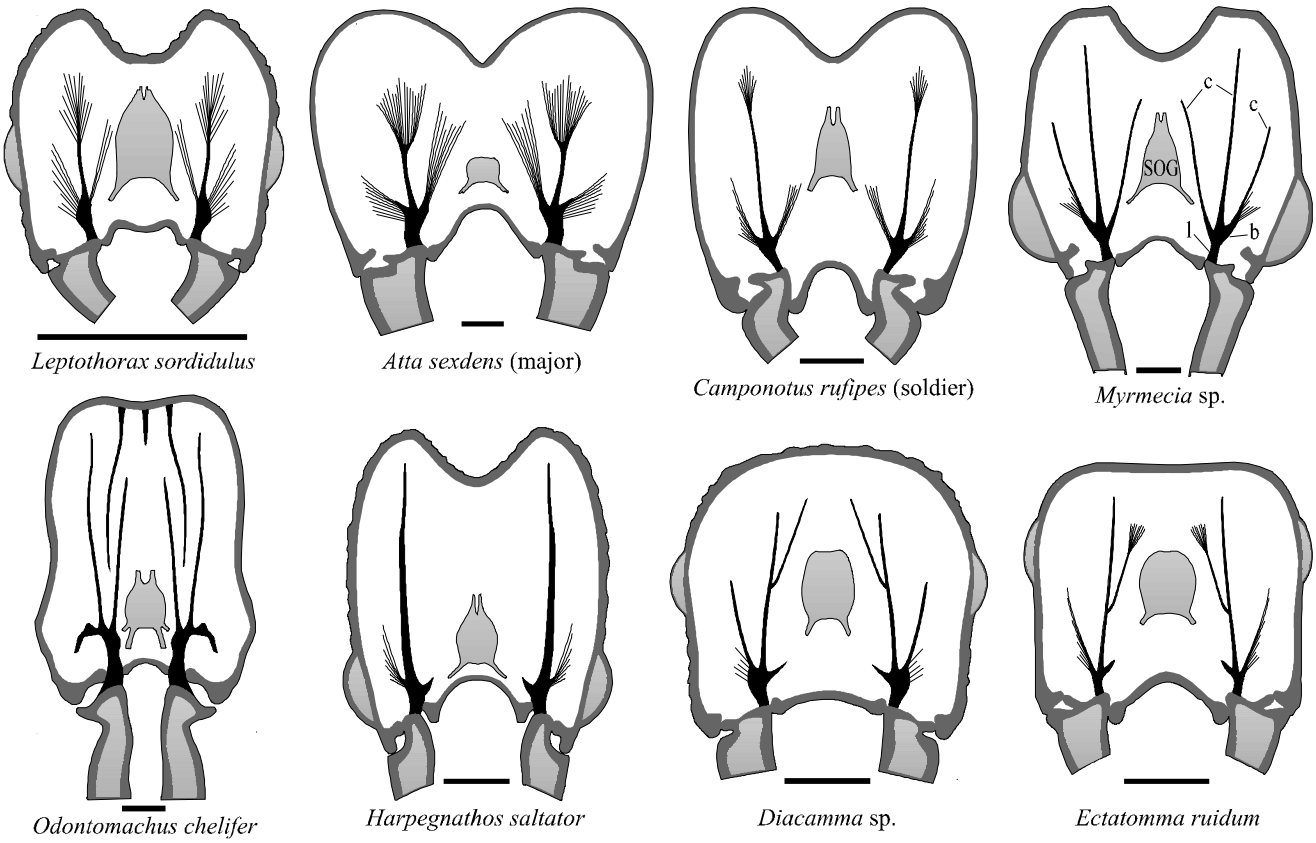



Fig. 2.2: Schematic dorsal views of heads of different ant species (mid-horizontal plane corresponding to the plane indicated by arrows in Fig. 2.3F) depicting the organization of the mandible closer apodemes (solid black); b, apodeme base; c, apodeme collateral branches; l, flexible apodeme ligament; SOG, suboesophageal ganglion. Scale bars, 500µm (modified from Paul, 1996).

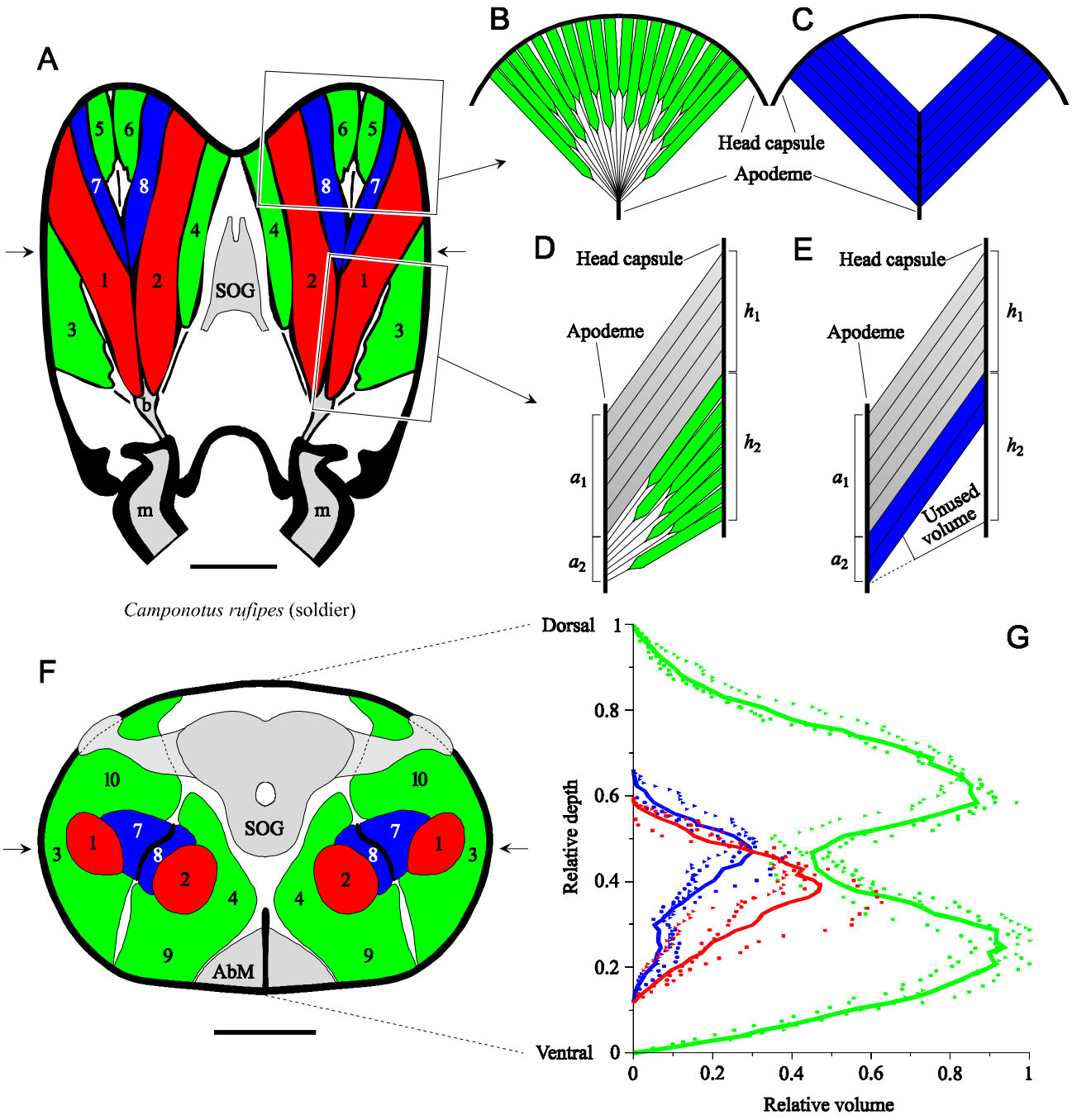



Fig. 2.3: Organization of mandible closer muscle fibers in the head of *Camponotus rufipes* (soldier). (A) Dorsal view in the plane indicated by arrows in F; (B-E) diagrams illustrating the advantage of filament-attached fibers in the regions marked by rectangles in A (for further details, see text); (F) anterior view in the plane indicated by arrows in A; (G) relative distribution of different closer muscle fiber types calculated from serial section of three specimens: red, fast fibers; blue, slow directly attached fibers; green, slow filament-attached fibers; individual values are represented by symbols; solid lines represent mean values. The *x* axis shows the relative volume of the respective fiber type in a single section (1 is the highest value found for filament-attached fibers in any section of that animal); the *y* axis shows the relative dorso-ventral depth (0 is ventral, 1 is dorsal, corresponding to the section in F). Symbols used in D and E: $a_1$, $a_2$, apodeme surface areas 1 and 2; $h_1$, $h_2$, corresponding head capsule surface areas 1 and 2. Muscle fiber types in A and F: red, 1, 2; fast; blue, 7, 8; slow directly attached; green, 3-6, 9, 10; filament-attached; AbM, mandible opener muscle; b, apodeme base; m, mandible; SOG, suboesophageal ganglion. Scale bars, 500µm.

The ponerine *Odontomachus chelifer* has the smallest percentage of filament-attached fibers (Table 2.1) which is probably a derived trait. *Odontomachus chelifer* possess a highly specialized trap-jaw mechanism which also involves other modifications of the closer apodeme (Fig. 2.2). Unlike in other ants, the lateral accessory apodeme branch is very strong and sclerotized. Instead of projecting postero-laterally it forms a hook-like structure that is bent anteriorly and is adapted for the introduction of lateral forces into the mandible. Similarly, the apodeme of *Acanthognathus rudis* features a massive rigid lateral arm allowing slight rotation of the mandible around its long axis in addition to the usual closing movement (Dietz and Brandão, 1993; Gronenberg *et al.* 1998a). Another feature of *Odontomachus chelifer* are three apodemes that originate from the rear of the head (Fig. 2.2). A large ribbon-like apodeme projects deeply into the head capsule on either side and a shorter one resides medially at the posterior head wall. The large apodemes run between and almost in parallel with the two mandible closer apodeme branches. Functionally, these additional apodemes enlarge the surface area of the head capsule substantially to allow the attachment of more closer muscle fibers.

**Positions and attachment angles of different fiber types**

According to the mode of attachment at the apodeme and to their contraction properties mentioned in the Introduction, muscle fibers can be subdivided into three basic types: fast or slow directly attached fibers and slow filament-attached fibers. No fast fibers attach to the apodeme *via* filaments; the reason for this will be discussed below. Filament-attached fibers are the only fiber type that occurs in the mandible closer muscle of all ants. The three fiber types (fast or slow directly attached and slow filament-attached fibers) are not arbitrarily distributed within the closer muscle but are organized in homogenous bundles of similar parallel fibers. In general, these fiber groups occupy specific positions, irrespective of the species examined, and are shown for a soldier of *Camponotus rufipes* in Fig. 2.3A, F. The ratio of the different fiber types varies among species but is very similar for all workers of a given species even if large size differences exist among them, giving species-specific distribution patterns of fiber types (Fig. 2.3G). Among the species examined, only for *Camponotus rufipes* does the closer muscle differ between soldiers and small workers. The closer muscles of the latter do not comprise any slow directly attached fibers.

If present, fast fibers occur in a similar position within the mandible closer muscle: they originate from the back of the head and project anteriorly over a long distance until they

attach at either side of the principal apodeme branch or at the apodeme base proper (fiber groups 1 and 2 in Fig. 2.3A). The fast fiber bundles are always the longest fibers; they form the center of the entire closer muscle and are surrounded by fibers of the other two types. The cross sections of the fast fiber bundles are almost circular (Fig. 2.3F). With the mandibles closed, the attachment angle is 24.8 ± 8.1° (mean ± S.D.; *N*=121) for the fast fibers of all species examined. Most species have a similar attachment angle (Table 2.2). However, some species feature extreme angles of attachment. In the fast predators *Harpegnathos saltator* and *Myrmecia* sp., the angle is very small (approximately 15°; Table 2.2). Among ants possessing 'regular' mandibles (as opposed to trap jaws), the largest attachment angles of fast fibers were found in *Atta sexdens* and *Pogonomyrmex* badius (Table 2.2), which do not perform fast mandible movements (Gronenberg *et al.* 1997).

The positions of slow directly attached fibers are more variable than those of the other two fiber types. As a consequence of their direct attachment at the apodeme, they are most often found close to the fast fibers. They may be assembled in fiber bundles as is the case in *Camponotus rufipes* (fiber groups 7, 8 in Fig. 2.3A,F). Their angle of attachment at the apodeme depends on their position and varies from 20° to 50° with a mean angle of 29.6 ± 8.9° (*N*=197) in all the species examined. For these fibers the largest angles of attachment were found in *Odontomachus chelifer* (46°; Table 2.2). In this genus, the mandible closer muscle is composed almost entirely of slow directly attached fibers which are relatively short compared to those of other genera. The same is true for *Acanthognathus rudis*, the other trap-jaw ant examined in the present study (Table 2.2).

Like the fast fibers, slow filament-attached fibers also are found in particular locations within the mandible closer muscle. In all species, they occupy peripheral positions and thus surround the fast fiber bundles dorsally, ventrally, laterally and (in *Atta sexdens*, *Camponotus rufipes*, and *Leptothorax sordidulus*) centrally (fiber groups 3, 4, 9, 10 in Fig. 2.3A,F). This organization is illustrated by the dorso-ventral distribution of different fiber types in *Camponotus rufipes* (Fig. 2.3G). Directly attached fibers are restricted to median head regions which coincide with the principal apodeme branch while filament-attached fibers are most abundant on the dorsal and ventral side of the head. In addition, in most myrmicines and formicines two groups of short filament-attached fibers are found at the distal end of the principal apodeme branch (Fig. 2.2; fiber groups 5 and 6 in Fig. 2.3A). Filament-attached fibers are generally short and feature the largest angles of attachment: up to 75° for lateral fiber groups (group 3 in Fig. 2.3A) when the mandibles are closed (closer muscle contracted; in the open position, no closer muscle fibers of any type feature

attachment angles greater than 40°). Some filament-attached fibers are oriented in the direction of pull (groups 5, 6 in Fig. 2.3A); for these fibers the attachment angle may be as small as 0°. Overall, variation in attachment angle is largest in slow filament-attached fibers.

| Species | N | Angle of attachment (°) |
|---|---|---|
| Directly attached fast fibers | | |
| *Myrmecia* sp. | 20 | 14.9 ± 2.8 |
| *Harpegnathos saltator* | 17 | 15.8 ± 3.6 |
| *Diacamma* sp. | 13 | 21.2 ± 2.7 |
| *Ectatomma ruidum* | 15 | 22.7 ± 3.4 |
| *Pachycondyla villosa* | 7 | 23.7 ± 6.2 |
| *Camponotus rufipes* | 22 | 24.6 ± 4.2 |
| *Atta sexdens* | 15 | 36.9 ± 9.3 |
| *Pogonomyrmex badius* | 12 | 38.4 ± 8.2 |
| Directly attached slow fibers | | |
| *Acanthognathus rudis* | 61 | 40.7 ± 6.6 |
| *Odontomachus chelifer* | 44 | 46.1 ± 9.2 |

Table 2.2: Angles of attachment of fast mandible closer muscle fibers at the apodeme in different ant species. Values are means ± S.D.; *N* = number of fibers examined; the trap-jaw ants *Acanthognathus rudis* and *Odontomachus chelifer* possess only slow fibers (the few specialized fast fibers in these ants serve a different function).

## Discussion

**Apodeme design: force and velocity require different angles of attachment**

The apodeme projects deeply into the closer muscle and the muscle fibers attach to it at varying angles. My results suggest that these angles are not arbitrary but are characteristic for a particular fiber type. What determines this angle of attachment and why is it different in different fiber types?

The optimal angle of attachment for any muscle fiber would be 0°, which is parallel to the principal direction of pull. Such a fiber arrangement is shown on the left of Fig. 2.4C, where all muscle fibers act in that optimal direction. However, this pattern is not found in any arthropod for two reasons. (1) The apodeme is composed of cuticle (Neville, 1975; Snodgrass, 1935) which, even if sclerotized (having cross-linked chitin filaments), is most stable in the direction parallel to the fibrils (Alexander, 1988; Neville, 1975). A thin sheet-like apodeme as shown in blue on the left of Fig. 2.4C would become bent upon contraction of the muscle rather than transmitting the force into the mandible. To function, a muscle fiber arrangement of this kind would require an extremely thick apodeme, taking up space and representing an additional load which would reduce the advantage given by the muscle

fiber's optimal angle of attack. (2) During contraction, such a muscle would swell considerably perpendicular to the direction of contraction because muscle volume remains almost constant while it shortens (Alexander, 1983; Baskin and Paolini, 1966). This, however, is not possible in the restricted space of the inflexible head capsule, which would either prevent the muscle from shortening or crack. In contrast, a muscle of the design shown on the right of Fig. 2.4C will not swell during contraction (Alexander, 1988).

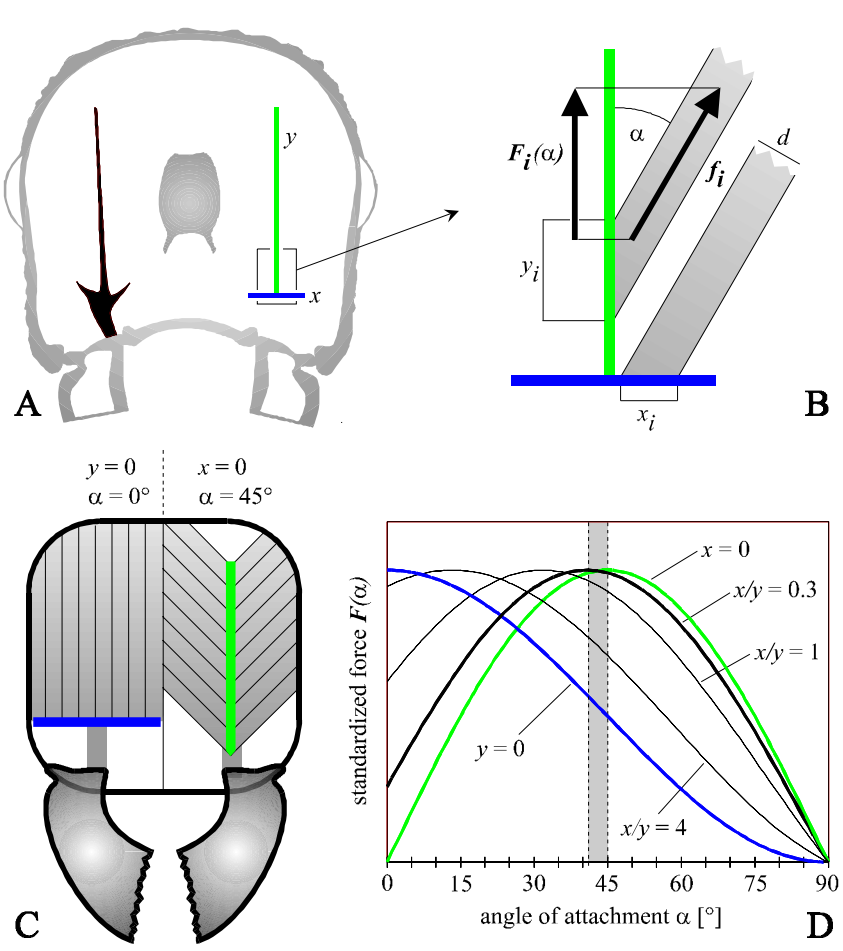



Fig. 2.4: The effects of apodeme design and fiber angle of attachment on force production. (A) Schematic dorsal view of an ant head mid-horizontal plane showing horizontal (blue, *x*) and vertical (green, *y*) apodeme components. (B) Muscle fiber forces produced in the apodeme components indicated by the rectangle in A. $f_i$, force generated by a single fiber; $F_i(\alpha)$, force in the principal direction of pull; $\alpha$, angle of fiber attachment; *d*, fiber diameter; $x_i$, $y_i$, attachment surface area of a fiber in the *x* and *y* direction, respectively. (C) Diagrammatic representation of head showing attachment angles $\alpha$ = 0° and 45°. (D) Force output (normalized to the maximum of the curve for *x* = 0) for apodemes with varying *x/y* composition; the range of *x/y* ratios found in most ants lies between the green and the thick black line; shaded area (*x/y* = 0-0.3) shows the corresponding range of optimal angles of attachment for maximal force output. The equation for the graphs in D is derived in the Appendix (equation 6).

To understand the design of arthropod muscles, we must first look at the geometrical requirements of single muscle fibers: a muscle will be fast if it consists of particularly long fibers composed of short sarcomeres (many units simultaneously shortening in series; Jahromi and Atwood, 1969; Lang *et al.*, 1977) that attach to the apodeme at small angles; the absolute amount by which the muscle shortens is thus maximized. An apodeme filament would shorten the contractile part of the muscle fiber and thus remove its fast properties. For this reason, fast fibers are never filament-attached. In contrast, a strong muscle requires many parallel fibers which may be short and are composed of long sarcomeres. In long sarcomeres, the many cross-bridges act in parallel even though, morphologically, they are

arranged in rows (Huxley, 1965, 1974; Jahromi and Atwood, 1969; Tregear and Marston, 1979).

The angle of attachment at the apodeme is crucial to the overall force generation of the entire muscle because the apodeme surface area is limited. The significance of the attachment angle can best be explained using a simple model for directly attached muscle fibers (model 1, see Appendix). The total force $\boldsymbol{F(\alpha)}$ of the muscle depends on the individual force of each single fiber $\boldsymbol{f_i}$, the fiber diameter $d$, the apodeme composition of horizontal $x$ and longitudinal $y$ components (Fig. 2.4A,B), and the angle of attachment $\alpha$ where $\boldsymbol{F(\alpha)} = (\boldsymbol{f_i} / d) (\cos \alpha) [2y (\sin \alpha) + x (\cos \alpha)]$ (see equation 6 from model 1 in the Appendix).

From this equation, it can be shown that the optimal angle of attack for maximum force output depends on the ratio of an apodeme's $x$ and $y$ components. Fig. 2.4D shows the standardized force produced by five different apodeme designs; the optimal angle of fiber attachment is that which gives maximum force production. If the apodeme has only an $x$ component ($y$=0; blue line in Fig. 2.4C) the optimal angle is 0° (see above for why this design is not feasible); if the apodeme has only a $y$ component ($x$=0; green line in Fig. 2.4C; representing species in which there is a principal apodeme branch only and no apodeme base) the optimal attachment angle would be 45°.

For real apodemes, all of which have a small $x$ component ($x/y < 0.3$), the optimum fiber attachment angle is between 41° and 45° for maximum force output (shaded region in Fig. 2.4D). However, in order to maximize the shortening velocity, the angle of attachment should approach 0° (see above). Hence, small mean attachment angles indicate fast muscle characteristics because the attachment angle is minimized at the expense of overall force output (at acute angles, fewer fibers can attach directly to the apodeme).

The smallest angles of attachment were found in *Harpegnathos saltator* and *Myrmecia* sp. (Table 2.2) whose fast fibers deviate by up to 30° from the predicted optimum angle for maximized force (45°). Ants of both genera are fast predators that snap at their prey (Gray, 1971a,b; Gronenberg *et al.* 1997) and are even able to snatch flying prey from the air (Ali *et al.* 1992; Tautz *et al.* 1994; Baroni Urbani *et al.* 1994). Hence, they depend on the speed of their muscles for their success as hunters. In *Camponotus rufipes*, the fast muscle fibers attach at 25°. These ants are not specialized in terms of mandible function and the attachment angle represents a compromise between speed and force of action. The herbivorous *Pogonomyrmex badius* and *Atta sexdens* had the largest angles of attachment. The fast fibers in these species attach at almost 40°, close to the predicted force optimum, indicating that they are specialized for the production of the high forces required to crack

seeds (*Pogonomyrmex badius*) or to cut leaves (*Atta sexdens*). In this respect, the mandible closer muscle of *Odontomachus chelifer* also is designed for maximum force production. It is composed of many short parallel fibers which attach at an angle of 46° (Table 2.2), almost exactly the angle for optimal force output. The apodeme of *Odontomachus chelifer* is almost exclusively composed of the *y* component which is possible because of the long internal processes of the head capsule and the elongated head (Fig. 2.2). A similar design is found in *Acanthognathus rudis*, the other trap-jaw ant in the present study. Hence, like the biochemical and physiological characteristics of muscle fibers, the angle of attachment of muscle fibers allows insights into their function in terms of speed *versus* force output.

**Muscle fiber attachment: why apodeme filaments?**

The presence of filament-attached muscle fibers in ants and even some aspects of their development has been known for a long time (Janet, 1905, 1907a,b) and has become textbook knowledge for insects in general (Gullan and Cranston, 1994). However, these muscle fiber attachments have always been treated as 'mere' idiosyncratic features of some insect taxa and no concepts regarding their functional significance have ever been published.

Ant heads, and elongated ones in particular, contain regions in which the apodeme surface area and the head capsule area are approximately equal (regions $a_1$ and $h_1$ in Fig. 2.3D,E). In these regions, muscle fibers preferentially attach directly to the apodeme because directly attached fibers make the best use of the head capsule volume relative to filament-attached fibers where part of the available space is taken up by filaments and the spaces between them rather than by contractile muscle fiber material.

The apodeme filaments are composed of unsclerotized cuticular material and thus are flexible and can easily follow movements of the apodeme. This is of particular importance for fibers such as the lateral fibers that attach at large angles (group 3 in Fig. 2.3A). Such fibers are subjected to larger angular changes when the apodeme moves during mandible closing. This explains why filament-attached fibers are found in this location in all ant species (Fig. 2.2).

The heads of almost all ants also contain regions where the inner surface of the head capsule provides more area for muscle attachment than does the apodeme. In my model (Fig. 2.3D,E), this is represented by $a_2 < h_2$. Such an arrangement often is found at the muscle periphery and where the curvature of the head capsule is large (most notably at the posterior end of the head; Fig. 2.3B,C). Whereas directly attached fibers require attachment surface areas of a similar size at the apodeme and at the head capsule, filament-attached fibers can

be found where the ratio of apodeme to head capsule surface area is small because they minimize the apodeme surface area required. They help to fill the head capsule with muscle fibers in regions that would otherwise remain unused because too little apodeme surface area would be available for the muscle fibers to attach directly. Filament-attached muscle fibers make better use of the available head capsule volume in these regions (Fig. 2.3D,E).

The distribution of filament-attached and directly attached muscle fibers with respect to the ratio of apodeme to head capsule surface area (*a/h*) is depicted by the curves in Fig. 2.5. The equation underlying these curves is derived from calculations describing the geometrical situations shown in Fig. 2.3D,E (see model 2, Appendix). For directly attached muscle fibers (relative length of filaments $f = 0$) the proportion of the head volume utilized may reach 100% if the *a/h* is 1. For $a/h < 0.5$, filament attachment becomes necessary in order to maximize the utilized volume (in Fig. 2.5 the maxima of the curves depicting *a/h* values of 0.35, 0.2 and 0.05, respectively, occur at increasing filament lengths). This is the case in peripheral head regions where there is not enough apodeme surface area available for muscle fibers to attach directly (Figs. 2.1C, 2.3B,D). The smaller the *a/h* ratio, the longer the filaments have to be to fill the space most efficiently with muscle fibers. This is the reason why the filaments vary in length within and between species and why in some cases they are not present at all (Fig. 2.1D-F). More efficient use of head volume means a larger overall power output or that the required muscular power can be generated within a smaller head capsule. An efficiently designed (hence smaller) head means that less energy has to be spent to move the body mass around, and a smaller head may also be more easy to manoeuvre.

Filaments need a much smaller apodeme surface area to attach to than do directly attached muscle fibers. Filament-attached fibers can thus make better use of the available head capsule surface area, resulting in a higher absolute number of muscle fibers (compare

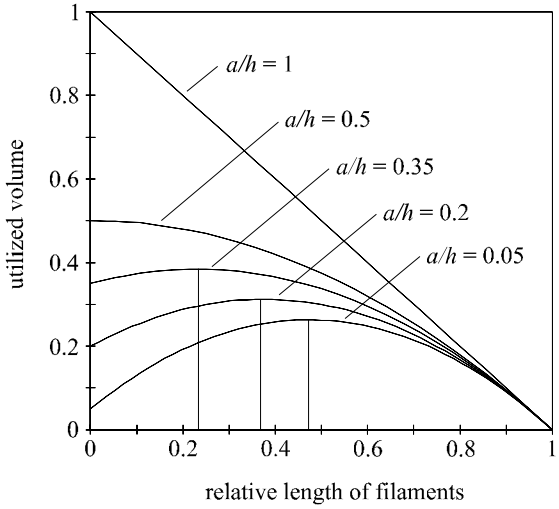



Fig. 2.5: The relationship between head capsule volume that can be used by muscle fibers ('utilized volume'; *y* axis) and the relative length of filaments *f* (*x* axis), for areas differing in the ratio of apodeme to head capsule surface area (*a/h*). The relationships were using the equation: $\eta = (a/h - 1) f^2 + (1 - 2\, a/h) f + a/h$ (for derivation see model 2 in Appendix). Directly attached fibers ($f = 0$) can use 100% of the head volume if $a/h = 1$. For $a/h < 0.5$, filament-attached fibers ($f > 0$) make better use of the available head capsule volume, hence more force can be generated. Vertical lines indicate the maxima of the three lower curves. See text for further details.

Fig. 2.3 B and D with C and E, respectively). Moreover, filament-attached fibers may insert at an optimal angle at the apodeme because there is no additional 'cost' in terms of apodeme surface area as is the case in directly attaching fibers (see above). Fig. 2.3B shows that in posterior head regions filament-attached fibers may insert at very small angles with respect to the overall direction of pull which results in a larger force vector in the required direction. Accordingly, their individual contribution to the resulting overall force is significantly larger than that of directly attached fibers for which the optimum angle is 45° (see above). The difference in overall power output due to the steeper attachment angle and the higher absolute number of muscle fibers acting in parallel (Fig. 2.3B,C) may be surprisingly large. The filament-attached fibers in the schematic example of Fig. 2.3B (20 fibers, mean angle of attachment 24.5°) would generate more than twice as much force as the directly attached fibers in Fig. 2.3C (12 fibers, angle of attachment 45°). Of course, on average the filament-attached fibers are shorter, meaning that fewer sarcomeres shorten in series and the resulting movement in Fig. 2.3B would not be as fast as the one in Fig. 2.3C.

**Species-specific differences: filaments or direct fiber attachment?**

It seems that the conditions can be defined relatively clearly under which filament-attached or directly attached fibers are advantageous. One might thus expect an optimal ratio of the two fiber types to be expressed in all ant species. Why, then, do we find among different species divergent designs and varying proportions of the fiber types ranging from virtual absence to almost sole presence of filament-attached fibers (Table 2.1)? Three tendencies emerge from my study which may account for this diversity.

First, the ratio of directly attached and filament-attached fibers depends on the behavior and living conditions of a given species, just as is the case for the proportions of fast and slow muscle fibers (Gronenberg *et al.* 1997). Species that perform fast mandible movements rely on fast muscles composed of long muscle fibers which attach directly at small angles at the apodeme. Hence, fast movements generally require long head capsules to accommodate the long muscle fibers (e.g. the predatory *Harpegnathos saltator*; Fig. 2.2). In contrast, species depending on particularly forceful mandible movements, such as seed-cracking harvesting ants or leaf-cutting ants, generally have broad heads (compare *Atta sexdens* and *Harpegnathos saltator* in Fig. 2.2). In a broad head, more muscle fibers can be accommodated in parallel. However, broad heads feature relatively shorter apodemes, and hence less apodeme surface area for the muscle fibers to attach to. For this reason, more muscle fibers are filament-attached in these species. Moreover, in posterior head regions,

filament-attached fibers have a force advantage over directly attaching fibers (see above). As a result, species requiring forceful mandible movements tend to have broad heads and many filament-attached fibers, whereas fast predators tend to feature longer heads and a greater proportion of directly attached muscle fibers in addition to a higher percentage of physiologically fast muscle fibers (Gronenberg *et al.* 1997). The relatively broad head of the ant *Myrmecia* sp. (Fig. 2.2) indicates that these ants can generate large forces with their long and sturdy mandibles. However, ants of the genus *Myrmecia* are also known for their fast mandibles snapping (Gray, 1971b). This seeming exception from the tendency of ants performing fast mandible movements to feature long heads can be explained by several facts: Firstly, ants of the genus *Myrmecia* have particularly large brains which probably require broad heads to fit into. Secondly, for unknown reasons, in the genera *Myrmecia*, *Amblyopone* and *Mystrium* the mandible bases are set widely apart and thus require broad heads. Thirdly, *Myrmecia* sp. have very large heads and mandible closer muscles in absolute terms. As with increasing head size the volume increases faster than the surface area, these ants need relatively larger attachment surfaces, hence broader heads and branched apodemes (Fig. 2.2). Nevertheless, *Myrmecia* sp. appears less specialized for speed of action than does *Harpegnathos* (Fig. 2.2) even though they do perform fast mandible movements.

Second, there is a general tendency for smaller species (*Leptothorax sordidulus*, *Technomyrmex* sp., *Gnamptogenys* sp.) and smaller individuals (*Camponotus rufipes* small workers *versus* soldiers) to feature more filament-attached fibers (Table 2.1; Paul *et al.* 1996). I do not understand the reasons for this tendency or understand its significance at present.

Third, the ranking of species according to their type of muscle fiber attachment (Table 2.1) to some extent reflects their phylogenetic relationships. The myrmeciine genus *Myrmecia* and the ponerine genera *Odontomachus*, *Diacamma, Harpegnathos* and *Ectatomma* all have substantially fewer filament-attached fibers and shorter filaments than the myrmicine or formicine genera studied (*Pogonomyrmex*, *Acanthognathos*, *Atta*, *Camponotus*), suggesting an evolutionary trend towards more and longer filaments. However, the former genera are predators relying mainly on rapid mandible action while the latter are herbivorous or omnivorous and probably less dependent on the speed of mandible movements. The apparent phylogenetic trend may thus only reflect different requirements in terms of mandible velocity.

To summarize, I conclude that muscle fiber attachment type in different species of ants depends both on body size and phylogeny. However, the most significant determinants

of the requirements for fast or forceful mandible movements in ant species are life style variables such as feeding habit.

## Appendix

**Model 1: optimal angle of attachment to maximize power output**

In principle, any real apodeme (left side of Fig. 2.4A) can be considered as being composed of two perpendicular surfaces, an *x* (horizontal) and a *y* (longitudinal) component (right side of Fig. 2.4A). In real apodemes, the *x* component is small (representing the apodeme base surfaces) while the *y* component is large (representing the long apodeme branches). Each directly attached fiber covers a portion of the apodeme surface. This portion ($y_i$, $x_i$ in Fig. 2.4B) depends on the angle of attachment $\alpha$ and (except the single case of $\alpha$ = 45°) is different for the *x* and the *y* component of the apodeme (Fig. 2.4B):

$y_i = d / \sin \alpha$ , (1)

$x_i = d / \cos \alpha$ , (2)

where $x_i$, $y_i$ are the attachment surface of a given fiber in the *x* or *y* directions, respectively; *d* is fiber diameter and $\alpha$ is angle of attachment.

The total number *i* of fibers that can attach to a given apodeme is calculated as the quotient of the apodeme surface (*x* and *y* component) and the attachment surface of a single muscle fiber. As fibers can attach to both sides of the longitudinal apodeme branches, the *y* component is doubled:

$i = (2y / y_i) + (x / x_i)$ , (3)

where *i* is the maximum number of muscle fibers and *x*, *y* are the apodeme expansions in the *x* and *y* directions, respectively.

The resultant force in the *y* direction (the principal direction of pull) of a single muscle fiber ($\boldsymbol{F_i(\alpha)}$) depends on the attachment angle:

$\boldsymbol{F_i(\alpha)} = \boldsymbol{f_i} \cos \alpha$ , (4)

where $\boldsymbol{f_i}$ is the force produced by a single fiber (see Fig. 2.4B).

The individual fibers act concurrently to generate the total resultant force of the muscle ($\boldsymbol{F(\alpha)}$) which is equal to the sum of the force produced by all fibers:

$\boldsymbol{F(\alpha)} = i\, \boldsymbol{F_i(\alpha)}$ . (5)

The optimal angle of attack between the apodeme and the muscle fiber should be 0° (equation 4; cos 0° = 1); however, the attachment surface for a given fiber increases with decreasing attachment angle (equation 1) and would become infinitely large at 0°. At 90°,

the attachment surface is at a minimum which would allow the maximum number of fibers to be attached to a given apodeme. However, in this case, the resulting force would be zero (equation 4; cos 90° = 0). Thus, the optimal mean angle of attachment for all fibers of a muscle will lie somewhere between 0° and 90°.

The total force $\boldsymbol{F(\alpha)}$ acting on the apodeme depends on fiber diameter, apodeme size, and angles of attachment and can be calculated by combining equations 3 and 5:

$$\boldsymbol{F(\alpha)} = (\boldsymbol{f_i} / d) (\cos \alpha) [2y (\sin \alpha) + x (\cos \alpha)] . \quad (6)$$

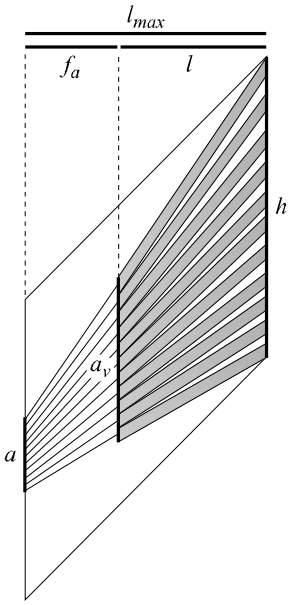



Fig. 2.6: Schematic illustrating the variables used to describe the use of head capsule volume by muscle fibers, and to derive equation 13 (for details see Appendix, model 2). $a$, apodeme attachment surface; $a_v$, virtual apodeme attachment surface; $f_a$, perpendicular component of the absolute filament length; $h$, head capsule surface; $l$, perpendicular component of fiber length; $l_{max}$, perpendicular component of maximal fiber length.

**Model 2: efficient use of head capsule volume and the mode of fiber attachment**

This model is applicable to any region of the head capsule which reflects the geometrical relationships shown in Fig. 2.3D,E. The 'utilized' volume $\eta$ is defined as the quotient of the volume $V$ which actually contains muscle fibers and the overall volume $V_{max}$ which could be filled with fibers:

$$\eta = V/V_{max} . \quad (7)$$

For simplicity, I treat $V$ and $V_{max}$ as areas rather than volumes. Thus, the volume $V_{max}$ corresponds to the area of the parallelogram in Fig. 2.6 which is defined by its base $h$ and its height $l_{max}$:

$$V_{max} = h\, l_{max} , \quad (8)$$

where $h$ is head capsule surface and $l_{max}$ is the distance between apodeme branch and head capsule.

The volume $V$ occupied by the muscle fibers is:

$$V = a_v\, l , \quad (9)$$

where $a_v$ is the virtual apodeme attachment surface and $l$ is the perpendicular component of the fiber length (Fig. 2.6).

The virtual apodeme attachment surface $a_v$ depends on the perpendicular component of the absolute filament length $f_a$ and the apodeme attachment surface $a$:

$a_v = m f_a + a$ , (10)

where $m$ is a linearity factor calculated from:

$m = (h - a) / l_{max}$ . (11)

Combining equations 11, 10 and 9, with equation 7 and substituting $l$ for $(l_{max} - f_a)$, equation 7 becomes:

$\eta = (l_{max} - f_a) \{[(h - a) / l_{max}] f_a + a\} / (h\, l_{max})$ . (12)

Defining the relative length of filaments $f$ as $(f = f_a / l_{max})$ and simplifying equation 12 yields:

$\eta = (a/h - 1) f^2 + (1 - 2\, a/h) f + a/h$ . (13)

The curves plotted in Fig. 2.5 are calculated using equation 13 for different $a/h$ ratios.

# A. THE MANDIBLES

## 3. Motor control of the mandible closer muscle in ants

## Introduction

In higher vertebrates as well as in arthropods, limb movements are of supreme importance in almost any behavioral context (e.g. escape, feeding, migration etc.). Most natural behavioral sequences involve more than one limb, and limbs are generally controlled by many muscles. It is therefore a daunting task to analyze even simple natural behaviors in terms of their underlying motor programs and muscle contractions. Because of their relative simplicity, however, limb movements of arthropods provide interesting models for studying the motor control of behaviorally relevant movements (e.g. locust leg: Wolf and Burrows, 1995; Ott et al. 2000; stick insect leg: Bässler and Büschges, 1998; Akay et al., 2001). Jaw movements of ants are a case in point, as their mechanical organization is simple yet the jaws contribute to a complex behavioral repertoire.

Ants use their jaws as universal tools for prey-catching, fighting, digging, leaf-cutting, seed-cracking or wood-scraping, as well as delicate routines such as grooming, brood care, and food exchange or communication among nestmates (Hölldobler and Wilson, 1990). Accordingly, ant mandibles perform many different kinds of movements in terms of velocity, force output, and precision (Gronenberg et al., 1997). Based on a simple design, the mandibles of many ants are large and powerful (Janet, 1905; Snodgrass, 1935): a single-segmented appendage is attached to the head capsule by a hinge joint and is operated by a single pair of antagonistic muscles (opener and closer) on each side of the head. Mandibular force, velocity and dexterity mainly depend on the muscles that control the jaws. The mandible closer muscle is the key to the versatility of mandible functions. Its behavioral significance is reflected by its anatomical design; it is much larger than the opener muscle and occupies up to two-thirds of the entire head capsule volume. It is the largest muscle in any ant worker.

Ant mandible closer muscles generally comprise two distinct muscle fiber types that are composed of either short (2-3 µm) or long sarcomeres (5-6 µm). Short sarcomeres suggest fast contraction properties while long sarcomeres imply slow yet forceful contraction of the respective muscle fibers (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul 2001). In ant jaw muscles, fast muscle fibers always directly attach to the mandible closer apodeme (an analogue to the vertebrate tendon) whereas slow fibers attach either directly or via thin cuticular filaments to the apodeme. The ratio of fast to slow muscle fibers as well as the proportion of filament-attached fibers and the angle of fiber attachment at the apodeme depend on the species-specific mandible use (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul 2001). Within the mandible closer muscle, the three morphologically distinct

fiber types (directly attached fast and slow fibers and the filament-attached slow type) are arranged in bundles that each consist of only a single fiber type (Fig. 3.1a, b; Gronenberg et al., 1997; Paul and Gronenberg, 1999). This is in contrast to the design of other arthropod muscles where functional properties (contraction characteristics, diameter, biochemical composition) may vary in a more graded fashion among fibers, and different fiber types may be arranged concentrically or interspersed within a muscle (Günzel et al., 1993: crayfish leg muscle; Hoyle, 1978, Müller et al., 1992: locust leg muscle; Maier et al., 1987: spider leg muscle; Ehmer and Gronenberg, 1997: ant antennal muscles). The segregation of different muscle fiber types within the ant mandible closer muscle hints at the possibility that those fiber bundles may represent functional units.

Little is known about the control of mandible closer muscles in insects, although they are a prominent muscles in all biting and chewing insect taxa. Some aspects of mandible control have been studied in several species of trap-jaw ants (Gronenberg, 1995a, b; Gronenberg and Ehmer, 1996; Gronenberg et al., 1993, 1997, 1998; Just and Gronenberg, 1998; Paul and Gronenberg, 1999), but the mandible mechanism of these ants is highly specialized. The innervation pattern of mandible muscles has been studied in locusts (Baines et al., 1990b), caterpillars of the hawk moth *Manduca sexta* (Griss, 1990), and the honeybee (Masuko, 1986; Rehder, 1989). Moth larvae only perform a reduced repertoire of mandible movements and honeybee mandibles are much reduced, reflecting phylogenetically derived sucking mouthparts. Hence bees, caterpillars or trap-jaw ants are not particularly well suited to study the general design of insect mandible control.

In the present study I investigate the control of the mandible closer muscles in two ant genera. Our previous studies of mandible muscles (Gronenberg et al., 1997; Paul and Gronenberg, 1999) were based on morphological characteristics. However, contractile properties, sarcomere length and other structural properties do not always accurately predict physiological performance of muscle fibers in arthropods (Costello and Govind, 1983; Silverman et al., 1987). I therefore try to establish a causal relationship between the mandible movement and the electrical activity of particular muscle fiber types. A major question addressed is whether distinct muscle fiber bundles within a muscle can be activated separately, and how their action affects the resulting mandible movement. Furthermore, I try to correlate the number of electrophysiologically distinct motor units with the number of morphologically identified motor neurons.

## Materials and Methods

Experiments were carried out on workers of the following ant species: *Pogonomyrmex rugosus and P. californicus* (Myrmicinae); *Camponotus rufipes, C. festinatus* and *C. laevigatus* (Formicinae). The ants were kept in plaster-of-Paris nests under a 12h : 12h L : D cycle at 25°C and 50% relative humidity. They were fed chopped cockroaches, crickets or wingless *Drosophila*, and honey-water (30%) or grains (*Pogonomyrmex*).

**Electrophysiology**

To assess the activity of the mandible closer muscle, extracellular muscle recordings were performed on *Camponotus rufipes*. For anesthesia, ants were subjected to enflurane vapors (Ethrane, Abbot) for not more than 5 seconds and waxed onto a support so that the mandibles could move freely. Electrolytically sharpened stainless steel minutien pins (0.1 mm diameter) served as muscle electrodes. For differential recording, two pins per recording site (distance ca. 100 μm) were inserted through the cuticle into the mandible closer muscle using micromanipulators. In any given experiment, I simultaneously recorded from two of the four sites (Fig. 3.1a, b) and changed the combination of recording sites for each experiment. Recording sites were chosen so as to sample homogeneous muscle fiber groups (fast, slow and filament-attached slow fibers; Fig. 3.1a, b). While it would have been desirable to record from all fiber groups simultaneously, the impairment of the animals was too large when more than two pairs of electrodes were inserted, and the resulting mandible movements were reduced and did not reflect natural activity.

Muscle potentials were amplified up to 1.000 x (DAM 50, World Precision Instruments) and stored together with the record of the mandible movement (see below) on magnetic tape (Biologic DTR 1800). Recordings were off-line computer-analyzed using appropriate software (Spike II, CED). After recording muscle activity, the recording sites were marked by two different methods: either by using the Prussian blue reaction (according to Wässle and Hausen, 1981; injection of positive current and precipitation of the ferric ions with potassium ferrocyanide solution) or by depositing a small amount of Congo-Red (Merck) at the electrode entry points followed by slightly moving the electrode backwards and forwards, thus transporting some of the dye into the muscle. After fixation (phosphate-buffered 4% formaldehyde or 2.5% glutaraldehyde, pH 6.9), the heads were dehydrated, embedded in Fluka Durcupan, and horizontally sectioned at 15 μm. Sections were then microscopically inspected.

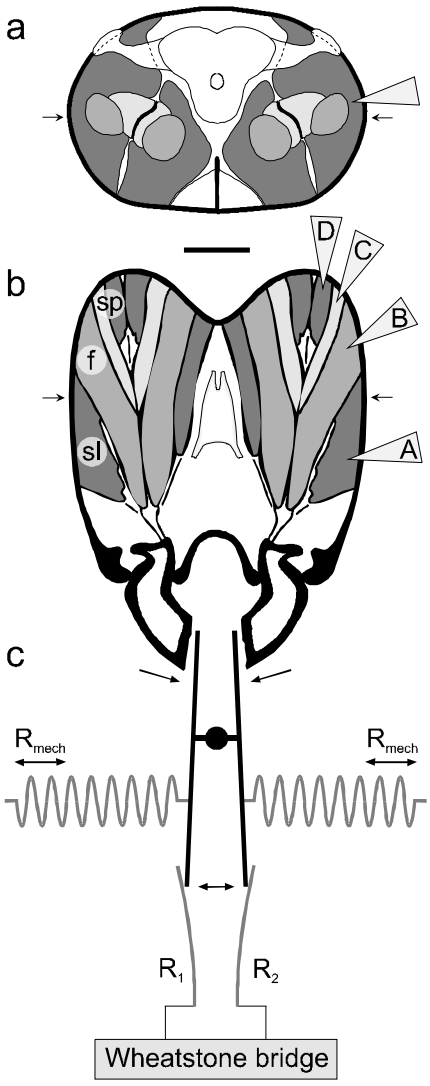



Fig. 3.1: Schematized composition of ant mandible closer muscle and experimental setup for electrophysiological experiments. Anterior view, **a** (plane indicated by arrows in **b**), and dorsal view, **b** (plane indicated by arrows in **a**), of a worker head of *Camponotus rufipes*; light grey directly attached slow fibers; medium grey directly attached fast fibers; dark grey filament-attached slow fibers; triangles A, B, C, D indicate electrophysiological recording sites corresponding to different muscle fiber groups [A: slow lateral (sl), B: fast (f), C/D: slow posterior (sp), C: slow posterior directly attached, D: slow posterior filament-attached]; scale bar 500 µm. **c**: Mechano-electrical transducer used to record mandible movements. Lever arms transmit mandible movements to two strain gages $R_1$, $R_2$ that change their electrical resistance upon bending. Strain gages are part of a balanced Wheatstone bridge. Mandible movements result in currents across the bridge that are amplified and recorded. The mandibles contract against mechanical resistance $R_{mech}$ which is set by adjusting two springs attached to the lever arms.

**Mandible movements**

Force and velocity of mandible movements were recorded simultaneously with the muscle fiber activity in workers (head width: 3.5 - 4.4 mm) of *Camponotus rufipes*. A mechano-electrical transducer was constructed from two strain gages (Measurements Group, Inc., CEA-06-250UN-350) that served as resistors in a Wheatstone bridge and were connected to the mandibles by spring-loaded adjustable lever arms (Fig. 3.1c). The system allowed measuring mandibular movement against two preset mechanical resistances and was calibrated using standard weights. The resulting electrical signal was 0 (baseline) when the mandibles were open; upward deflection indicated mandible closing (cf. Fig. 3.3). The slope and amplitude of the transducer signal allowed calculating the mandible velocity and force, respectively. The slope was calculated from movement episodes that covered at least 20° (up to 40°) of mandible movement (see Fig. 3.3).

**Neuroanatomy**

To examine the mandible closer motor neurons' input regions within the suboesophageal ganglion, the ants were anaesthetized and immobilized in wax. A small window was cut into the head capsule of the ant laterally, posteriorly, or dorsally to give access to a particular group of mandible closer muscle fibers. Some muscle fibers were then slightly damaged in order to disrupt the motor neuron terminals, and a tiny crystal of a fluorescent tracer (Fluoro Ruby, Molecular Probes) was placed onto the damaged parts using minutien pins or drawn-out glass capillaries. The hole in the head capsule was then sealed and the dye was allowed to be retrogradely transported by the damaged neurons into the central nervous system for 15-20 hours. The ants were then decapitated, their heads fixed in phosphate-buffered 4% formaldehyde, dehydrated with dimethoxy propane, embedded in Fluka Durcupan, and horizontally sectioned at 10-20 μm. Labeled motor neurons and stained muscle fibers were viewed under an epi-fluorescence microscope (Zeiss Axiophot) equipped with the appropriate filter combinations. Images were digitized (SPOT 2, Diagnostic Instruments) and graphically reconstructed using Adobe Photoshop software.

## Results

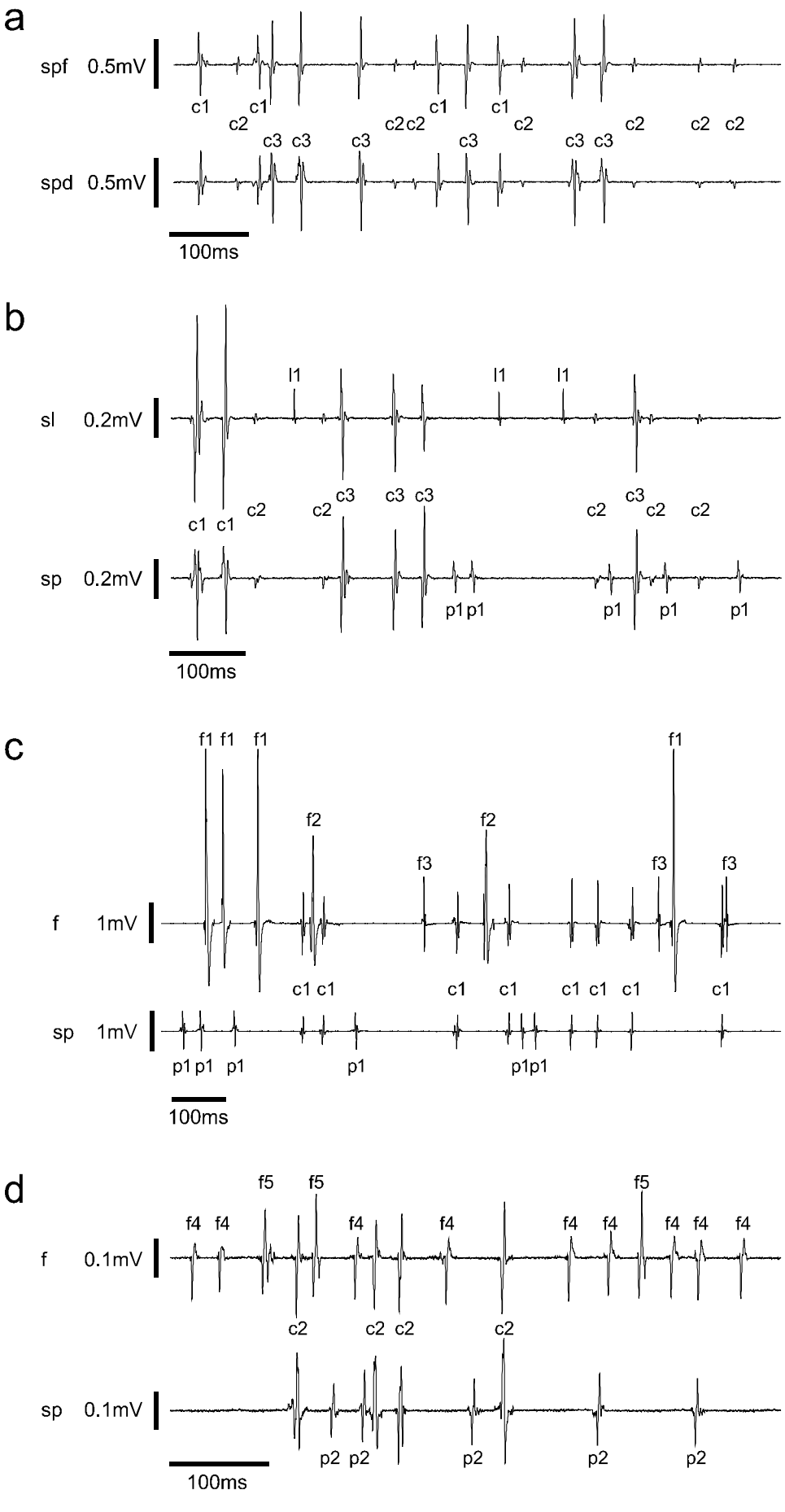
**Muscle fiber groups and motor units**

Ant mandible closer muscles comprise three types of morphologically distinct muscle fibers: directly attached fast fibers with short sarcomeres, slow filament-attached fibers, and slow directly attached fibers (the latter two have long sarcomeres). In ants these different fiber types are segregated into bundles of like fibers. In all ants studied, the mandible closer muscle is thus composed of sub-units that each comprise only a single muscle fiber type and occupy characteristic positions within the head capsule. This general design is shown in Figure 3.1a, b, which is based on the muscle fiber arrangement found in *Camponotus*. While the relative and absolute size of each muscle fiber group differs across species and in some cases between individuals of different body size, the relative position of the different muscle fiber bundles is very similar in different species. The electrical activity of a particular muscle fiber type can therefore be assessed by inserting recording electrodes through the head capsule at specific locations (Fig. 3.1b). In each experiment, I simultaneously recorded from two of the three fiber types (e.g. recording sites A and B in Fig. 3.1b) or from the same fiber type in two different locations (sites A and D in Fig. 3.1b both probe filament-attached slow fibers). The dye marking procedures (Congo Red and Prussian Blue reaction) allowed the

determination of the actual electrode position. In all cases the assumed electrode position coincided with the location of the dye precipitation.

During any particular recording experiment, potentials of different shape and amplitude were recorded within the same as well as across separate muscle fiber bundles. The amplitude varied considerably, particularly among potentials recorded from the fast muscle fiber type (Fig. 3.2c, d). These junctional potentials lasted 6 - 11 ms and have a spike-like appearance at low temporal resolution (as in Figs. 3.2, 3.3). I will refer to the potentials as 'spikes' even though they do not represent classical action potentials. Spikes in the same recording trace that were of identical or very similar shape and amplitude are referred to as a single spike type (each particular spike type is thought to reflect the activity in a single motor neuron). Thus, several spike types recorded with the same electrode indicate that the respective muscle fiber group is controlled by several motor neurons. An example is shown in the two traces of Figure 3.2a, that were recorded from filament-attached (spf) and directly attached slow posterior muscle fibers (spd) corresponding to electrode positions D and C in Figure 3.1b. Three spike types can be discriminated (Fig. 3.2a: c1-c3). The same potentials occur simultaneously in both recording traces. This temporal coincidence of spikes in two different muscle fiber groups is here interpreted as originating from activation of the same motor neuron that supplies both muscle fiber groups. Another potential explanation for the simultaneous occurrence of spikes in different recording channels is electrical crosstalk between the electrodes. However, I did not find any evidence for this artifact in my experiments. I found simultaneous spikes not only in adjacent muscle fiber groups, but also in ones much further apart from each other (e.g. recording location 'A' and 'C' in Fig. 3.1; see Fig. 3.2b). There, potential crosstalk should be much reduced. Likewise, the large spikes (f1, f2) in Figure 3.2c do not coincide with any potentials in the adjacent muscle fiber group (lower trace in Fig. 3.2c). If crosstalk were present, the strongest artifacts (synchronous spikes in the lower trace) would be expected under these conditions.

In general, no differences were found when recording activity from directly attached and filament-attached slow muscle fibers in the posterior adjacent fiber groups. The functional similarity of these fiber types (directly attached and filament-attached slow fibers) is also suggested by functional morphology (Paul and Gronenberg, 1999). I will therefore only refer to the three functionally distinct fiber groups: fast fibers, lateral slow fibers and posterior slow fibers.


a
spf 0.5mV
spd 0.5mV
100ms
b
sl 0.2mV
sp 0.2mV
100ms
c
f 1mV
sp 1mV
100ms
d
f 0.1mV
sp 0.1mV
100ms

◀ Fig. 3.2: Electromyograms of workers of *Camponotus rufipes* simultaneously recorded from two different muscle fiber groups of the mandible closer. **a**, **b**: examples from different animals showing slow posterior filament-attached (spf) and slow posterior directly attached (spd) fibers (**a**), and slow lateral (sl) and slow posterior (sp) fibers (**b**), respectively. **c**, **d**: two recording sequences from the same animal showing fast (f) and slow posterior (sp) muscle fibers. Several unique spike types are labeled: ones exclusively recorded from fast muscle fibers f1-f5, from slow posterior fibers p1-p2, or from slow lateral fibers l1, respectively; and types simultaneously recorded from both traces c1-c3 (the label 'c' therefore always corresponds to both the potential in the upper trace and that in the lower trace). N = 3 (number of evaluated animals) for the combinations of electrode positions spf/spd, sl/sp, and f/sl, respectively, and N = 4 for f/sp; average recording time that was analyzed per animal = 25 min.

I found seven distinct spike types when simultaneously recording from the two separate packets of slow muscle fibers (the posterior and the lateral fiber group). One spike type was exclusively associated with the lateral muscle fibers (Fig. 3.2b: l1). Two spike types were exclusively recorded from the posterior muscle fiber bundle [only one type (p1) is shown in Fig. 3.2b]. In addition, four spike types occurred simultaneously in both fiber groups (three of them, c1-c3, are shown in Fig. 3.2b).

The top traces of Figure 3.2c, d were recorded from a fast muscle fiber group in the same animal (corresponding to electrode position B in Fig. 3.1b). The two sequences shown in 2c and 2d, respectively, reflect different mandible movements. Several spike types can be discriminated from the activity of this fast muscle fiber bundle. Of these, five unique spike types (Fig. 3.2c, d: f1-f5) did not coincide with any spikes in the bottom traces of Figure 3.2c, d, which were recorded from slow posterior muscle fibers. Recording from fast muscle fibers generally revealed 4-5 unique spike types, indicating that this part of the muscle is controlled by at least five exclusive motor neurons that do not supply other parts of the muscle. In addition, spike types were found to occur simultaneously in fast and slow muscle fibers (e.g. the unmarked spike type in the upper trace of Fig. 3.2c that coincides with 'c1' in the lower trace of Fig. 3.2c).

The posterior group of slow muscle fibers generally featured fewer spike types than the fast fiber bundle. In Figure 3.2c, d (respective bottom traces), two exclusive spike types could be discriminated (p1, p2). In addition, three spike types (c1-c3) always occurred simultaneously in the upper and lower traces (c3 not shown in Fig. 3.2c, d), indicating that they originated from the same set of motor neurons in both traces. The recordings in Figure 3.2c, d therefore show that at least five motor neurons exclusively control the fast fibers, at least two motor neurons exclusively control the posterior slow muscle fiber group, and in addition three other neurons supply both muscle fiber groups simultaneously.

Different experiments did not always reveal the exact same number of spike types in a given muscle fiber group. The results of the ten experiments that showed the best resolution (highest number of spikes discriminated, high spike amplitudes and recording quality stable for at least one hour) are combined in table 3.1. These results indicate that the fast muscle fiber group is supplied by 5 distinct motor neurons, the posterior group of slow muscle fibers receives exclusive input from 2 neurons, and the lateral group of slow fibers is supplied by only one specific motor neuron. In addition, 4 motor neurons supply more than one muscle fiber group simultaneously. Judging by the number of recorded spike types, the entire mandible closer muscle therefore appears to be controlled by 12 motor neurons.

| | **Spike types recorded in** | | | |
|---|---|---|---|---|
| positions of electrodes | fast fibers exclusively | slow post. fibers exclusively | slow lat. fibers exclusively | more than one location simultaneously |
| B + C/D | 5 | 1 - 2 | | 3 |
| A + B | 4 - 5 | | 1 | 4 |
| A + C/D | | 1 - 2 | 1 | 4 |

Table 3.1: Number of unique spike types recorded in different muscle fiber groups of workers of *Camponotus rufipes*. Electrode positions correspond to Fig. 3.1b; n = 10 animals (3-4 for each combination of electrode positions).

**Force and velocity of mandible movements**

When recording activity from the fast muscle fiber group, at least one particularly large spike type was found in every experiment. The amplitude of this particular spike type (2-6 mV; Figs. 3.2c, 3.3) was considerably higher than the amplitude of any spike recorded from the slow muscle fibers (less than 1 mV) or other spike types in the fast muscle fiber bundle. Whenever these large potentials occurred in the fast muscle fibers, the resulting mandible movement velocity was significantly higher compared to movements generated without the recruitment of this particular motor neuron (Fig. 3.3). In Figure 3.3, the mandible velocity (the slope of the upper trace that represents the mandible movement) is much higher during the first movement episode (0.64°/ms) than during the second one (0.15°/ms). The first movement is preceded by, and probably results from, the high amplitude spikes in the fast muscle fibers. In contrast, the slower movement is only associated with small amplitude spikes in both, the fast and the slow muscle fiber group. Movement episodes preceded by high amplitude spikes in the fast muscle fibers were always faster than movements lacking this particular spike type, even if the overall spiking frequency (number of total spikes per time unit) was higher than for movements accompanied by these large potentials.

The correlation between mandible movement velocity and the occurrence of the large spikes is depicted in Figure 3.4. Those mandible movements that were preceded by the large amplitude spikes were significantly faster ($p < 0.0001$, compare grey with black bars in Fig. 3.4, respectively) than mandible movements that were only accompanied by smaller spikes in the fast muscle fibers or that were not accompanied by fast muscle fiber activity at all. In the latter cases, the observed movements supposedly resulted from exclusive activation of slow muscle fibers. No significant correlation was found between the mandible movement velocity and the occurrence of particular spike types in any of the slow muscle fiber groups. This indicates that activation of fast muscle fibers by high amplitude spikes is essential for generating maximum mandible closing movement velocities.

The fast mandible movements resulting from activation of the large amplitude spikes are highly susceptible to increased load (Fig. 3.4). The mandible closing velocity correlated with the large spike activity in the fast muscle fibers is significantly reduced when the mandibles close against a higher mechanical resistance ($p < 0.0001$; compare grey bars in Fig. 3.4). In contrast, within the range of mandibular loads tested, no statistically significant differences were found for the slower mandible movements that result from activation of small amplitude spikes in the fast and slow mandible closer muscle fibers (compare black

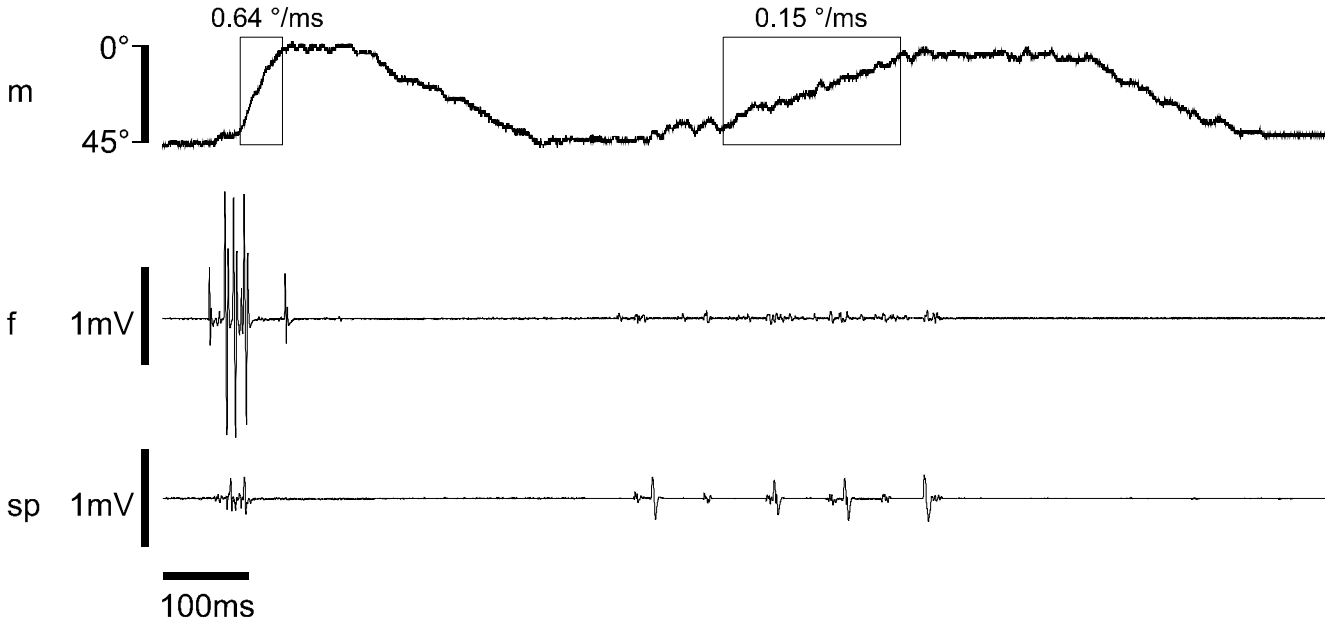



Fig. 3.3: Electro-myogram and corresponding mandible movements of a worker of *Camponotus rufipes*. Top trace (m) represents mandible movements (baseline: mandibles open, inter-mandibular angle about 90°; maximum: mandibles closed; slope corresponds to movement velocity [°/ms]); rectangles indicate fast (0.64°/ms) and slow (0.15°/ms) movements and the respective integration times. Simultaneous muscle recordings from fast (middle trace, f) and slow posterior fibers (bottom trace, sp).

bars in Fig. 3.4). For lower mandibular resistances (average load approximately 17 mN), the minimum mandible closing velocity at which the high amplitude spikes started to occur was 0.37 °/ms (mean velocity under these conditions = 0.49 ± 0.07 °/ms, n = 14; cf. Fig. 3.4).

If the motor neuron(s) that generate the large amplitude spikes are specialized for fast contractions, how then is mandibular force output generated under conditions that require graded or maximum power output rather than ultimate velocity? In analogy to other motor systems, one would expect the spike frequency to control the contraction state of the muscle. An overall comparison of the mean spike frequency with the mandibular force (the amplitude of the mechano-electrical transducer output) shows a significant correlation between spike frequency and force generation (Fig. 3.5a). This correlation holds for both fast and slow muscle fibers.

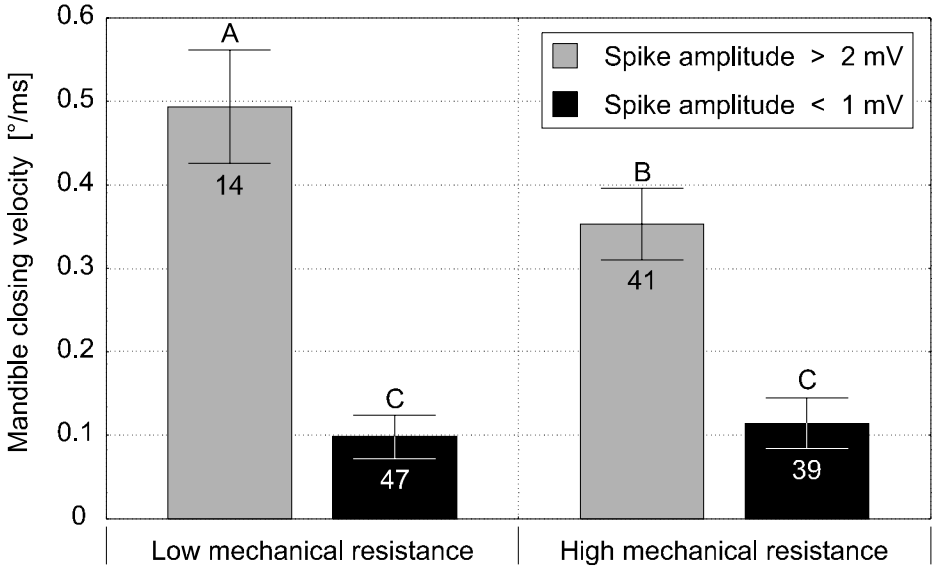



Fig. 3.4: Correlation between mandible closing velocity and electrical activity in muscle fibers with short sarcomeres ("fast fibers") in *Camponotus rufipes* at two different mechanical loads. Grey bars represent mandible movements preceded by high amplitude spikes (> 2 mV), black bars represent movements preceded by smaller (< 1 mV) spikes or not associated with activity in the fast muscle fibers at all; mean ± standard deviation; n = number of movement episodes evaluated; N = 3 animals; statistically significant differences (t-test; $p < 0.01$) expressed by different letters.

When comparing the activity in fast versus slow muscle fibers in each recording experiment (spike frequency in fast fibers divided by spike frequency in slow fibers) with respect to the resulting mandibular force, I did not find any significant correlation (Fig. 3.5b). The slope of the respective linear regression is almost 0, the mean ratio of fast to slow muscle fiber activity is approximately 1. This indicates that both fast and slow muscle fiber units increase their spike frequency simultaneously and proportionately when higher forces are generated. It therefore appears that both muscle fiber types contribute in a similar way to the muscle's force production. However, slow muscle fiber groups were sometimes activated exclusively without activating fast muscle fibers. For example, the four data points in Figure 3.5b that show an activity ratio of 0 indicate that fast fibers were completely inactive during

these movement episodes. The large spikes in the fast muscle fibers occur only correlated with fast contractions (large spikes were not evaluated separately in Fig. 3.5b).

As the mandible pivots around its axis in the mandible joint, the supposed lever arm and the relative direction of pull of the entire closer muscle at the apodeme basis might change during the mandible closing movement. It therefore seems possible that particular motor units may be activated depending on the mandible's actual angular position in order to maximize contraction efficiency. For example, lateral muscle fibers might reach their

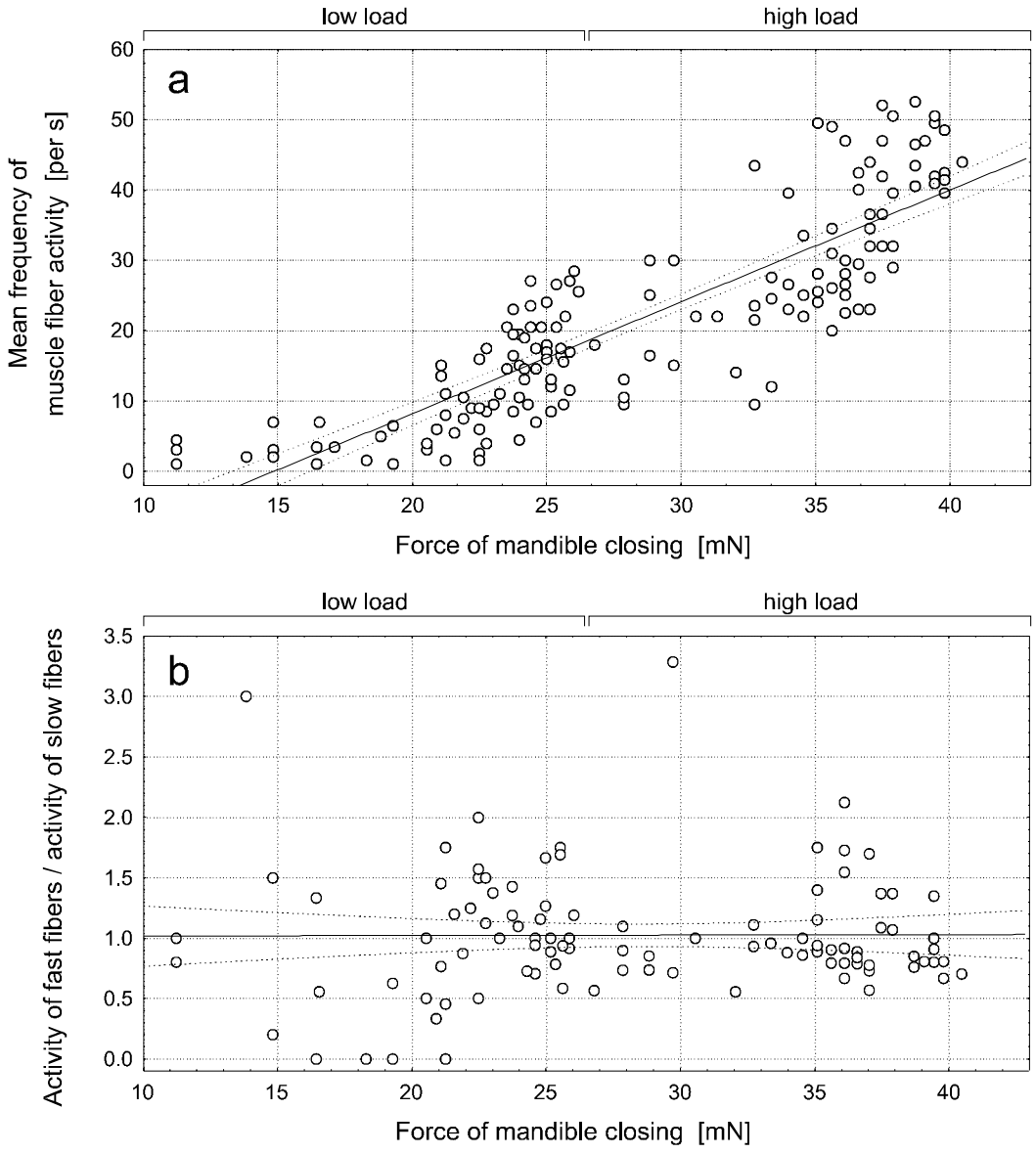



Fig. 3.5: Correlations between electrical activity and force developed by the mandible closer muscle. Each animal (*Camponotus rufipes* workers) was tested at two different mechanical resistances represented by the left (low load) and right half (high load) of the graphs. **a**: Each data point represents the mean spiking frequency recorded from two muscle fiber groups simultaneously (n = 171 movement episodes; N = 4 animals; r = 0.85706; p < 0.0001). **b**: Data points depict the activity ratio of fast vs. slow mandible closer muscle fibers (n = 112; N = 2; r = 0.00595; p = 0.95051).

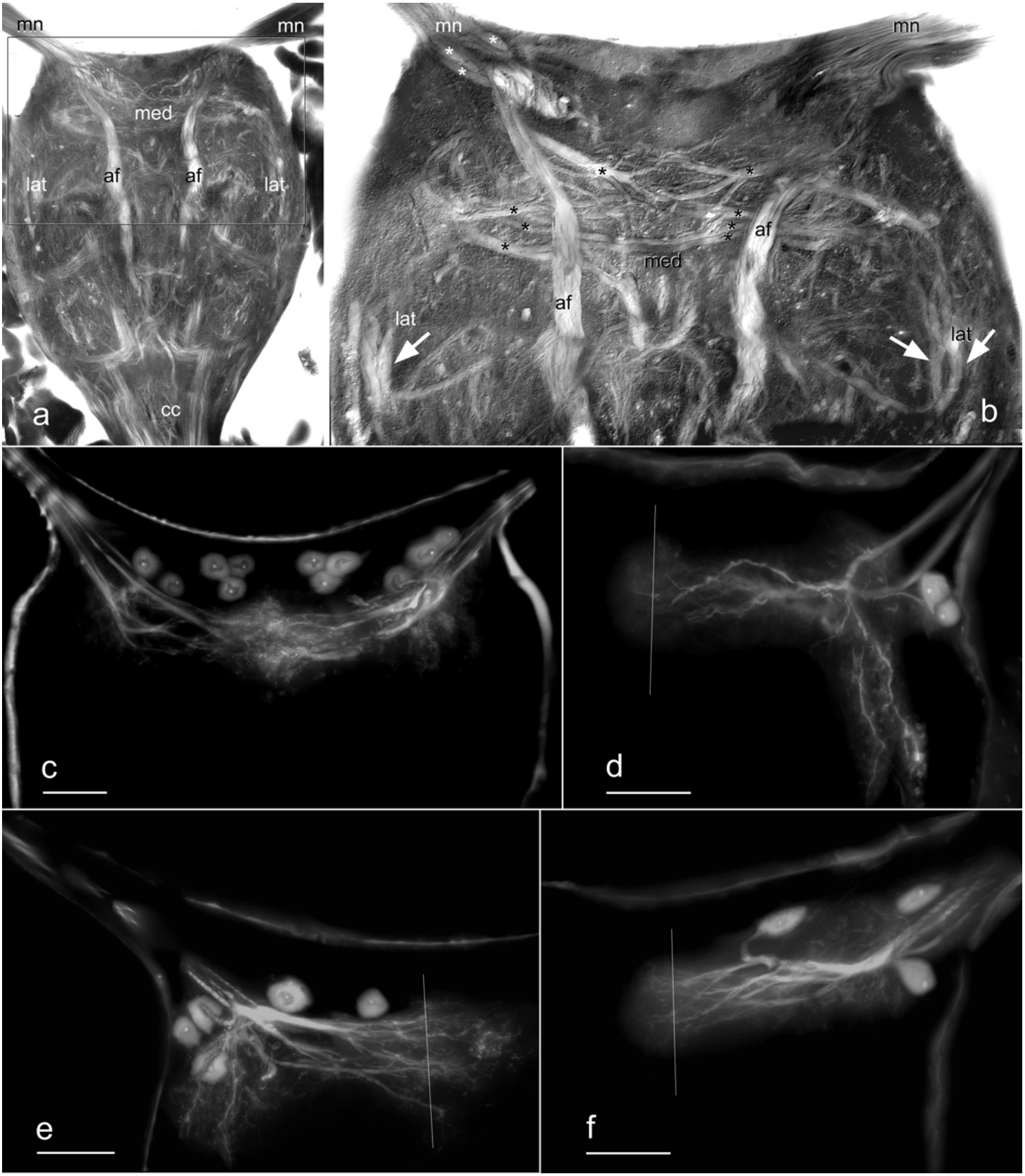
optimum angle of pull when the mandibles are almost closed whereas posterior muscle fibers might contribute much less force under these conditions. To test this hypothesis, I compared the spiking activities in posterior and lateral muscle fibers for different mandibular angles over a range of 45° (covering most of the mandibles' working range in *Camponotus*). No significant correlation was found when plotting the ratio of spike frequencies in posterior fibers to lateral fibers against the mandibular angle ($p > 0.05$; $r = -0.18961$; $n = 186$; $N = 4$). This indicates that posterior and lateral muscle fibers are not recruited differentially under these experimental conditions. I did not find any indication for predictable successive activation pattern of the different muscle sub-units, although distinct muscle fiber groups were activated independently of each other in some individual cases. Rather, the entire muscle generally appears to contract as a single unit, irrespective of the mandibular position.

**Neuroanatomy**

I found some mandible closer motor neurons to be activated independently of each other, hence they probably receive different synaptic input, therefore morphological differences in their dendritic input regions may exist. I have analyzed the anatomy of mandible closer motor neurons from eight successful tracing preparations of *Camponotus* (*C. festinatus* and *C. laevigatus*) and nine preparations of *Pogonomyrmex* (*P. californicus* and *P. rugosus*). No substantial differences (number of neurons, position of cell bodies, primary arborization pattern of dendrites) have been found among these species.

Mandibular motor neurons originate in the suboesophageal ganglion and their axons project through the mandibular nerves (Fig. 3.6a, b) toward the mandible closer muscle. The mandibular nerves are the most prominent nerves of the suboesophageal ganglion. They are mixed nerves and, in addition to the mandible closer motor axons, carry the opener muscle motor axons and the sensory axons that originate from sensilla on the mandible (indicated in Fig. 3.6a, b). Our ethylgallate preparations show that the mandible closer motor axons are

Fig. 3.6 (opposite): Ethylgallate-stained (**a**, **b**) and Fluoro-Ruby-stained mandible closer motor neurons in the suboesophageal ganglion of *Camponotus laevigatus* (**a**-**c**, **e**) and *Pogonomyrmex rugosus* (**d**, **f**); boxed area in **a** enlarged in **b**; motor neurons stained by tracer application in fast muscle fibers (left side of **c**), in fast and slow posterior fibers (right side of **c**), in slow lateral fibers (**d**), in fast and slow lateral fibers (**e**) and in slow posterior muscle fibers (**f**); af mandibular sensory afferents; cc cervical connectives; lat lateral and med medial mandibular motor neuropil; mn mandibular nerve; arrows point at lateral motor neuron dendrites, black asterisks indicate motor neuron primary dendrites, white asterisks indicate motor axons in nerve; midlines indicated by white lines in **c**-**f**; scale bars 50 µm; anterior is up (a, b, ethylgallate-stainings by W. Gronenberg).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the thickest neurons entering the suboesophageal ganglion (5-8 μm) and they can be identified by their axon diameter in ethylgallate-stained material (Fig. 3.6a, b). The mandible motor neurons are restricted to the mandibular neuromere, the ventral-most part of the ganglion (the suboesophageal ganglion is composed of the labial, the maxillary and the mandibular neuromeres). The cell bodies of the mandible closer motor neurons are large (diameter 20-25 μm) and reside in anterior and antero-lateral parts of the cell body rind of the ganglion (Fig. 3.6c-f). The dendrites are restricted to the anterior half of the ganglion where they overlap with sensory afferents from the mandibles. All mandibular sensory afferents together supply a larger part of the suboesophageal ganglion than do the motor neurons. Unlike the motor neurons, some of the mandible afferents pass through the cervical connectives into the thoracic ganglia (not shown in the figures).

Mandible closer motor neurons are mainly restricted to the ipsilateral side of the ganglion. In most neurons, only some fine dendritic branches cross the midline and reach into contralateral neuropil for a short distance (Fig. 3.6d-f). This central region of bilateral overlap is largest in those motor neurons that supply the fast closer muscle fibers (Fig. 3.6c). In addition, preparations in which the fast muscle fibers were labeled usually showed the highest number of cell bodies (five and six, respectively, in Fig. 3.6c). However, in some cases (n = 5) up to 3 of the labeled motor neurons were not specific for this particular muscle fiber group but would also be stained when labeling a different set of muscle fibers, indicating that these motor neurons supply more than one muscle fiber type.

When the lateral set of slow muscle fibers was stained, generally only two motor neurons were labeled (Fig. 3.6d). These particular motor neurons are characterized by lateral dendritic branches in addition to the central branches common to all mandible closer neurons. The cell bodies of these neurons with processes in the lateral neuropil are situated in a lateral soma cluster close to the root of the mandible nerve (Fig. 3.7).

In Figure 3.6e, slow lateral muscle fibers were stained together with a few of the adjacent fast fibers. Only two of the 'fast' motor neurons were stained together with three neurons that control the lateral slow muscle fiber group. The two 'fast' motor neurons give rise to the dendrites that characteristically cross the midline. Two of the 'slow' motor neurons are characterized by their posterolateral dendritic arborizations and by their lateral soma location as seen in Figure 3.6d and described in the previous paragraph (the third lateral cell body probably belongs to a neuron that supplies all slow muscle fibers, as it was also found after tracer application to the posterior muscle fiber group shown in Fig. 3.6f).

Staining from posterior muscle fibers revealed three motor neurons that lack the postero-lateral dendrites and whose central dendritic branches barely cross the midline (Fig. 3.6f). Two of their cell bodies are found in the anterior clusters and a third one resides more laterally close to the mandibular nerve root. The latter is probably identical with one of the cell bodies in Figure 3.6e and represents a motor neuron that supplies all the slow mandible closer muscle fibers simultaneously.

The anatomical data are summarized in Figure 3.7. Together, at least ten different somata were identified to supply the mandible closer muscle. Four of these (number 5, 6, 8, 9) were reliably associated with the fast muscle fibers. One or two somata give rise to motor neurons supplying lateral slow muscle fibers (number 2 and one of its neighbours, either number 1 or 3), and 1-2 cell bodies exclusively supply posterior slow muscle fibers (number 10 and one soma in the lateral cell body cluster). In addition, I found 3-4 cell bodies (among them number 4 and 7) that were revealed in many preparations irrespective of the muscle fiber group stained. These motor neurons supposedly supply all muscle fiber types simultaneously. Cell bodies number 1 and 3 were hard to differentiate because they were stained in different combinations with other neurons. They supply either lateral or posterior slow muscle fibers, or both groups.

The dendrites of motor neurons supplying fast muscle fibers are situated slightly more anterior compared to those of the other motor neurons, and they project deeper into contralateral neuropil. Neurons supplying lateral slow muscle fibers, in addition to their

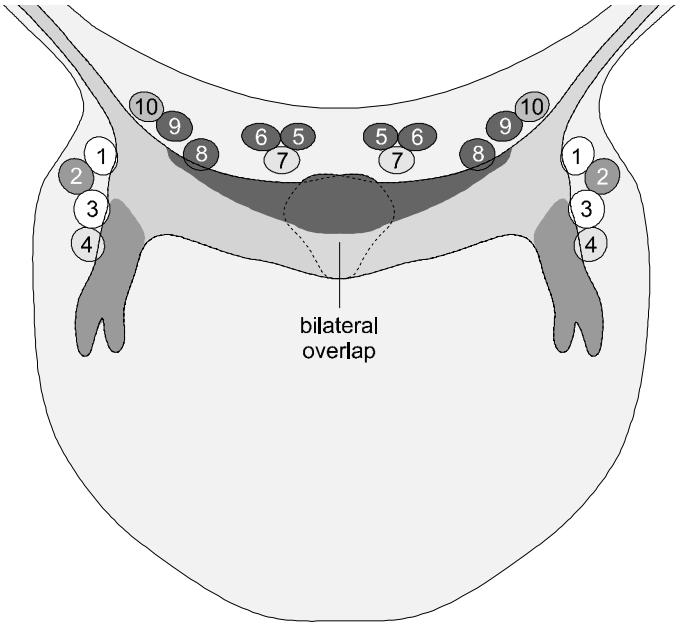



Fig. 3.7: Schematic showing suboesophageal ganglion with dendritic regions and cell body locations of mandible closer muscle motor neurons (horizontal plane, anterior is up). Cell body numbers 5, 6, 8, 9 supply fast muscle fibers exclusively; 2, 10 supply slow muscle fibers (2 lateral, 10 posterior ones); 1, 3 supply either lateral or posterior slow muscle fibers, or both groups; 3-4 motor neurons innervate all muscle fiber types simultaneously (among them 4 and 7). Different sets of motor neurons share most of their input region (light grey); dark grey distinct input regions of fast muscle fibers, medium grey of slow lateral fibers; bilateral overlap is largest in fast motor neurons.

central dendritic collaterals, feature postero-lateral dendrites. However, the dendrites of motor neurons that supply posterior slow muscle fibers could not be discriminated from the central dendrites of motor neurons that supply the entire muscle (Fig. 3.7). In conclusion, all mandible closer motor neurons strongly overlap in the antero-median mandibular neuropil. I found additional, distinct dendritic branches only in motor neurons that supply either fast or lateral slow muscle fibers.

## Discussion

**Motor units and their input regions**

Although similar principles apply to the motor control of vertebrates and invertebrates (Pearson, 1993), there are some important differences in structure and function between vertebrate and invertebrate muscles and their motor neurons. In arthropods, the final integration occurs on the muscle fiber rather than at the motor neuron level as is the case in the vertebrate spinal cord. Moreover, arthropod motor axons generally form multiple synaptic sites as they run along a muscle fiber (Hoyle, 1974), in contrast to the typical vertebrate motor endplate. As most arthropod muscle fibers do not generate action potentials, this design ensures a rapid activation of the muscle fiber by the motor neurons. Therefore, the potentials recorded from an insect muscle reflect the activity of the motor neurons that supply it. This allows the identification and characterization of the motor neurons supplying a given muscle by its electromyogram (Hill and Govind, 1983; Rathmayer and Erxleben, 1983; Clarac et al., 1987; Bauer and Gewecke, 1991; Kawasaki and Kita, 1995; Rathmayer, 1996).

The number of muscle junctional potentials recorded from the mandible closer muscle in different recordings was slightly variable in the present study. I was able to discriminate 9-12 types of potentials (table 3.1), indicating the number of motor neurons supplying the muscle. In any given experiment I was only able to record from two of the three muscle fiber groups simultaneously (see Materials and Methods). Therefore, some ambiguity remains when assigning types of potentials (motor neurons) to specific muscle fiber groups. The electrophysiological results are summarized in table 3.1. The most likely explanation for my recording results is that the closer muscle is supplied by 9-12 motor neurons, as described in the results. Other possible explanations would require fewer motor neurons, but would exclude the existence of a motor neuron that exclusively supplies the lateral slow muscle fibers. This would contradict my neuroanatomical data (Figs. 3.6d, 3.7). My neuroanatomical results suggest very similar numbers of motor neurons. At least 10

motor neurons could be discriminated by their cell body location and dendritic arborization pattern (Fig. 3.7). By superimposing morphological and electrophysiological data, the following arrangement of innervation emerges for the mandible closer muscle: 4-5 motor neurons exclusively supply the fast muscle fibers, 1-2 neurons control the posterior group of slow muscle fibers, and 1-2 neurons supply the anterolateral slow fibers. In addition to these specific neurons, 3-4 motor neurons innervate the entire closer muscle. These motor neurons therefore control all types and locations of muscle fibers.

If all mandible closer motor neurons of ants received the same input, they might yet differ in their thresholds and could be activated successively, depending on the strength of the input. This would provide a means of modulating mandible power output by varying input strength. I additionally found that the motor neuron dendritic fields do not completely overlap in the suboesophageal ganglion. The partial separation of the motor neurons' receptive fields could indicate that they receive common as well as individually distinct input in specific regions of the neuropil. To unambiguously answer this question, detailed physiological and ultrastructural analyses would be required. However, differences in the motor neurons' dendritic input regions suggest that the different closer muscle fiber groups could be activated independently by some of the motor neurons. This is supported by the occurrence of unique muscle potentials specific for each of the respective muscle fiber bundles (Fig. 3.2) and by individual mandible movements where particular muscle fiber groups were completely inactive. The supply by motor neurons reflects the separation and specialization of the three muscle fiber groups. I conclude that functionally distinct (fast and slow) and spatially separated (slow posterior and lateral) muscle fibers can be recruited individually. However, for forceful movements, all muscle fibers are recruited simultaneously (Fig. 3.5; see below).

The larger region of bilateral overlap found in motor neurons supplying the fast muscle fibers indicates that bilateral input may be more important for the control of fast movements. The slow and precise 'manipulation' of objects may not require a precisely synchronized bilateral action and may depend more on feedback from ipsilateral mandibular sensory input (hair receptors, campaniform sensilla and mandibular muscle receptor organs; Gronenberg et al., 1998). The idea that grasping movements that are too fast for sensory feedback depend more on bilateral input is supported by findings in trap-jaw ants. In *Odontomachus*, the fast mandible motor neurons receive bilateral sensory input and always act in synchrony (Gronenberg et al., 1993; Gronenberg, 1995b; Just and Gronenberg, 1998). The somata of the trap jaw ants' fast motor neurons occupy a position similar to that of cell

bodies 5 and 6 in *Camponotus* and *Pogonomyrmex* (Fig. 3.7). This similarity of fast mandible closer motor neurons supports the idea that the trap-jaw mechanisms are homologous to, and can easily be derived from, the general design of mandible muscles and motor neurons in less specialized ants such as *Camponotus* or *Pogonomyrmex* (Gronenberg and Ehmer, 1996).

**Modulation and multineuronal innervation**

Some insect muscles are controlled by only a single motor neuron (Strausfeld et al., 1987; Rathmayer, 1996). The classical insect muscle paradigm, the locust jump muscle (extensor tibiae) is controlled by 3 motor neurons, a fast and a slow excitatory and a common inhibitory neuron (Hoyle, 1974). Up to five motor neurons have been found that control particular locust antennal muscles (Bauer and Gewecke, 1991), and at least six motor neurons control the retractor unguis muscle in locusts (Walther, 1980), which is composed of three widely separated parts that probably react independently of each other (Burrows, 1996).

The relatively high number (10-12) of motor neurons supplying ant mandible closer muscles may reflect the importance of this muscle for the behavior of insects. Large numbers of mandible closer motor neurons have also been found in other insects [6-8 in the honeybee *Apis mellifera* (Rehder, 1989), 12 in the tobacco hornworm *Manduca sexta* (Griss, 1990)]. This high number of motor neurons may also be required to generate different forces and velocities in this unisegmental limb. In other insect limbs (e.g. other mouthparts or legs), which are composed of several segments, the movement is controlled by many muscles which together can be orchestrated by a larger pool of motor neurons (about 70 motor neurons in the locust hind leg; Burrows, 1996).

Why would a single muscle or a single group of similar muscle fibers be supplied by several motor neurons? The contraction properties of arthropod muscle fibers do not only depend on their morphological and biochemical properties but also on the type of neuron by which they are activated (Hoyle and Burrows, 1973). In crickets, a muscle has been shown to be controlled by four motor neurons with very similar properties and effects (Consoulas et al., 1993). However, different motor neurons supplying the same muscle fibers generally serve to modulate the contraction properties of those fibers. The two mandible closer muscle fiber types in ants ('fast' and 'slow') can thus probably be finely tuned by activity in the different motor neurons supplying them. I assume that some of the mandible closer motor neurons in ants release modulatory transmitters. Locust mandibular muscles receive

innervation from serotonergic neurons (Tyrer et al., 1984; Baines et al., 1990b), occupying similar positions within the suboesophageal ganglion as the somata of some motor neurons stained in the present study. Moreover, some locust mandible closer motor neurons release proctolin (Baines et al., 1990a). Muscle contraction may be enhanced by proctolin without an increase in spike frequency (Allgäuer and Honegger, 1993; Bartos et al., 1994). Insect skeletal muscles may also be modulated by dorsal unpaired median (DUM) neurons that contain octopamine (Hoyle et al., 1974; Orchard et al., 1989). Such neuromodulatory substances may be involved in fine tuning of mandibular movements in ants as well, but no immunocytochemical data are available for ant nervous systems.

The common innervation of the ant mandible closer by 3-4 motor neurons might also suggest that a common inhibitor motor neuron (Pearson and Bergman, 1969) contributes to the fine tuning of mandibular movements. Such inhibitory neurons supply more than one muscle and modulate muscle properties in crustaceans and insects (e.g. locust flight steering muscle: Wolf, 1990; antennal innervation of crickets: Honegger et al., 1990; Allgäuer and Honegger, 1993). Common Inhibitor motor neurons release GABA as their neurotransmitter. However, a preliminary study did not reveal any evidence for GABA-like immunoreactivity of mandibular muscles and motor neurons in the ant *Odontomachus* (Stefan Just, personal communication). Likewise, mandibular motor neurons in the larva and adult of the tobacco hornworm, *Manduca sexta*, do not contain GABA (Homberg et al. 1987; Griss 1990). I therefore assume that mandible muscles in ants, and probably in insects in general, are not controlled by common inhibitory motor neurons.

The excitatory junctional potentials of fast motor neurons, i.e. neurons whose activation results in fast contraction of a particular muscle fiber, are considerably larger than those of slow motor neurons (Rathmayer, 1996). This is also true for the potentials recorded from the fast lateral muscle fiber group in ants in the current study (Figs. 3.2c, 3.3). It is possible that the electrical current of several motor neurons is required in order to release a sufficient amount of transmitter fast enough to maximally depolarize the entire set of fast muscle fibers simultaneously. I do not know if each of the fast motor neurons supplies every single muscle fiber because I always injured and stained larger groups of muscle fibers. The homologous fast muscle fibers of trap-jaw ants (genera *Odontomachus* and *Anochetus*) are supplied by two particularly thick and specialized motor neurons. Each of these two fast neurons makes synapses with only half of the fast muscle fibers even though the entire fast muscle comprises less than 50 fibers which always act in a highly synchronized way (Just and Gronenberg, 1998). This design may be common to ants in general. I therefore assume

that not all of the 4-5 fast motor neurons found in the present study converge on the same muscle fibers. Each individual fast muscle fiber may be supplied by only a subset of the fast motor neurons.

**Force and velocity of mandible movements**

Morphological and ultrastructural characteristics indicate that muscle fibers with short sarcomeres are able to contract faster (Gronenberg et al., 1997), hence I refer to them as fast muscle fibers. The current results show that the fastest mandible movements always coincide with particular activity in the fast muscle fibers. When no high-amplitude junctional potentials were present during a recording sequence of fast muscle fibers, the resulting mandible velocity was reduced by a factor of five (Fig. 3.4), even if small potentials were present in the fast muscle fibers. Fast mandible movements therefore result from rapid contraction of muscle fibers with short sarcomeres, which corroborates the use of the term "fast" muscle fibers. The maximum angular velocities determined in the current study were about 0.5 °/ms (Fig. 3.4), similar to values previously published for *Camponotus rufipes* (Gronenberg et al., 1997). In those experiments (Gronenberg et al., 1997) we used opto-electronic measurements without any mechanical constraints. The fact that both techniques showed very similar velocities indicates that the current mechanical probing method did not significantly affect the performance of the animals.

To generate forceful movements, all muscle fiber types are activated simultaneously. As mandibular force increases, the spike frequency increases proportionately in all muscle fiber types (Fig. 3.5). This does not indicate, however, that all muscle fibers contribute equally to the overall force of the mandible movement. Based on morphological and ultrastructural data (Gronenberg et al., 1997) and on my movement velocity measurements (Fig. 3.4), I conclude that muscle fibers with long sarcomeres generate slower yet more forceful movements. Therefore, slow muscle fibers contribute relatively more force to the mandible's overall force output. The maximum forces reached 41 mN in the current experiments (head width of *Camponotus rufipes* workers: 3.5 - 4.4 mm). While these are considerable forces, these values do not reflect the maximum forces possible. As I was interested in assessing the mandible movements, my mechanical loads (Fig. 3.1) were such that the ants were always able to readily close their mandibles. I therefore assume that the maximum forces that the ants can generate are considerably higher than is reflected by my values (large workers of *Camponotus rufipes* can penetrate human skin and draw blood even though their mandibles are relatively ‘dull’).

It has been suggested from cursory muscle recording experiments that particular muscle fiber groups may be activated successively (Just and Gronenberg, 1998). Lateral muscle fibers were assumed to contract first, and later, when the mandibles are in a more closed position, posterior muscle fiber activity would take over. However, this is unlikely from a biomechanical point of view (see results, and Paul and Gronenberg, 1999). Moreover, in the current study I did not find any evidence suggesting sequential activation of different muscle fiber groups (Fig. 3.5). Rather, activity was found to occur simultaneously in anterior and posterior muscle fibers during complete mandible closing movements. This does not exclude the possibility that under certain conditions only a part of the muscle is activated, but I was not able to consistently evoke such movements in my experiments. During my recording conditions the ants were in a stressful situation (heads stuck down, animals not able to run away). They were probably more prone to biting and escape behavior than to engage in social actions such as brood care, grooming or communication, all of which probably involve particularly sensitive and exacting mandible control. Some of the slow movement episodes that I recorded were associated by activation of only one muscle fiber group (e.g. some data points in Fig. 3.5b reveal inactivity of fast fibers during these movement episodes), indicating that different muscle fibers can be controlled independently. I assume that such weak activation of particular muscle fibers supports the fine control of movements such as required for social interactions. In addition, several modulators may contribute to the fine tuning of mandibular movements (see above). Besides, the co-activation of mandible closer and opener muscle has been demonstrated (Gronenberg et al., 1998). This co-activation probably further increases the precision of the resulting force output.

To summarize, I conclude that the ant mandible closer muscle is supplied by 10-12 motor neurons which allows the ant to precisely control the velocity and force of mandible movements. Some motor neurons supply the entire muscle whereas others control specific subsets of muscle fibers. Particularly fast movements result from activation of at least two fast motor neurons which exclusively supply fast muscle fibers. When maximum force is required, all motor neurons are activated strongly and synchronously. In contrast, slow and delicate movements that require little yet precisely controlled force result from independent activation of only a few motor units.

## B. THE LABIOMAXILLARY COMPLEX

### 4. How do ants put out their tongue?

## Introduction

The mouthparts of insects are known as very versatile tools. They are prominent examples in biology demonstrating how homologous organs can be shaped very differently for various uses. According to their homology, they can be traced back to the same basic design (Snodgrass, 1935). While the adaptive differences in external morphology of mouthparts among diverse insect taxons became textbook knowledge (Wehner and Gehring, 1990; Chapman, 1998), important questions about the mechanisms underlying mouthpart movements are still unanswered. Understanding movement mechanisms could be substantial for the evaluation and interpretation of physiological, behavioral, and ecological data. The following three paragraphs give examples.

In honeybees, the fluid-intake rate decreases with increasing sugar concentration and bees maximize their net energy uptake rate at 60% sucrose solution (Núñez, 1966; Roubik and Buchmann, 1984). Although Kingsolver and Daniel (1995) pointed out that intake rates are strongly affected by feeding mechanics, very little is known about the exact mechanism of proboscis extension in the honey bee. Since glossa protractor muscles are absent, Snodgrass (1956) postulated two alternative mechanisms: A protraction driven by hemolymph pressure analogous to lepidopterous proboscis extension (Schmitt, 1938; Bänzinger, 1971; Krenn, 1990), or a movement as a result of specific elastic features of the involved structures. But the question, which of both mechanisms applies, remained unanswered.

Several studies on bumble bees dealt with the effects of the external morphology of the mouthparts, such as tongue length, on flower handling time (e.g. Heinrich, 1979; Harder, 1983). Other experiments showed that individual bumble bees maintain constant lapping rates regardless of concentration and viscosity of the offered sucrose solution (Harder, 1986); morphologically dissimilar bumble bees drank at different rates because glossa length affects lapping rate. But also in these investigations movement mechanisms have not been analyzed, although they could be crucial for flower handling time and could explain the constant individual lapping rates as well.

Gotwald (1969) studied in an extensive comparative work the external morphology of ant mouthparts. Janet (1905, 1907) gave an overview of the head anatomy of *Lasius niger*. But except for the mandibles (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul, 2001), functional morphology, biomechanics and movement mechanisms of the other mouthparts (maxillae and labium) have not been addressed. However, the maxillae and the labium are essential for food intake. Besides, some parts of the labiomaxillary complex are

also used for other tasks such as communication (e.g. maxillary palpi for begging behavior during trophallaxis, and social grooming, Hölldobler and Wilson, 1990). Particularly the glossa, the distal end of the labium, is very important for feeding liquids. For the ant species *Camponotus mus*, it has recently been shown that the liquid food intake rate depends on sucrose concentration and colony starvation (Josens et al., 1998; Josens and Roces, 2000). Experiments with *Pachycondyla villosa* demonstrated that the licking frequency during liquid feeding is almost constant, independently of concentration and viscosity of sugar solution (Dasch, 1998), similar to the constant lapping rates of bumble bees (see above). Also some ecological aspects of liquid feeding in ants were studied: Particularly for intruders in comparison to territory owners of food resources, the ingestion rate per ant is crucial for foraging success and could modify foraging strategy (Dreisig, 1988, 2000). But nothing is known about the movement mechanisms during feeding. They could have an impact on licking frequencies, on intake rates and therefore on foraging strategies as well. So, how do ants move their tongue?

In the present study I investigated the anatomical design of the labiomaxillary complex in different ant species with particular reference to movement mechanisms. To get an idea of the physiological performance of the labial and maxillary muscles, I measured sarcomere lengths, muscle fiber lengths and diameters, and the relative muscle volumes. Furthermore I compared the design of the labiomaxillary complex of ants with the general insect design, and finally provided a concise overview of nonmuscular movements in arthropods. Since my results demonstrate that glossa protraction in ants is a nonmuscular movement, the major question addressed was how ants put out their glossa.

## Materials and Methods

Experiments and measurements were carried out on workers of the following ant species: *Ectatomma ruidum*, *Diacamma* sp., *Harpegnathos saltator*, *Pachycondyla villosa*, *Rhytidoponera impressa*-complex (Ponerinae), *Myrmecia* sp. (Myrmeciinae), *Atta sexdens*, *Leptothorax sordidulus* (Myrmicinae), and *Camponotus rufipes* (Formicinae). The ants were kept in plaster-of-Paris nests under a 12h:12h L:D cycle, at 25°C and 50% relative humidity. They were fed chopped cockroaches, crickets or wingless *Drosophila*, and honey-water (30%) or fresh leaves (*Atta sexdens*).

**Histology**

To examine the anatomical design of the labiomaxillary complex, ants were anesthetized with enflurane (Abbot Ethrane) and decapitated. After fixation (buffered 4% formaldehyde or 2.5% glutaraldehyde, or ammoniacal ethanol), the heads were stained either with Methylene Blue or with osmium/ethylgallate (Gronenberg, 1995), or silver-impregnated (Gronenberg et al., 1997). The heads were then dehydrated, embedded in Fluka Durcupan, and horizontally, sagittally, or vertically sectioned at 10-15 µm.

**Morphometry**

Schematic drawings were made from light-microscopic images using a calibrated camera lucida attachment to the microscope (Zeiss Axiophot). For volume measurements, light-microscopic images were videorecorded and digitally evaluated with a computer equipped with a video card (Screen Machine, Fast Electronic) and appropriate software (courtesy of Reinhard Wolf). From each microscopic slide, the outlines of the respective structures were traced on the computer screen and the areas computed. Volumes were calculated considering the section thickness. Muscle and head capsule volumes were measured of all species examined. Glossa and hypopharynx volumes (see results) were measured in *Pachycondyla villosa*. Sarcomere length, muscle fiber length, and fiber diameter were measured using a calibrated camera lucida attachment to the microscope. Sarcomere length was established by counting all sarcomeres of an entire muscle fiber.

**ATP-experiments**

To test the hypothesis of an elastic mechanism underlying glossa protraction, ants were anesthetized with enflurane (Abbot Ethrane) in most cases. Then, their head capsules were opened at several places, and the heads of the decapitated ants were immediately put into Calcium-free Ringer-solution (8.8 g/l NaCl; 0.2 g/l KCl; 2.3 g/l TES = N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; 8.6 g/l sucrose; 10 mmol/l ATP; 25 mmol/l EDTA = ethylenediamintetraacetic acid; pH = 7.2). The additional ATP and EDTA ensured muscle relaxation. Relatively large holes in the head capsule guaranteed a rapid diffusion of the Ringer-solution into the head and prevented the possibility of an increase of hemolymph pressure within the head. The position of the labiomaxillary complex and of the glossa were checked every minute during the first ten minutes and every five minutes for additional 30 minutes. Muscles were still active for several minutes in some cases and the labiomaxillary complex then frequently moved in- and outwards. In these cases, the

amplitude of glossa movement decreased continuously until the glossa stopped moving at a completely protracted position. 'ATP-experiments' were carried out on workers of *Atta sexdens*, *Camponotus rufipes*, and *Pachycondyla villosa*.

## Results

### Anatomical design

In ants, the labiomaxillary complex is located between the two sturdy mandibles (Fig. 4.1a-c). The strongly sclerotized shovel-like mandibles are the largest mouthparts in ants. They are moved by one very large mandible closer and one much smaller opener muscle on each side of the head (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul, 2001). When retracted, the more fragile labiomaxillary complex is protected by the sclerotized labrum (Fig. 4.1b, c) and the bottom sides of the stipes and the mentum (Fig. 4.1a, c). In contrast to the mandibles, both the maxillae and the labium contain several joints and are therefore controlled by various muscles with different functions.

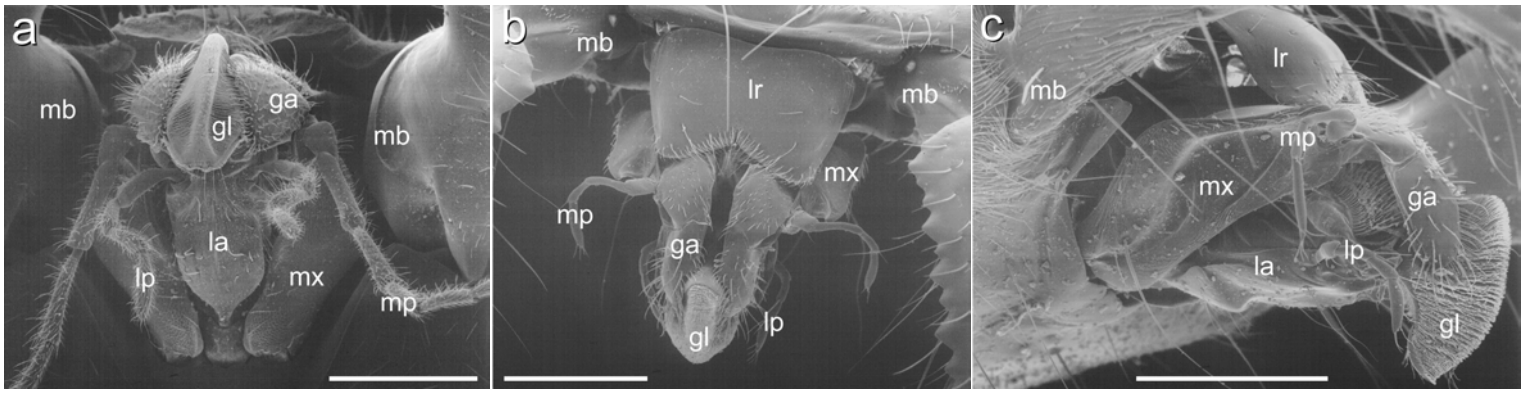
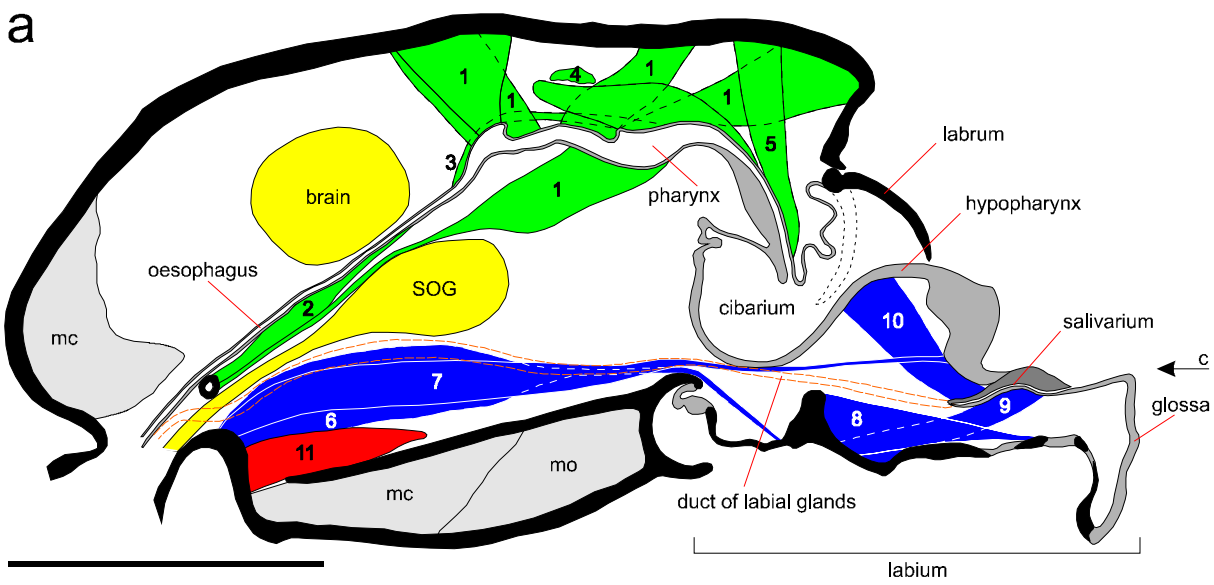



Fig. 4.1: Scanning electron microscopic images of the labiomaxillary complex of ants. (**a**) frontal view, *Camponotus rufipes*; (**b**) dorsal and (**c**) lateral view, *Pachycondyla villosa*; scale bars = 500 µm; (ga) galea, (gl) glossa, (lp) labial palpus, (la) labium, (lr) labrum, (mb) mandible base, (mx) maxilla, (mp) maxillary palpus.

In all species examined, I found the same basic anatomical design. Figure 4.2a-c give an overview of the diverse structures and muscles within the head of an ant. Six distinct paired muscles are associated with the labium (5 of them are shown in Fig. 4.2a-c, blue, numbers 6-10). The whole labiomaxillary complex is moved by two antagonistic muscles: one opener (6, labial abductor) and one closer (7, labial adductor). While the bilateral pairing of the labial opener can still be seen in posterior head regions, its muscle fibers attach to the same central apodeme from both sides of the head (Fig. 4.2c). This central apodeme conveys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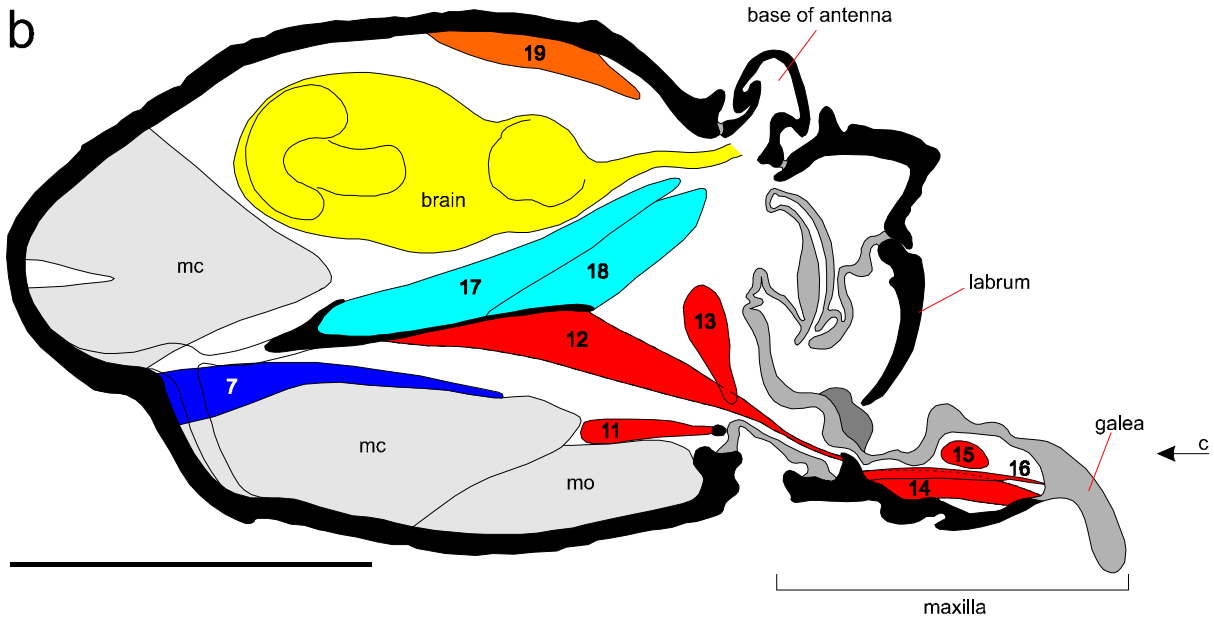
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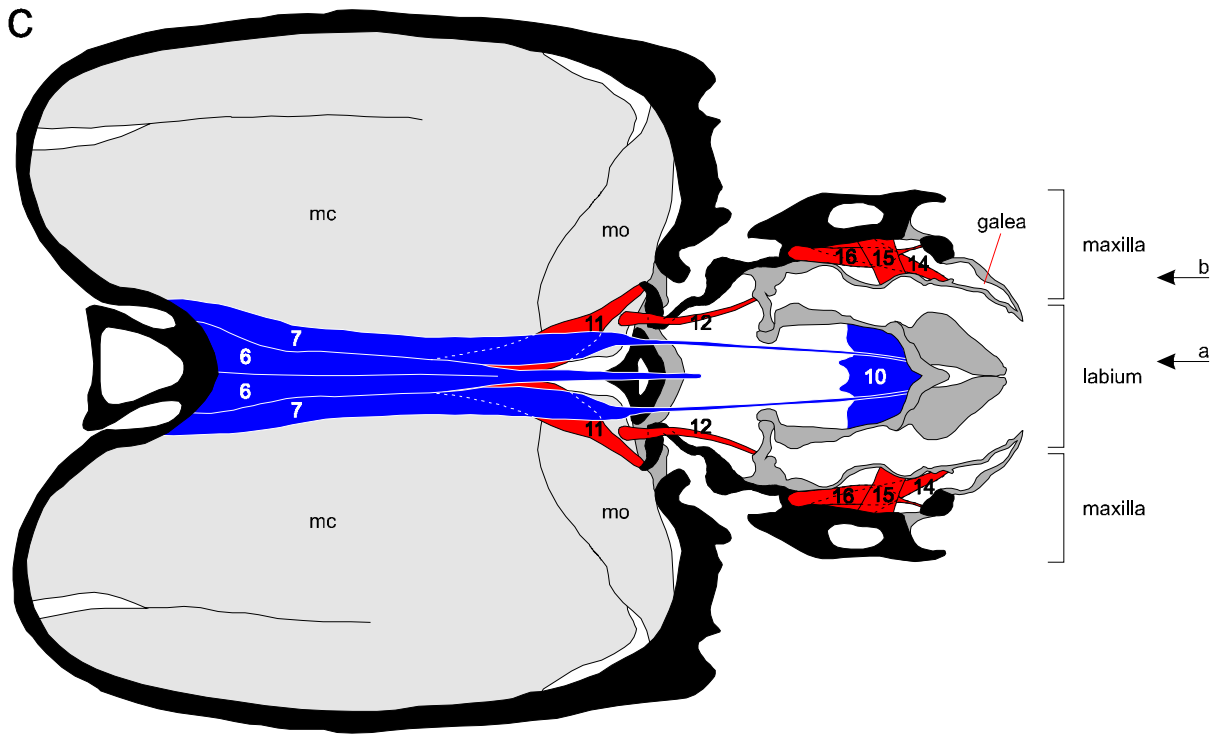
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◀ Fig. 4.2 (opposite): Schematic drawings of a head of *Pachycondyla villosa*; (**a**, **b**) sagittal and (**c**) horizontal sections at the levels indicated by arrows. The drawings represent several layered sections of at least two different preparations, respectively. Yellow: brain and suboesophageal ganglion (SOG). Green: pharynx muscles; (1) pharynx dilator, (2) pharynx retractor, (3) pharynx longitudinal muscle, (4) pharynx transversal adductor, (5) retractor of buccal tube. Blue: labial muscles; (6) labial abductor, (7) labial adductor, (8) glossa muscle, (9) paraglossa muscle, (10) hypopharynx muscle, labial palpus muscle not shown. Red: maxillary muscles; (11) maxillary abductor, (12) maxillary adductor, (13) maxillary levator, (14) galea muscle, (15) lacinia muscle, (16) maxillary palpus muscle. Cyan: antennal muscles; (17) antennal abductor, (18) antennal adductor. Orange: (19) labral adductor muscle. Black: sclerotized head capsule or tentorium. Grey: not or only slightly sclerotized cuticular structures, or mandible muscles: (mc) mandible closer, (mo) mandible opener; scale bars = 1 mm.

the muscle power to the bottom of the labium (Fig. 4.2a). If the labial opener contracts, the ventral part of the labium is pulled backwards and therefore becomes slightly shorter than the dorsal part. Accordingly, the labiomaxillary complex extends. The apodeme of the labial closer deeply projects into the labium and is dorsally attached at the base of the hypopharynx (Fig. 4.2a, c). Upon contraction of the labial closer, the dorsal part of the labium is pulled backwards, and the entire labiomaxillary complex is folded up and retracted.

Except for the labial closer and opener, the other four labial muscles are located within the labium. The glossa muscle (8) as well as the paraglossa muscle (9) work as glossa retractors. Their function will be described below in more detail ("glossa protraction mechanism"). According to its position, I refer to muscle number 10 as hypopharynx muscle. Its muscle fibers directly attach to a cuticular structure at the salivarium and run dorsolaterally to the top of the hypopharynx (Fig. 4.2a, c). Similar to the labial opener, hypopharynx muscle fibers converge to one central area of attachment from both sides of the head. A contraction of the hypopharynx muscle causes a dilation of the salivarium. Probably driven by the resulting underpressure the secretory products of the labial glands are thus pumped out of the duct (Fig. 4.2a). The sixth labial muscle is the palpus muscle (not shown in Fig. 4.2). It lies laterally next to the glossa muscle, and controls the movements of the labial palpus.

The maxillae are moved by six different paired muscles as well (Fig. 4.2a-c, red, numbers 11-16). Two antagonistic muscles move one maxilla in the horizontal plane, respectively. The maxillary opener (11, maxillary abductor) attaches posteriorly to the head capsule and anteriorly to a sclerotized stud of the maxilla (Fig. 4.2a-c). Since the virtual turning point of the maxilla lies closer to the bilateral midline than this stud does, the maxilla rotates outwards during contraction of the maxillary opener (Fig. 4.2c). The maxillary closer

(12, maxillary adductor) is posteriorly affixed to a rigid apodeme that arises from the posterior head capsule (Fig. 4.2b). The manoeuvrable apodeme of the maxillary closer attaches to the inner median side of the maxilla (Fig. 4.2c). If the maxillary closer contracts, the whole maxilla rotates inwards and simultaneously moves slightly upwards. In addition, a third maxillary muscle (13) is located within the head capsule (Fig. 4.2b). Its muscle fibers attach to the dorsal tentorium, laterally to the antenna, and run across the anterior head capsule to a ventral maxillary stud. This stud lies in a small hollow close to the ventral coalescence of labium and maxilla. The function of this muscle is not completely clear. Upon contraction it probably causes a rotation of the maxilla around its longitudinal axis and lifts the maxilla upwards. Therefore I called it maxillary levator (Fig. 4.2b).

Three additional maxillary muscles are located within the maxilla, respectively (Fig. 4.2b, c, numbers 14-16). The muscle fibers of two of them (14, galea muscle; 16, palpus muscle) run almost parallel to the longitudinal axis of the maxilla. If the galea muscle (14) contracts, the median side of the galea, the distal end of the maxilla, is pulled backwards and therefore bent. Accordingly, the galea moves inwards (Fig. 4.2c). The palpus muscle (16) controls the movements of the maxillary palpus. Upon contraction of the transversal lacinia muscle (15) the median side of the maxilla is moved laterally. Possibly, the ventral part of the galea could thus be rotated inwards around the longitudinal axis of the maxilla.

Besides the two mandible muscles and the different muscles that move the labiomaxillary complex, there are several other muscles within the head of an ant. Most of the pharynx muscles are pharynx dilator muscles (Fig. 4.2a, green, number 1). Except for one, all these muscles arise from the dorsal head capsule and directly attach to the upper part of the pharynx (Fig. 4.2a). The exception lies beneath the pharynx. The muscle fibers of this pharynx dilator attach to a long apodeme that runs parallel to the oesophagus and is posteriorly connected to a rigid tentorial structure (Fig. 4.2a). All the pharynx dilator muscles contribute to the proper function of the pharyngeal sucking pump. Four additional muscles move or deform different parts of the pharynx (Fig. 4.2a). These muscles are the pharynx retractor (2), the pharynx longitudinal muscle (3), the pharynx transversal adductor (4), and the retractor of the buccal tube (5). One antenna is controlled by two antagonistic muscles, respectively (Fig. 4.2b), the antennal abductor (17), and the antennal adductor (18). I only found one paired labral closer muscle (Fig. 4.2b, orange, number 19). It is affixed to the dorsal head capsule and its long apodeme projects to the base of the labrum. An antagonistic labral opener muscle does not exist.

**Muscle morphology**

To get an idea of the physiological performance of a specific muscle I measured sarcomere length, muscle fiber length and diameter, and the relative muscle volume. I compared all morphological data of the maxillary and labial muscles with data of the mandible closer muscle as reference, because the functional significance of the mandible closer is already well known (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul, 2001).

In previous studies, I found three morphologically distinct muscle fiber types for the mandible closer of ants (Gronenberg et al., 1997; Paul and Gronenberg, 1999). I confirm this finding for the two new ant species that were additionally examined in the present study (*Pachycondyla villosa*, *Rhytidoponera impressa*-complex). The three muscle fiber types of the mandible closer differ in two morphological characteristics: the sarcomere length and the mode of attachment at the apodeme. Fast contracting fibers have short sarcomeres (2-3 µm, closed mandibles) and are always directly attached to the apodeme whereas slow but forceful fibers have long sarcomeres (5-6 µm, closed mandibles) and are either directly attached or attach to the apodeme via individual thin thread-like filaments (filament-attached). Since the sarcomere lengths of mandible closer muscle fibers of *Pachycondyla* in Table 4.1 were measured for opened mandibles (intermandibular angle about 45°), both fast and slow muscle fibers feature slightly longer sarcomeres.

In contrast to the mandible closer, the maxillary as well as the labial muscles do not contain distinct muscle fiber types within one muscle. This is true for all investigated species. The three measured parameters (sarcomere length, fiber length and fiber diameter) slightly deviates from a muscle-specific mean value, respectively (e.g. *Pachycondyla villosa*, Table 4.1). Except for two cases, all maxillary and labial muscle fibers directly attach to their apodemes or the exoskeleton. The galea muscles of *Camponotus rufipes* and *Atta sexdens* consist of filament-attached muscle fibers. Both species have broad maxillae that accommodate many galea muscle fibers acting in parallel. Accordingly, there is relatively less surface area at the base of the galea for the converging muscle fibers to attach to. Therefore filaments become necessary, because they minimize the needed attachment surface. This finding keeps with the biomechanical model of the advantages of filament-attached muscle fibers under specific geometrical conditions (Paul and Gronenberg, 1999).

Table 4.1 summarizes morphological muscle data for *Pachycondyla villosa*. The sarcomere lengths of the labial opener and closer and of all maxillary muscles lie between 5.01 µm and 6.77 µm. This fits to slow mandible closer muscle fibers. The glossa,

paraglossa, and hypopharynx muscles tend towards shorter sarcomeres (3.90-4.65 µm). And shorter muscle fibers tend to have shorter sarcomeres in some cases as well. But these trends can not be found over all muscles and species examined. The sarcomere lengths of the respective muscle fibers of other species were approximately same to those of *Pachycondyla* (e.g. *Camponotus rufipes*; labial opener: 4.94 ± 0.88 µm; labial closer: 5.45 ± 0.70 µm; glossa muscle: 4.87 ± 0.19 µm; paraglossa muscle: 3.24 ± 0.18 µm).

| muscle / fiber type | sarcomere length | | | fiber length | | | fiber diameter | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean ± st.dev. [µm] | n | N | mean ± st.dev. [µm] | n | N | mean ± st.dev. [µm] | n | N |
| (6) labial abductor | 5.30 ± 0.24 | 1098 | 2 | 894.3 ± 59.7 | 21 | 3 | 18.36 ± 2.24 | 102 | 2 |
| (7) labial adductor | 6.77 ± 0.54 | 948 | 2 | 1296.0 ± 86.6 | 20 | 3 | 17.42 ± 1.91 | 116 | 2 |
| (8) glossa muscle | 4.65 ± 0.39 | 1299 | 2 | 486.0 ± 65.0 | 20 | 2 | 13.49 ± 1.68 | 87 | 3 |
| (9) paraglossa muscle | 4.13 ± 0.39 | 1680 | 2 | 482.3 ± 67.8 | 20 | 2 | 14.75 ± 1.87 | 83 | 4 |
| (10) hypopharynx muscle | 3.90 ± 0.35 | 1148 | 3 | | | | | | |
| (11) maxillary abductor | 6.66 ± 0.39 | 905 | 2 | 440.6 ± 90.6 | 40 | 3 | 16.25 ± 1.53 | 101 | 2 |
| (12) maxillary adductor | 6.76 ± 0.47 | 938 | 2 | 668.5 ± 46.0 | 20 | 2 | 21.22 ± 1.94 | 88 | 3 |
| (13) maxillary levator | 5.62 ± 0.54 | 1076 | 2 | | | | | | |
| (14) galea muscle | 6.73 ± 0.48 | 772 | 3 | 214.1 ± 51.9 | 33 | 3 | 14.17 ± 2.02 | 68 | 3 |
| (15) lacinia muscle | 6.19 ± 0.54 | 565 | 3 | 155.3 ± 8.2 | 34 | 3 | 15.03 ± 0.61 | 52 | 3 |
| (16) max. palpus muscle | 5.01 ± 0.24 | 935 | 3 | | | | | | |
| mand. cl., direct-fast | 3.29 ± 0.22 | 1308 | 2 | 1323.5 ± 129.7 | 20 | 2 | 44.42 ± 4.29 | 81 | 3 |
| mand. cl., direct-slow | 7.37 ± 0.51 | 1042 | 2 | 766 ± 136.9 | 30 | 2 | 34.28 ± 4.67 | 130 | 3 |
| mand. cl., filament-slow | 6.31 ± 0.36 | 995 | 2 | 466 ± 71.5 | 30 | 2 | 37.85 ± 3.79 | 75 | 3 |

Table 4.1: Comparison of morphological characteristics of labial, maxillary, and mandible closer muscle fibers of *Pachycondyla villosa*. All values are means ± standard deviation; n = number of measured sarcomeres or muscle fibers, respectively; N = number of different preparations; mand. cl. = mandible closer muscle; direct-fast = directly attached fast fibers; direct-slow = directly attached slow fibers; filament-slow = filament-attached slow fibers. All data are related to extended labiomaxillary complex, protracted glossa, and slightly opened mandibles.

The longest muscle fibers among labial and maxillary muscles were found in the labial closer (Tab. 4.1, labial adductor). Its muscle fibers were almost as long as the fast muscle fibers of the mandible closer. These fast muscle fibers always occupy two specific positions within the mandible closer to be as long as possible. The longer a muscle fiber, the faster the resulting movement, because more sarcomeres can be accommodated and therefore more contractile units act simultaneously in series. But the long sarcomeres of the labial closer muscle fibers abolish fast muscle characteristics that result from fiber length. In general, larger muscles consist of longer muscle fibers. An exception is the maxillary opener. This muscle belongs to the four large labiomaxillary muscles (see below, cf. Fig. 4.3) but

features relatively short muscle fibers (Tab. 4.1). Accordingly, many muscle fibers act in parallel, suggesting forceful muscle properties. The most important constraints determining muscle fiber length result from the anatomical design (see above; e.g. distance of muscle attachment points).

Intuitively, forceful muscle fibers have larger diameters. This is not true for mandible closer muscle fibers. Fast fibers were significantly thicker than slow ones (t-test, $p < 0.001$; Tab. 4.1). This does not mean that fast fibers comprise more contractile material (per area unit of cross-section) because they also feature wider central tubes than slow muscle fibers and these central tubes lack myofilaments. Relatively thick central tubes seem to be typical for fast muscle fibers (Gronenberg and Ehmer, 1995). In general, larger muscles consist of thicker muscle fibers (Tab. 4.1). An exception is again the maxillary opener. Considering its volume (see next paragraph, cf. Fig. 4.3), this muscle contains relatively short and thin muscle fibers (Tab. 4.1).

The maxillary opener and closer, and the labial opener and closer have approximately the same size (Fig. 4.3a-d). These four muscles are the largest maxillary and labial muscles in any ant worker. All the muscles that are located within the labiomaxillary complex and the maxillary levator are considerably smaller. The relative volume (in relation to the head capsule volume) of all maxillary and labial muscles decreases with increasing head capsule volume (Fig. 4.3a-d), whereas the mandible closer is relatively larger in larger head capsules (Fig. 4.3e). Although the mandible closer is much larger in any case, maxillary and labial muscles become more important in small individuals. Obviously, a specific minimum maxillary and labial muscle volume is necessary to move the labiomaxillary complex properly.

Taken together, all maxillary and labial muscles feature rather slow than fast muscle characteristics, and seem to be less specialized for specific tasks and movements than the mandible closer muscle. Maxillary and labial muscle fibers do not morphologically differ within one muscle. Comparing distinct maxillary and labial muscles, morphology mainly depends on the anatomical design and does not seem to be determined by specific movement requirements. This suggests that maxillary and labial muscles control simpler movements than the mandible closer does. The importance of the mandible closer muscle is also reflected by its immense volume. On the other hand both the labium and the maxillae are moved by several muscles in contrast to only two mandible muscles. This allows more variety of movements and compensates less specialized muscle fibers to some extent.

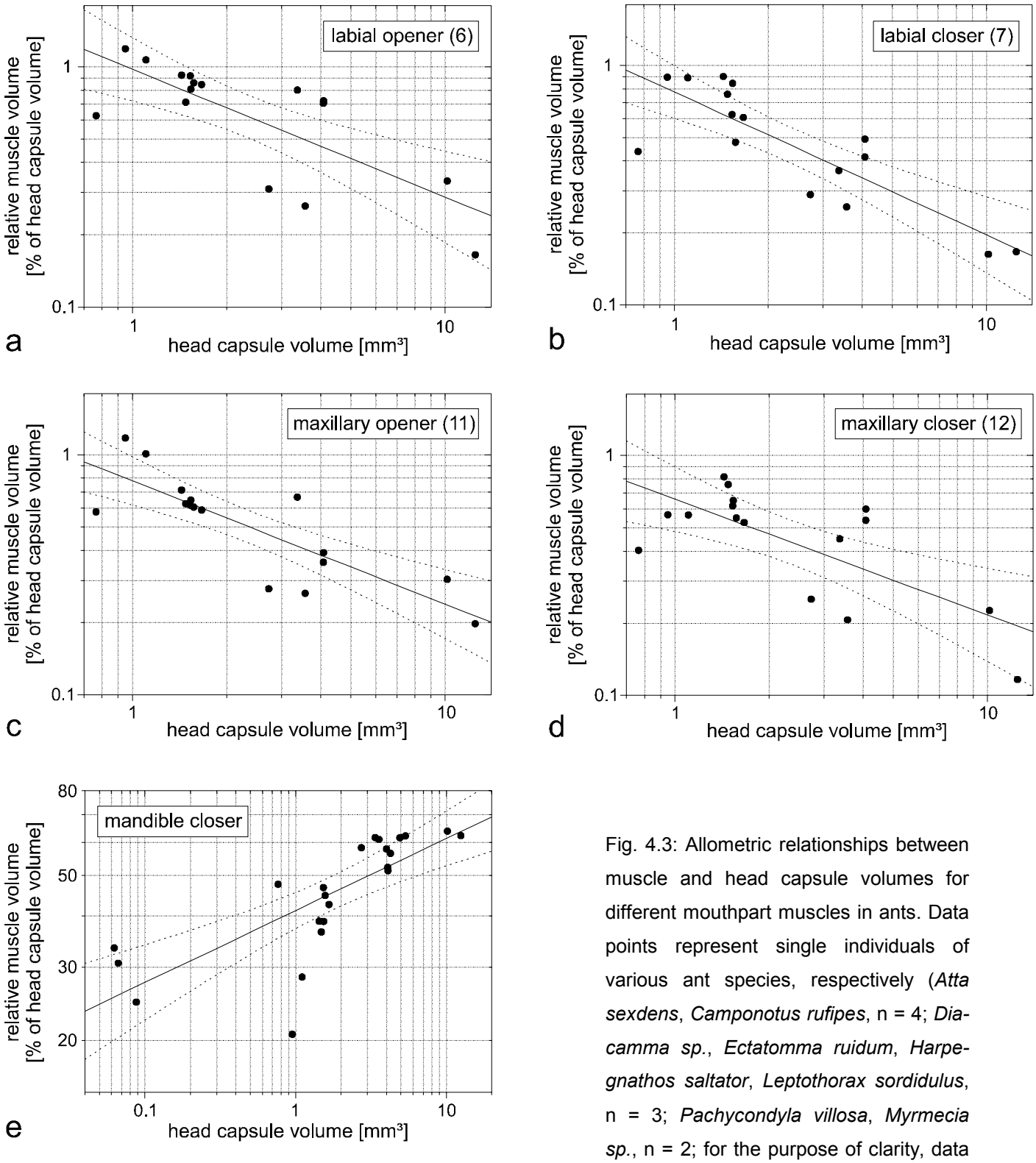



Fig. 4.3: Allometric relationships between muscle and head capsule volumes for different mouthpart muscles in ants. Data points represent single individuals of various ant species, respectively (*Atta sexdens*, *Camponotus rufipes*, n = 4; *Diacamma sp.*, *Ectatomma ruidum*, *Harpegnathos saltator*, *Leptothorax sordidulus*, n = 3; *Pachycondyla villosa*, *Myrmecia sp.*, n = 2; for the purpose of clarity, data of different species are presented using the same symbols). (**a**-**d**) The relative volumes of the labial abductor (**a**; r = -0.75485; p = 0.00072), labial adductor (**b**; r = -0.83955; p < 0.0001), maxillary abductor (**c**; r = -0.82538; p < 0.0001), and maxillary adductor (**d**; r = -0.71461; p = 0.00187) decrease with increasing head capsule volume. (**e**) The relative volume of the mandible closer muscle increases with increasing head capsule volume (r = 0.76315; p < 0.0001).

**Glossa protraction mechanism**

The glossa, the distal end of the labium, is essential for feeding. During licking, the glossa works as an up and down moving shovel. This glossa movement is supported and amplified by a slight synchronous movement of the entire labium. Figure 4.4 illustrates two specific positions of the glossa. When the glossa is retracted, both the glossa and the paraglossa muscle are contracted (compare Fig. 4.4a and b). This is corroborated by measurements of sarcomere length. The sarcomeres of glossa and paraglossa muscle fibers were significantly shorter when the glossa was retracted compared to protracted glossa (Fig. 4.5). Accordingly, both muscles are glossa retractor muscles. The only muscle left within the labium that could work as a glossa protractor is the hypopharynx muscle. But its sarcomeres are only slightly shorter when the glossa is protracted (Fig. 4.5). The hypopharynx muscle is responsible for transporting the labial glandular products outwards (see "anatomical design") and may be relaxed when the glossa is retracted because then it may lose its specific function. Also its location hardly suggests that it works as a direct glossa protractor muscle, since the necessary lever arms and sclerotized cuticular structures are absent (Fig. 4.4). Therefore, a glossa protractor muscle does not exist. Two alternative mechanisms for glossa extension are thus possible: A protraction driven by hemolymph pressure or a movement as a result of specific elastic features of the involved structures.

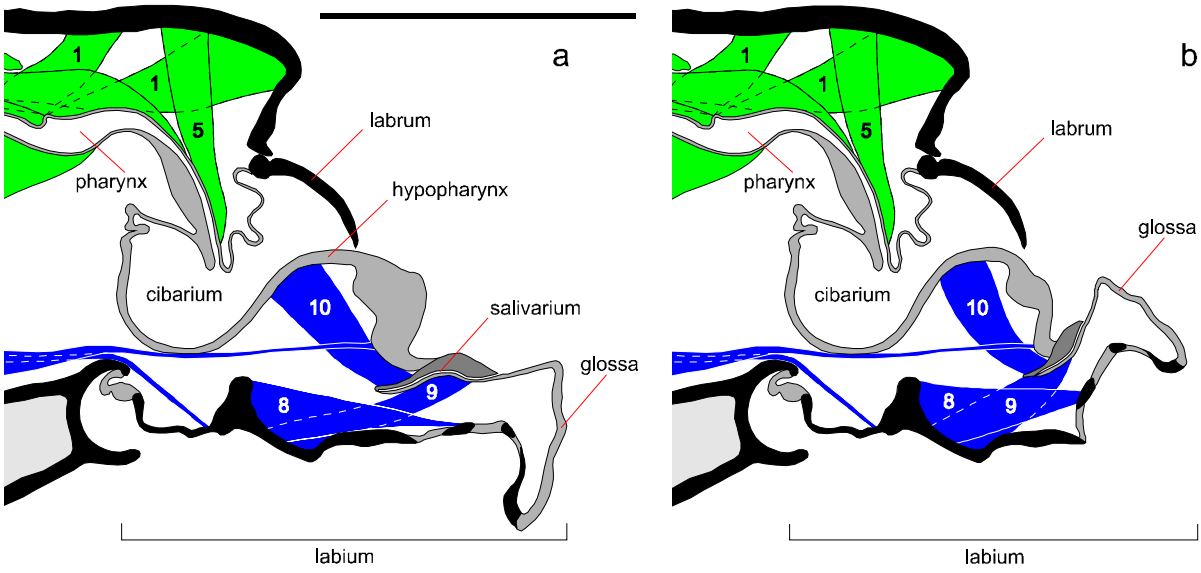



Fig. 4.4: Specific positions of the glossa during glossa movement (e.g. licking). (**a**) protracted and (**b**) retracted glossa. Color-code and numbers as in Figure 2. When the glossa is retracted, both the glossa muscle (8) and the paraglossa muscle (9) are contracted. Scale bar = 1 mm.

Assuming a pressure-driven mechanism, the hemolymph pressure would have to be increased for glossa extension. This increase could be produced either within the entire head capsule (cf. leg movement as a result of hemolymph pressure increase within the prosoma of spiders; Foelix, 1982) or locally only within the labium. There are neither muscles that, upon contraction, could decrease the entire head capsule volume, nor any structures that could serve as valves for precisely controlling the flow of hemolymph into the respective head appendages (Fig. 4.2). Furthermore, the head capsule of ants is relatively rigid and it takes such a powerful muscle as the mandible closer to deform the head capsule like trap-jaw ants do in order to store potential energy for their fast mandible strikes (Gronenberg et al., 1993; Gronenberg, 1995; Paul, 2001). So the only possibility of a pressure-driven mechanism would be a local one. Conceivably, upon contraction of the hypopharynx muscle, the volume of the hypopharynx vault decreases and the hemolymph partly streams, diverted by the ventral cuticular stud, anteriorly into the glossa (Fig. 4.4a). This hemolymph flow would increase the local hemolymph pressure within the glossa and drive the glossa to protract. The local increase of hemolymph pressure would last only shortly, since hemolymph would also stream posteriorly into the head capsule and an efficient valve is absent (Fig. 4.4a), but would possibly suffice for one complete glossa protraction.

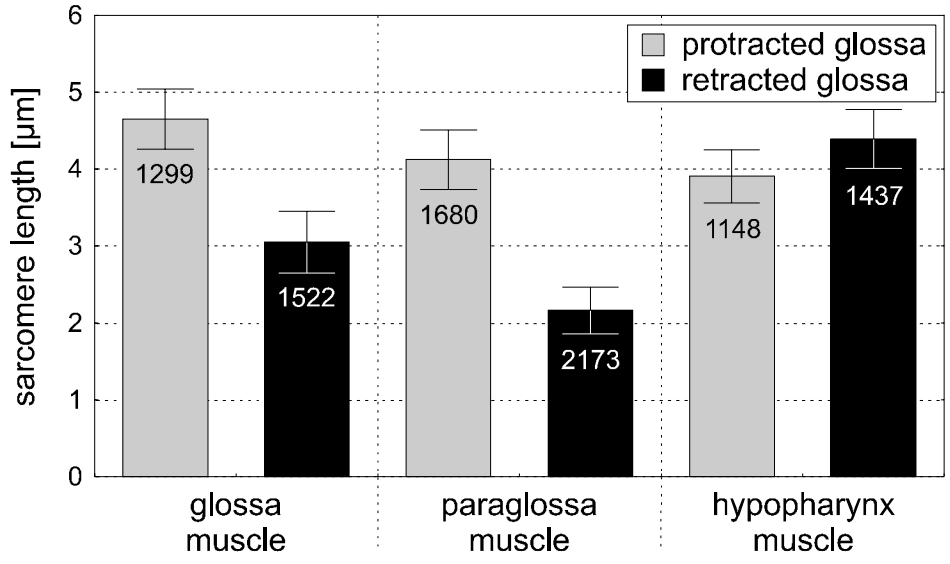



Fig. 4.5: Sarcomere lengths of labial muscles of *Pachycondyla villosa* (protracted labiomaxillary complex). Values are means ± standard deviation; n = number of measured sarcomeres, represented by numbers below the top of the bars, N = 2-3 different preparations / individuals, respectively.

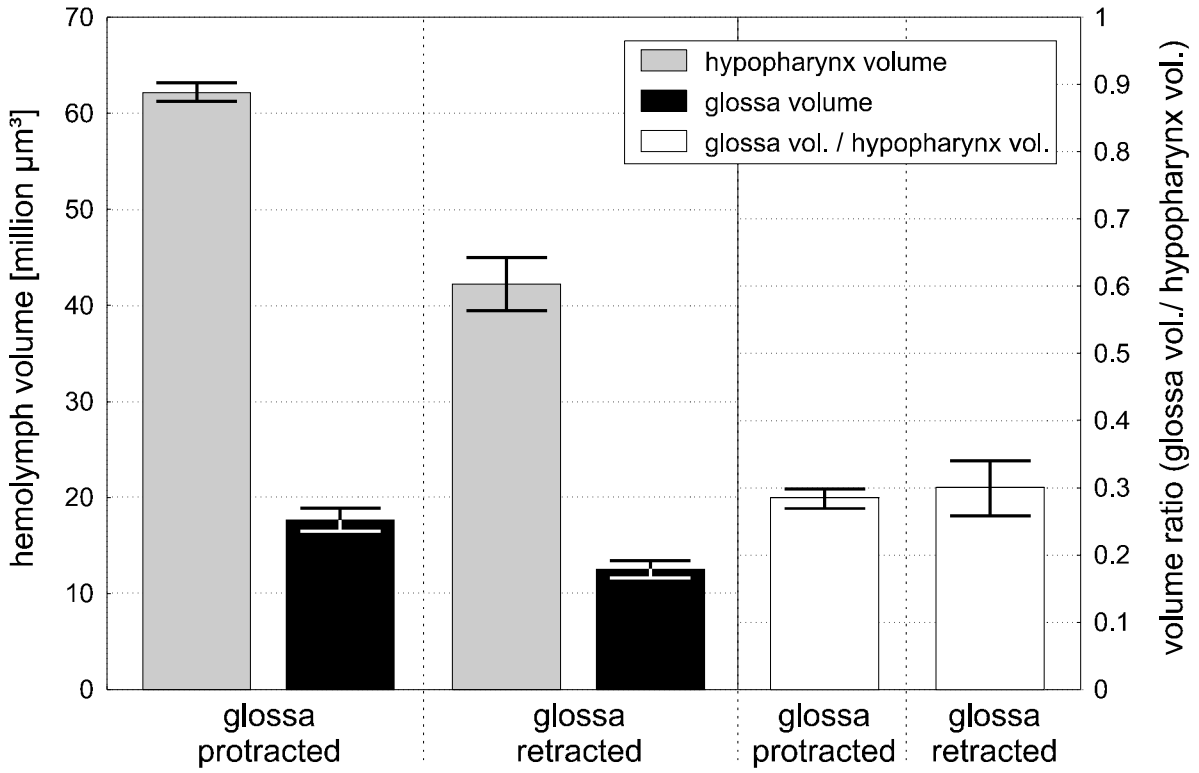



Fig. 4.6: Volume changes as a result of glossa movement (*Pachycondyla villosa*, protracted labiomaxillary complex). All values are means ± standard deviation; n = 4 animals.

If such a pressure-driven mechanism applied, respective hemolymph volume changes should occur within the labium as a result of glossa movement. To test the hypothesis of a pressure-driven mechanism, I measured histologically the hemolymph volumes of the hypopharynx vault and of the glossa for exposed and retracted glossa (Fig. 4.6). Both the hypopharynx and the glossa volume were smaller when the glossa was retracted (Fig. 4.6). The ratio of glossa to hypopharynx volume was constant of about 0.3 (Fig. 4.6). If there exists a pressure-driven mechanism, the volume changes should have been different. During glossa extension, the hypopharynx volume should have decreased while the glossa volume should have increased. But this was not the case. Volumes changed proportionately. Accordingly, I exclude a hemolymph pressure-driven mechanism for glossa extension.

The only remaining possibility would be an elastic mechanism: When the two retractor muscles (glossa and paraglossa muscle) relax, the glossa protracts elastically and automatically. To test this hypothesis I investigated what happens to the glossa when all muscles relax. Freshly preparated ant worker heads were put into a Ringer solution without Calcium and with additional ATP and EDTA. This Ringer solution caused the desired muscle relaxation (see materials and methods, "ATP-experiments"). If the labiomaxillary

complex was completely closed before putting the head into the Ringer solution, only one animal protracted its glossa after Ringer contact (Fig. 4.7; 11%). When the labiomaxillary complex was exposed and the glossa was retracted, 95% of the tested animals protracted their glossa after muscle relaxation (Fig. 4.7). In one case, the position of the glossa did not change. The position of the glossa never changed, when the labiomaxillary complex and the glossa were already completely protracted (Fig. 4.7). If the position of the glossa changed in any way, it resulted always in a glossa protraction (Fig. 4.7). These findings strongly suggest that the glossa protracts elastically and automatically when the glossa retractor muscles relax, and when the labiomaxillary complex is not completely retracted.

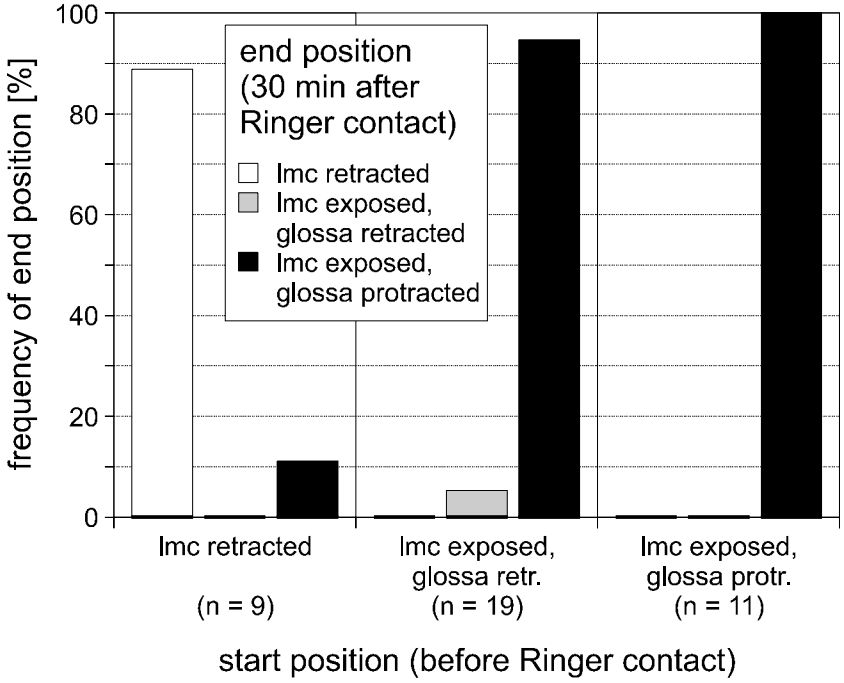



Fig. 4.7: Summarized results of 'ATP-experiments' (see text for further details). lmc = labiomaxillary complex; retr. = retracted; protr. = protracted; n = number of tested animals (*Atta sexdens*, *Camponotus rufipes*, *Pachycondyla villosa*).

In several experiments I narcotized the ants with enflurane before preparation (see materials and methods). One effect of enflurane is a relaxation of all muscles (Lüllmann et al., 1996). In most cases of narcotization the glossa protracted completely. Although the experimental conditions have not been controlled as well as for the ATP-experiments (e.g. concentration of enflurane vapors), the result of this side effect also hints at an elastic mechanism of glossa protraction. Taken together, I exclude a glossa protraction as a result of muscle activity or increased hemolymph pressure but support an elastic movement mechanism.

## Discussion

### Anatomical design

The mouthparts of insects provide several excellent examples of how a group of structures that are fundamentally simple in their generalized condition (Snodgrass, 1935) can be integrated and individually elaborated to form complex organs capable of various activities (e.g. fly: Szucsich and Krenn, 2000; butterfly: Krenn, 1990; honey bee: Snodgrass, 1956). Adaptive differences of mouthparts among insect taxa are not only reflected by their external morphology. Changes in the sclerotization of the cuticular integument at which the muscles attach can result in entirely different movements produced by the same muscles (Snodgrass, 1956). Moreover the elasticity of the skeleton obviates the necessity for antagonistic muscles in some cases, so that movement in two directions is accomplished by only one muscle (Snodgrass, 1956). This leads to an almost tangling variety of differences among distinct insect taxa (e.g. unlike functions of homologous muscles, loss of muscles). I therefore compare the anatomical design of the maxillae and the labium between ants, honey bees and a generalized insect focusing on muscles and their functions.

One maxilla of a generalized insect is moved by two 'ventral adductor muscles' that are both attached to the tentorium (Chapman, 1998; Snodgrass, 1935: Fig. 78, muscles KLt). This muscle of both that is more anteriorly attached to the tentorium and is inserted on the posterior margin of the stipes is homologous to the maxillary closer of ants (12, Fig. 4.2). The second 'ventral adductor muscle' corresponds to the maxillary opener of ants (11, Fig. 4.2). In ants, its muscle fibers arise more ventrally and posteriorly from the tentorium. Comparing ants with a generalized insect, the function of this muscle converted to the opposite, because the articulation of the cardo, the proximal part of the maxilla, changed. In ants, the articulation of the cardo to the cranium lies more distally. As a result, muscle fibers of the ant maxillary opener attach behind the articulation. Accordingly, upon contraction, the maxilla is moved outwards as described in the results. The same change is found in the honey bee, where the extreme end of the cardo projects a little beyond the articular condyle as well, and on it is inserted the single cardinal muscle from the head wall (Snodgrass, 1956: Fig. 25, muscle 10). The second 'ventral adductor muscle' of a generalized insect that corresponds to the maxillary closer of ants is absent in the honey bee (Snodgrass, 1956).

The maxillary 'posterior rotator muscle' of a generalized insect (Chapman, 1998; Snodgrass, 1935: Fig. 78, muscle J) corresponds to the maxillary levator of ants (13, Fig. 4.2). This is the only maxillary muscle in ants that takes its origin on the tergal wall of the head. Ants lack the 'anterior rotator muscle' (Chapman, 1998; Snodgrass, 1935: Fig. 78,

muscle rtmxa). In the honey bee, three similar large muscles insert on the posterior (ventral) part of the maxilla and arise from the clypeus; these muscles work as protractors of the maxilla (Snodgrass, 1956: Fig. 25, muscles 11-13). Because of the close connection of the labium with the maxillae by means of the lorum in the honey bee, the protraction of the maxillae protracts also the labium and therefore the entire proboscis. This protraction mechanism of the whole labiomaxillary complex could apply for ants as well, in addition to the movement upon contraction of the labial opener (6, Fig. 4.2).

The flexor muscle of the galea, the flexor muscle of the lacinia, and the levator muscle of the maxillary palp in a generalized insect (Chapman, 1998; Snodgrass, 1935: Fig. 78, muscles fga, flcs, O) are homologous to the three intrinsic maxillary muscles of ants (14-16, Fig. 4.2). In both ants and honey bees, the second muscle of the maxillary palp (depressor of palp) and the cranial flexor of the lacinia (Snodgrass, 1935: Fig. 78, muscle flcc) of a generalized insect are absent. The maxilla of the ant thus corresponds fundamentally in its structure with that of the maxilla of a generalized insect, except for the lack of several muscles and some changes of the function of the remaining muscles.

In a generalized insect, three labial muscles arise from the tentorium within the head capsule. Snodgrass (1935) termed two of them as ‘labial adductors’, because they clearly correspond to the tentorial adductors of the maxilla, although in their actual function they may produce various movements of the labium. Accordingly, Chapman (1998) called them more generally as ‘muscles to prementum from tentorium’. The first ‘labial adductor’ is absent in ants. The labial opener of ants (6, Fig. 4.2) is homologous to the second ‘labial adductor’ that attaches to the bottom of the prementum (Snodgrass, 1935: Fig. 84, muscle 2adlb). Like the maxillary opener (see above), the labial opener of ants changed its original function and became a labial abductor muscle. The labium of ants is retracted by the labial closer (7, Fig. 4.2) that can be derived from the ‘retractor of the hypopharynx’ (Snodgrass, 1935: Fig. 84, muscle rhphy). This muscle is attached at the base of the hypopharynx and is the third labial muscle of a generalized insect that arises from the tentorium.

The labial closer of ants is homologous to and has a similar function as the ‘anterior labial head muscle’ of the honey bee (Snodgrass, 1956: Fig. 27, muscle 17). This muscle is prominent in insect electrophysiology, since its electromyogram is often used as an indicator for the proboscis reflex in honey bees (Rehder, 1987; Smith and Menzel, 1989). In ants, the glossa movement during licking is supported by a synchronous movement of the entire labium. This labial movement probably results from alternate contractions of the labial opener and closer. Therefore the electromyogram of the labial closer should correlate with

the movement of the glossa in ants as well as in honey bees, though the labial closer is not actually responsible for glossa movement in ants.

The flexor muscle of the glossa, the flexor muscle of the paraglossa, and the levator muscle of the labial palp in a generalized insect (Chapman, 1998; Snodgrass, 1935: Fig. 84, muscles fgl, fpgl, lplp) can also be found in ants (8-9, Fig. 4.2, palp muscle not shown). Although the paraglossae are strongly reduced or completely absent in ants (Gotwald, 1969), the respective muscles of ants clearly correspond to the original muscles of the paraglossae. Nevertheless, the paraglossa muscles work as glossa retractors as well as the glossa muscles do (see results). Both ants and honey bees (Snodgrass, 1956) lack the second muscle of the labial palp (depressor of palp) and the 'retractor of the prementum' of a generalized insect (Chapman, 1998; Snodgrass, 1935: Fig. 84, muscles dplp, rst). Ants possess only one of the three salivary muscles of a generalized insect (Snodgrass, 1935: Fig. 84, muscles 1s, 2s, 3s). In my study I called it hypopharynx muscle (10, Fig. 4.2) which is homologous to muscle '1s'. In addition to this dilator muscle of the salivarium, honey bees have a salivary compressor muscle on each side of the head (Snodgrass, 1956: Fig. 27, muscle 24) that is absent in ants.

While ants lack several labial muscles in comparison to a generalized insect, some muscles changed their original function, and the labium, particularly the glossa and the paraglossa, seem to be more derived than the maxillae, one can still see the fundamental structures of a generalized insect. Honey bees and ants share most of the differences compared to a generalized insect concerning muscles and their functions. Since glossa protractor muscles are also absent in honey bees (Snodgrass, 1956), I thus suggest an elastic mechanism for glossa protraction in honey bees as I have shown for ants.

**Non muscular movement mechanisms**

Several other examples of mechanisms of non muscular movements are known among arthropods. In general, they can be divided into two groups: on the one hand mechanisms driven by hemolymph pressure and on the other hand elastic mechanisms. One of the most prominent pressure-driven mechanisms is that underlying leg movement of spiders (Foelix, 1982). Most leg joints of spiders are equipped with several muscles that either bend the joint (flexors) or stretch it (extensors). Two remarkable exceptions are the femur-patella joint and the tibia-metatarsal joint, both of which lack extensors (Petrunkevitch, 1909). The extension of these joints is caused by a hydraulic mechanism, by an increase of the hemolymph pressure (Ellis, 1944; Wilson, 1970, 1973; Anderson and

Prestwich, 1975). A contraction of the 'musculi laterales', which traverse the carapace vertically on both sides, leads to a reduction in the volume of the prosoma and thus to increased pressure there (Foelix, 1982). This hemolymph pressure has been measured directly in several spiders (Parry and Brown, 1959a, b).

Another well investigated pressure-driven mechanism is the lepidopterous proboscis extension (Schmitt, 1938; Bänzinger, 1971; Hepburn, 1971; Krenn, 1990). Uncoiling of the proboscis is a result of a step-by-step increase of the hemolymph pressure in the galeae created by compression movements of the stipites. The uncoiled position and its passively formed bend region is maintained by the valve function of the stipites. The elasticity of the proboscis cuticle tends to an intermediate slightly coiled position. After the proboscis was tightly coiled beneath the head by its musculature, the cuticular processes interlock between the coils and keep the coiled position without further muscle action (Krenn, 1990).

Some other movements in insects are based on pressure-driven mechanisms as well. Dragonfly larvae catch their prey with a fast-moving elongated labium. This predatory strike is caused by a hydraulic mechanism (Olesen, 1972; Tanaka and Hisada, 1980). Fecal firing in a skipper caterpillar is pressure-driven (Caveney et al., 1998). Adult beetles of the genus *Stenus* have a highly modified labium that works as an adhesion-capture apparatus and can be rapidly protruded by hemolymph pressure (Betz, 1996).

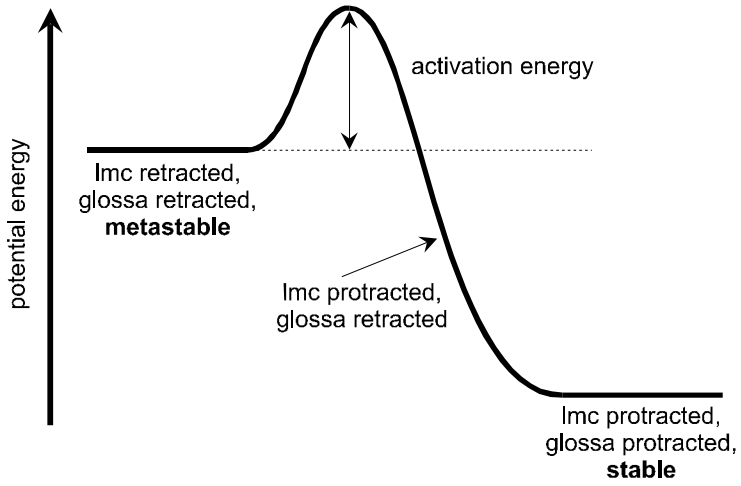



Fig. 4.8: Hypothetical levels of potential energy in dependence on the respective positions of the labiomaxillary complex (lmc) and the glossa. A specific activation energy is necessary to protract the labiomaxillary complex. If this activation energy is generated, the glossa protracts automatically according to the low-energy state for the whole system at a protracted glossa (see text for further details).

Beside pressure-driven mechanisms, movement can also be generated by elasticity. In general, the elastic materials and elements work like springs that act as muscle antagonists, energy stores, or catapults (Alexander, 1988). Most of the fast actions in arthropods are based on catapult and click mechanisms (Gronenberg, 1996). To overcome the temporal limitations of muscular contraction, muscles slowly load spring-like structures with potential energy that is later released instantaneously by various mechanisms. Such spring-loaded systems are widely employed by insects (e.g. the jumps of fleas, Bennet-Clark and Lucey, 1967; springtails, Christian, 1979; click beetles, Evans, 1973; flea beetles, Furth et al., 1983; grasshoppers and locusts, Heitler, 1974, Bennet-Clark, 1975; predatory mandible strike of trap-jaw ants, Gronenberg, 1995, Paul, 2001). The energy for the jump of the mite genus *Zetorchestes*, that is also released by a click mechanism, results from a combination of increased hemolymph pressure and strain energy stored in elastic elements (Krisper, 1990). In all these elastic mechanisms, a set of antagonistic muscles exist nevertheless.

Only few examples are known where elasticity really works as muscle antagonist (e.g. bivalve shells, mesogloea of sea anemones; Alexander, 1988). The elastic properties of the insect cuticular exoskeleton precludes the need for antagonistic muscles in some cases, so that bi-directional movement is achieved by only one muscle or a group of muscles with the same function (Snodgrass, 1935, 1956). Such a movement apparatus is represented by the glossa of ants. If the labiomaxillary complex is exposed, the glossa of ants protracts elastically and automatically when the antagonistic muscles, the glossa retractors, relax (see results). Accordingly, one could conclude that an ant, in order to hold the glossa retracted, has to permanently contract its glossa retractor muscles. This would not be very economical considering that an ant spends much time for other tasks than using its glossa for food intake. When an ant does not use its glossa, the entire labiomaxillary complex remains completely retracted. My results show that in this case the glossa or the whole labiomaxillary complex do not protract automatically when all muscles relax (Fig. 4.7). I therefore think that the totally retracted labiomaxillary complex represents a biomechanically metastable position in terms of the level of potential energy (Fig. 4.8). To overcome the necessary activation energy, the labial opener muscles contract, so that the labiomaxillary complex protracts. Then, the glossa protracts automatically as well, if the glossa retractor muscles are still relaxed. This would be the lowest-energy state and therefore the stable state of the labiomaxillary complex (Fig. 4.8). To close the whole complex again, first the glossa retractors contract, after that the labial closer muscles are activated, and finally the labrum is closed. The labiomaxillary complex is thus held in a metastable position and no muscle

activity is needed (Fig. 4.8). This retracted position of the labiomaxillary complex of ants is comparable to the lepidopterous tightly coiled proboscis (see above) that is kept in the coiled position without further muscle activity (Krenn, 1990).

I conclude that ant glossa protraction is based on an elastic mechanism where elasticity works as an actual antagonist to glossa retractor muscles (muscles of glossa and paraglossa). Since glossa protraction is therefore a passive movement, it can not be precisely controlled in terms of force output and movement velocity. This explains why licking frequency is almost constant during liquid feeding and independent of concentration and viscosity of sucrose solution over a wide range (Dasch, 1998; cf. constant lapping rates of bumble bees, Harder, 1986). The mechanism of glossa movement could set an upper limit to licking frequency, thus influencing food intake rates and ultimately foraging behavior.

## B. THE LABIOMAXILLARY COMPLEX

## 5. Liquid food intake in ants: I. Feeding techniques and intake rates

## Introduction

Social foraging is a complex process due to individual decisions influenced by interactions among members of the whole group or society. Two currencies, 'efficiency' (net energy gain per unit energy expenditure) and 'rate' (net energy gain per unit time), have been widely discussed to predict foraging behavior of different animals more precisely (e.g. Schmid-Hempel et al., 1985; Kacelnik et al., 1986; Schmid-Hempel, 1987; Ydenberg et al., 1992; Ydenberg and Hurd, 1996). In addition to time and energy calculations at the level of an individual, models developed to understand social foraging have to consider the characteristics of social life such as communication (Ydenberg and Schmid-Hempel, 1994). In social insects, workers might exploit a food patch less efficiently, as measured on an individual basis, but allocate more effort to information exchange, thus increasing the performance of the entire colony (Núñez, 1982). For leaf-cutting ants, it has been shown that workers increase the recruitment of nestmates to more profitable food resources at the expense of individual performance as carriers (Roces, 1993; Roces and Núñez, 1993; Roces and Hölldobler, 1994). However, efficiency seems to be less important for ants than rate, since the energy gain of a foraging worker is up to 1000 times higher than the energy spent, depending on type of food and species (Fewell, 1988; Weier and Feener, 1995; Fewell et al., 1996). For ants, it is essential to exploit a discovered food source rapidly. Accordingly, a scout should quickly collect some food and recruit nestmates immediately (Mailleux et al., 2000) by offering them a portion of food of the new source. After establishing the recruitment process, workers should collect food as fast as they can in order to increase the overall intake rate of the whole colony. Therefore, the ability of an individual to be fast in collecting food should be advantageous for both scouts and workers recruited to a newly-discovered source. In liquid-feeding ants, this ability is represented by the fluid intake rate (amount of collected liquid per unit time).

Fluid intake rates depend on the species-specific mechanics of feeding as well as on nectar characteristics such as sugar concentration and viscosity (Josens et al., 1998). The dynamic of nectar intake was studied in various species (honeybees: Núñez, 1966; Roubik and Buchman, 1984; bumblebees: Harder, 1986; butterflies: May, 1985; nectar-feeding bats: Roces et al., 1993). Theoretical models have been developed to understand the intrinsic mechanisms underlying nectar uptake rates (Kingsolver and Daniel, 1979, 1983, 1995; Harder, 1983, 1986; Heynemann, 1983). For ants, bumblebees, honeybees, and other fluid-feeding insects, the glossa, the distal end of the labium, is essential for food intake. The specific mechanism of glossa movement could influence food intake rates (Paul et al., 2001),

depending on the actual feeding technique. The respective feeding technique is thus important for evaluation of intake rates.

Several ant species are ubiquitous visitors of liquid food sources, which are typically extrafloral nectaries on plants or honeydew produced by aphid colonies. The ants obtain the food, and pay for this by protecting the aphids or plants against predators or herbivores (Way, 1963; Bentley, 1977; Beckmann and Stucky, 1981; Dreisig, 1988; Rico-Gray, 1993). Both aphid colonies and plants provide solutions that mainly contain sugars but also some additional substances such as amino acids, which clearly increase the acceptance of carbohydrate solutions (Lanza, 1991). Carbohydrate-protein ratios of ant rewards may control the identities of ant associates as well as the quality of ant-rendered services (Davidson, 1997). While various ant species are specialized on collecting fluids, others rarely feed on nectar sources. Indeed, the latter also take the opportunity and feed on liquid food sources if they have found one, but focus on other food or material such as seeds (harvesting ants), leaves (leaf-cutting ants), or living prey (predatory ants).

In the present study I first investigated the techniques of liquid food intake employed by foragers of several ant species by video analysis, and then measured the fluid intake rates of workers of four particular species adapted to different ecological niches (*Camponotus rufipes*, *Pachycondyla villosa*, *Atta sexdens*, and *Rhytidoponera impressa*-complex). *Camponotus rufipes* represents a typical nectar-feeding ant species, and workers of *Pachycondyla villosa* very often collect liquids as well. In contrast, the leaf-cutting ant *Atta sexdens* and the predatory ant *Rhytidoponera impressa* are not specialized in collecting liquids. Workers of these four species were presented with pure sucrose solutions of a wide range of concentrations, and intake rates were recorded under controlled feeding conditions in the laboratory. The aim of these measurements was to analyze to what extent species-specific feeding techniques lead to different effects of sucrose concentration on fluid intake rates. For interspecific comparison, I measured intake rates of individuals of different size for the two polymorphic species (*C. rufipes* and *A. sexdens*), and used the linear regressions, relating intake rate and head width, to scale the intake rates to the worker size of the two non-polymorphic species (*P. villosa* and *R. impressa*). Hence, intake rates could be quantitatively compared across species independently of worker size, but related to species-specific feeding habits.

## Materials and methods

Experiments were carried out on workers of the following ant species: *Ectatomma ruidum*, *Pachycondyla villosa*, *Rhytidoponera impressa*-complex (Ponerinae), *Myrmecia* sp. (Myrmeciinae), *Atta sexdens*, *Messor barbarus*, *Pogonomyrmex californicus*, *P. rugosus* (Myrmicinae), *Camponotus rufipes*, *C. festinatus*, and *C. laevigatus* (Formicinae). The ants were kept in plaster-of-Paris nests under a 12h:12h L:D cycle at 25°C and 50% relative humidity. They were fed chopped cockroaches, crickets or wingless *Drosophila*, honey-water (30%), and fresh leaves (*Atta sexdens*) or grains (*Messor*, *Pogonomyrmex*).

For analysis of feeding techniques, the behavior of ants and particularly the movements of the labiomaxillary complex during feeding were videotaped at 50 frames per second (Panasonic F15 HS), or were observed with a dissection microscope (Wild M3Z). Ants could move freely in small arenas with free access to the feeding site.

Measurements of intake rates were performed with colonies of *Atta sexdens*, *Camponotus rufipes*, *Pachycondyla villosa*, and *Rhytidoponera impressa*-complex, all of them being deprived of food for approximately two days. Each assay was commenced by connecting the nest to a foraging arena (19 x 19 cm²; main food source, Fig. 5.1) by a wooden bridge (1 m long; main trail, Fig. 5.1). The ants had free access to the offered food there. After this main food source was established and foraging activity was stable, a small group of foragers (about 5 ants) were allowed to pass another wooden bridge (20 cm long; mobile bridge, Fig. 5.1) that led to a balance (Mettler AE 200). This way directed further to an additional food source that was placed at the end of the wooden side trail (liquid food source, Fig. 5.1), where a large droplet (ca. 1 cm in diameter) of sucrose solution was presented. Once the foraging group returned to the main trail carrying sucrose solution, only single ants were allowed to pass the mobile bridge (Fig. 5.1).

For each individual worker that foraged on the side trail, I measured its body mass without load (before drinking, on the way to the food source, $m_b$), the feeding time ($t$), its

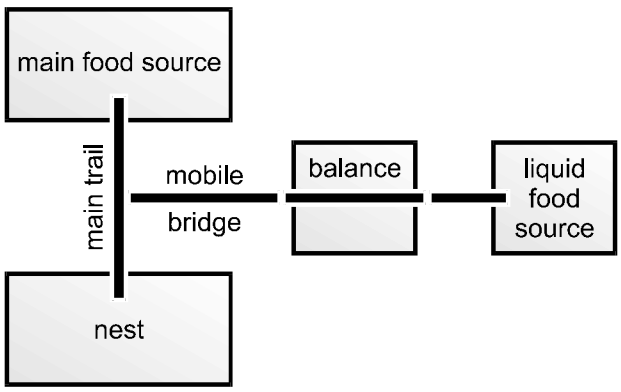



Fig. 5.1: Experimental setup for measuring fluid intake rates of individual ants (see materials and methods for further details).

loaded body mass (after drinking, on the way back, $m_a$), and its head width ($w_h$). For the latter, I captured the forager on its way back to the main trail, and determined its head width by using a calibrated scale (Zeiss) and a dissection microscope (Wild M3Z). Then, the worker was separated from the rest of the colony to avoid repeated measurements. These four variables were measured for concentrations of sucrose solution of 30, 40, 50, 60, and 70% w/w. For *Camponotus rufipes*, I additionally tested 10 and 20% w/w sucrose solutions. The load (in mg) was obtained as the difference between $m_a$ and $m_b$. The volume of solution taken ($V$) was calculated by dividing the collected load by the density of the solution drawn from tables (Wolf et al., 1984). The time actually spent in contact with the solution droplet (sucking), or spent on licking cycles at the droplet, respectively, was recorded as feeding time ($t$). Possible interruptions were excluded. Fluid intake rates were calculated for each individual by dividing the volume of sucrose solution taken ($V$) by the respective feeding time ($t$).

For interspecific comparison, I plotted the calculated intake rates against the corresponding head width of individual workers of the two polymorphic species (*C. rufipes*, *A. sexdens*). Linear regressions revealed scaling equations for the respective sugar concentration and species. Using these equations, I could calculate the intake rate for any head width within a particular range. The head widths of the two non-polymorphic species (*P. villosa*, *R. impressa*) lay within the measured range of head widths of the two polymorphic species. Intake rates could thus be compared across species independently of worker size. In contrast to the body mass that depends on variable parameters such as foraging load or content of the digestive tract, the head width is a constant morphological size parameter of any individual worker. I therefore used the head width for scaling ant size. However, I found a linear correlation between unloaded individual body mass and the respective head width (*Camponotus rufipes*: $r = 0.94661$, $p < 0.001$, $n = 236$; *Atta sexdens*: $r = 0.96189$, $p < 0.001$, $n = 117$).

## Results

### Feeding techniques

Our videoanalysis of the behavior of ants and the movements of the labiomaxillary complex during liquid feeding showed that two different techniques for liquid food intake are used. The glossa, the distal end of the labium (Fig. 5.2a), works either as a passive duct (sucking), or as an up- and downwards moving shovel (licking).

For sucking, ant workers deeply put their protracted glossa and some neighboured parts of the labiomaxillary complex into the droplet of sugar solution. Figure 5.2b shows a worker of *Camponotus rufipes* that additionally put its mandibles into the drop. Workers of species that possess large mandibles did not use them during sucking (e.g. *Myrmecia*). Workers kept this position of the glossa below the surface level of the solution until they stopped sucking spontaneously or the droplet was almost completely ingested (Fig. 5.2c shows a worker just before it stopped sucking). Probably, capillary tension ensured a continuous flow of the solution over glossa and hypopharynx to the actual mouth of the ant. Driven by the underpressure produced by the pharynx dilator muscles (see chapter B.6.), the liquid was transported to the pharynx and, after passing the oesophagus, was finally stored within the crop. During sucking, only a slight rhythmic pulsing of the labiomaxillary complex could be noticed (see Josens and Roces, 2000, for quantitative measurements in *Camponotus mus*) that probably resulted from the activity of the pharynx dilator muscles. If the worker continued drinking when the droplet was almost completely ingested, it switched to licking behavior, since the remaining fluid built a flat film in which the glossa could not be introduced.

In contrast, workers of *Pachycondyla villosa* licked the offered droplet of sugar solution from the beginning. For licking, the protracted glossa only touched the surface of the solution (Fig. 5.2d), whereby the glossa was loaded with liquid according to its capillary pressure. The loaded glossa was then retracted, after which the contact to the solution was interrupted. During retraction of the glossa, the two galeae moved ventrally, without touching the glossa (cf. Fig. 5.2a). During the following protraction of the glossa, the galeae wiped the liquid upwards along the downwards-moving glossa, before the glossa again got into contact with the surface of the droplet. This opposite movement of galeae and glossa ensured the upwards transport of the liquid. The entire labium contributed to the licking process. The movement of the glossa was supported and amplified by a synchronous movement of the whole labium. About four to five complete licking cycles per second were performed. Workers of ponerine species accumulated and transported the fluid as a drop between their mandibles according to capillary tension and adhesion (Fig. 5.2d, e).

Workers of all ant species examined were able to employ both, either licking or sucking, for liquid food intake. But workers of different species preferred one of the two different feeding techniques described. During collecting fluid food at *ad libitum* sources, workers of *Pachycondyla villosa* always licked, whereas workers of *Camponotus rufipes* always sucked the offered sugar solution. Table 5.1 gives an overview of the used feeding

techniques for the investigated ant species. Workers of the ponerine species licked fluids at *ad libitum* food sources, whereas workers of the other species sucked it. The used technique depends on whether liquid food transmission among nestmates occurs and on how workers transport the liquid food to the nest (see discussion).

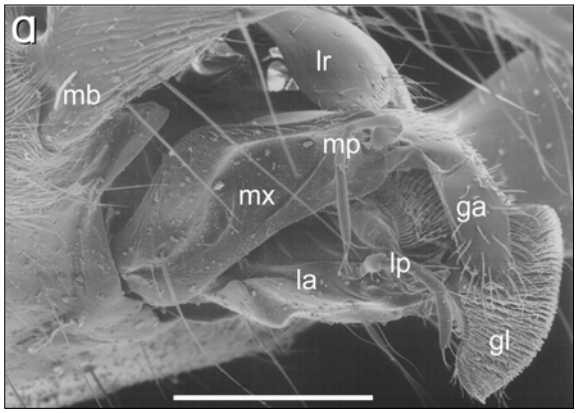







Fig. 5.2: (**a**) Scanning electron microscopic image of the labiomaxillary complex of a worker of *Pachycondyla villosa*, lateral view; scale bar = 500 µm; (ga) galea, (gl) glossa, (lp) labial palpus, (la) labium, (lr) labrum, (mb) mandible base, (mx) maxilla, (mp) maxillary palpus. (**b**-**e**) Different techniques of liquid feeding at an *ad libitum* food source. (**b**, **c**) Sucking worker of *Camponotus rufipes*, and (**d**, **e**) licking worker of *Pachycondyla villosa* (see text for further details).

**Intake rates and ant size**

I measured fluid intake rates of individual workers of two polymorphic species (*Atta sexdens* and *Camponotus rufipes*) for various concentrations of sucrose solution. For a given species, intake rates varied considerably among workers of different size. Figure 5.3a shows,

as an example, the intake rates of *Camponotus rufipes* workers for a concentration of 30% sucrose. At this concentration, small workers (head width: 1.0 - 1.5 mm) ingested liquid food at a rate of about 4 µl/min, whereas large workers (head width: 3.0 - 3.5 mm) reached a rate of about 9 µl/min. As in *Camponotus rufipes*, smaller workers of *Atta sexdens* also drank at lower intake rates than larger ones did (Fig. 5.3b). The fluid intake rate increased with increasing head width for both polymorphic species. Comparing individuals of the same head width, workers of *Camponotus rufipes* showed intake rates approximately ten times higher than workers of *Atta sexdens* (Fig. 5.3a, b). This enormous difference is analyzed in more detail in the next chapter (interspecific comparison).

| Subfamily | Species | Feeding technique |
|---|---|---|
| Ponerinae | *Ectatomma ruidum* | Licking |
| | *Pachycondyla villosa* | Licking |
| | *Rhytidoponera impressa*-complex | Licking |
| Myrmeciinae | *Myrmecia gulosa*-complex | Sucking |
| Myrmicinae | *Atta sexdens* | Sucking |
| | *Messor barbarus* | Sucking |
| | *Pogonomyrmex californicus* | Sucking |
| | *P. rugosus* | Sucking |
| Formicinae | *Camponotus laevigatus* | Sucking |
| | *C. festinatus* | Sucking |
| | *C. rufipes* | Sucking |

Table 5.1: Feeding techniques of workers of different ant species at an *ad libitum* liquid food source.

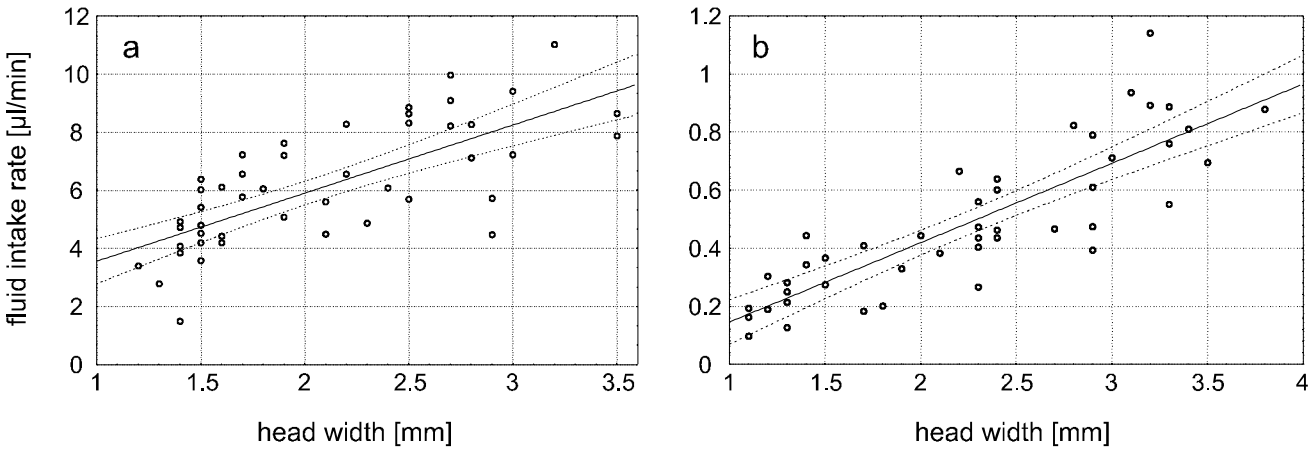



Fig. 5.3: Examples of correlations between fluid intake rate (ordinate) and head width (abscissa) of ant workers drinking a solution of 30% sucrose concentration. Each data point represents an individual of *Camponotus rufipes* (**a**, left side; r = 0.73472; p < 0.0001; n = 50) and of *Atta sexdens* (**b**, right side; r = 0.85114; p < 0.0001; n = 45), respectively.

| species | conc. | a | b | r | p | n | y for x = 1.29 mm | y for x = 2.37 mm |
|---|---|---|---|---|---|---|---|---|
| *C. rufipes* | 10% | 0.966 | 2.487 | 0.900 | <0.0001 | 39 | 4.173 | 6.859 |
| | 20% | 0.782 | 2.446 | 0.931 | <0.0001 | 39 | 3.937 | 6.579 |
| | 30% | 1.210 | 2.346 | 0.735 | <0.0001 | 50 | 4.237 | 6.771 |
| | 40% | -0.564 | 2.701 | 0.851 | <0.0001 | 43 | 2.920 | 5.837 |
| | 50% | 0.764 | 0.954 | 0.605 | <0.0001 | 50 | 1.994 | 3.024 |
| | 60% | 0.151 | 0.546 | 0.644 | <0.0001 | 53 | 0.855 | 1.445 |
| | 70% | 0.073 | 0.266 | 0.681 | <0.0001 | 40 | 0.417 | 0.704 |
| *A. sexdens* | 30% | -0.127 | 0.273 | 0.851 | <0.0001 | 45 | 0.225 | 0.520 |
| | 40% | -0.150 | 0.197 | 0.838 | <0.0001 | 42 | 0.105 | 0.318 |
| | 50% | -0.093 | 0.113 | 0.758 | <0.0001 | 30 | 0.053 | 0.176 |

Table 5.2: Correlations between fluid intake rate (y) and head width (x) of workers for the two polymorphic species *Camponotus rufipes* and *Atta sexdens* at different concentrations of sucrose solution (= conc.). I used the equations of the linear regressions (y = a + b * x) for scaling intake rates to the head width of the two non-polymorphic species, respectively (mean head width of workers of *Pachycondyla villosa*: 2.37 mm; and of *Rhytidoponera impressa*-complex: 1.29 mm). r = correlation coefficient; p = probability of error; n = number of tested individuals; y = intake rate [µl/min]; x = head width [mm]; x-range of *Camponotus rufipes*: 1.1 - 3.6 mm; x-range of *Atta sexdens*: 1.1 - 4.2 mm.

Within the two polymorphic species, I found linear correlations between intake rate and head width for all tested sucrose concentrations (Tab. 5.2), as it is graphically shown in Figure 5.3 for 30% sucrose concentration. Plotting intake rate (y) against head width (x), the slope (b) of the respective linear regression was always positive. At higher sucrose concentrations, the absolute values of intake rates decreased for a given ant size, while the difference between smaller and larger individuals clearly persisted (Tab. 5.2, right two columns). In order to correct for the effects of worker size when comparing different species, I used the equations of the linear regressions (Tab. 5.2) for scaling intake rates to the respective worker size of the two non-polymorphic species (mean head width of workers of *Rhytidoponera impressa*-complex: 1.29 mm, and of *Pachycondyla villosa*: 2.37 mm).

**Interspecific comparison**

The intake rate of liquid food at an *ad libitum* food source did not only depend on worker size but also on the concentration of sucrose solution as well as on the species. In all investigated ant species, workers collected low-concentrated solutions at higher intake rates. The highest intake rates were reached by workers of *Camponotus rufipes* at sucrose concentrations of 10% to 30% (Fig. 5.4a); workers of this species with a head width of 2.37 mm ingested liquid food at about 6.7 µl/min. At these low concentrations, the intake rate was

independent of the respective sucrose concentration. At higher sucrose concentrations, intake rates of *Camponotus rufipes* workers began to decrease continuously, approaching 0.7 µl/min at 70% sucrose (Fig. 5.4a).

The maximum intake rate of *Pachycondyla villosa* workers was about 4.2 µl/min and thus lower than in *Camponotus rufipes* (Fig. 5.4a). These high intake rates, however, were constant for increasing sucrose concentration until 50%, and decreased rapidly for higher concentrations. The intake rates of *Camponotus rufipes* and *Pachycondyla villosa* were statistically different at 30%, 40%, 50%, and 60% (t-test, $p < 0.01$). Hence, workers of *Camponotus rufipes* drank faster at low sugar concentrations, whereas workers of *Pachycondyla villosa* collected liquids relatively faster at high concentrations (Fig. 5.4a).

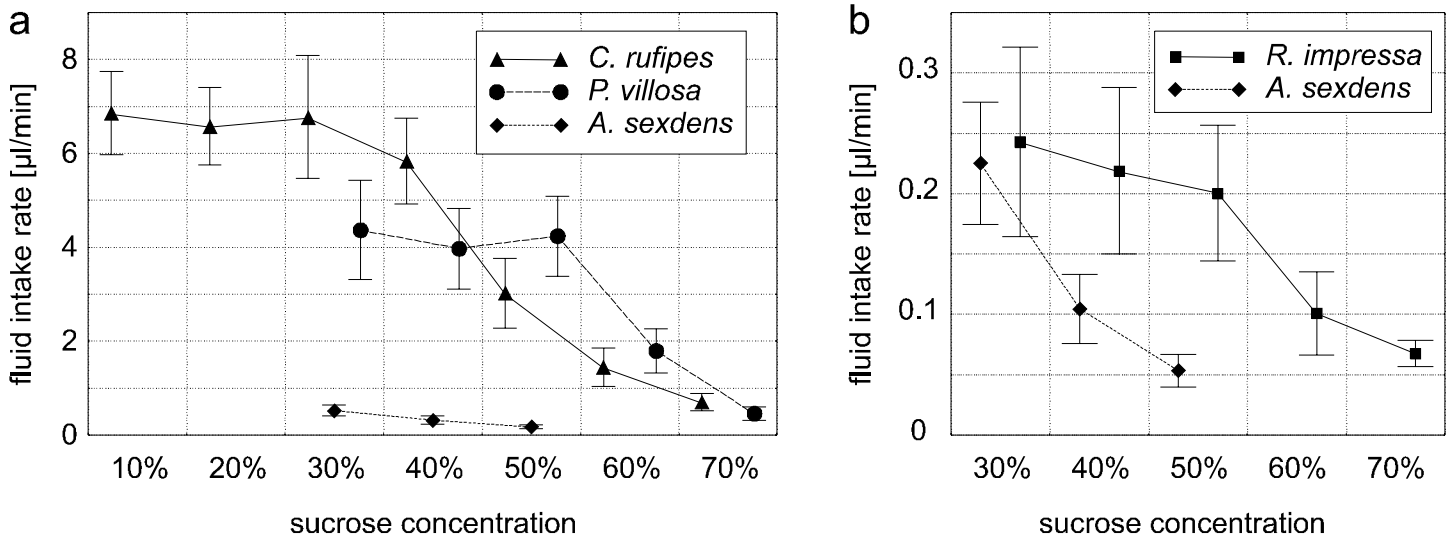



Fig. 5.4: Fluid intake rates (ordinate) of workers of *Camponotus rufipes*, *Pachycondyla villosa, Atta sexdens*, and *Rhytidoponera impressa*-complex, at different sucrose concentrations (abscissa). (**a**) Intake rates for *C. rufipes* and *A. sexdens* are scaled to the head width of *P. villosa* (2.37 ± 0.07 mm, n = 115); (**b**) intake rates for *A. sexdens* are scaled to the head width of *R. impressa* (1.29 ± 0.06 mm, n = 112). Values of intake rates are means ± standard deviation of n individuals, respectively (n = between 20 and 30 for *P. villosa* and *R. impressa*; see Table 2 for n-numbers of the two polymorphic species).

Compared to *Camponotus rufipes* and *Pachycondyla villosa*, workers of *Atta sexdens* with a head width of 2.37 mm ingested fluid food at considerable lower intake rates, not exceeding 0.6 µl/min (Fig. 5.4a; Tab. 5.2, right column). In many cases when offering highly concentrated sucrose solution (60%, 70%), either individuals of *Atta sexdens* did not drink at all or only collected very tiny amounts so that the load could not be precisely determined. At

high sucrose concentrations, the intake rates were thus too small to be detected accurately if the *Atta* worker ingested the solution. Therefore, no data for *Atta sexdens* at 60% and 70% sucrose solution are available (Fig. 5.4a). As in *Camponotus rufipes*, the intake rates of *Atta sexdens* workers decreased with increasing concentration between 30% and 50% (Fig. 5.4a, b). The curves for these two species had a similar shape, compared to that of *Pachycondyla* workers within this range of sucrose concentration (Fig. 5.4a, b). In absolute numbers, intake rates of *Camponotus rufipes* foragers were more than ten times higher than those of *Atta sexdens* (Fig. 5.4a; Tab. 5.2, right column).

Since workers of the two non-polymorphic species featured distinct head widths, I did not illustrate the intake rates for all four species in a single graph (Fig. 5.4a, b). The intake rates of *Rhytidoponera* workers were approximately at the low level observed for *Atta sexdens*, although significantly higher at 40% and 50% sucrose concentration (Fig. 5.4b; t-test, $p < 0.01$). Between 30% and 50%, the intake rates for *Rhytidoponera* only tended to decrease, before decreasing rapidly between 50% and 60%. Therefore, the shape of this curve was similar to that of *Pachycondyla* (Fig. 5.4a, b). Over the whole range of sucrose concentration, the mean intake rates of individuals of *Rhytidoponera* lay between 0.24 µl/min at 30% and 0.067 µl/min at 70%. Workers of *Camponotus rufipes* with a head width of 1.29 mm ingested liquids at much higher intake rates at any concentration (Tab. 5.2).

## Discussion

### Licking and sucking

When collecting liquid food during a foraging bout, workers of different ant species employed different techniques (Tab. 5.1), although they were able to use both, either licking or sucking. So why do workers of one species prefer a specific feeding technique?

Fluid-feeding ants use various techniques for transporting the liquid food. Workers of most ant species collect and store the liquid within their crop, which is capable of considerable distension in many species such as *Camponotus rufipes*. Ants of the genus *Camponotus* possess a highly developed proventriculus, a morphological adaptation for the retention of food in the crop (Eisner, 1957). When a forager comes back to the nest with a loaded crop, the fluid is regurgitated and delivered to nestmates that distribute it over large portions of the whole colony (Hölldobler and Wilson, 1990). Thus, the ensemble of crops of all workers taken together serve as a ‘social stomach’ from which the colony draws nourishment. Workers of some ponerine ant species transport the liquid food as a drop between their mandibles, as in *Pachycondyla villosa*. These ponerine species employ an

external social bucket, which is functionally involved in social food exchange as the internal social crop of phylogenetically more advanced species (Hölldobler, 1985). An extraordinary kind of nectar transport was found in the African ponerine ant *Platythyrea conradti*: for transportation, nectar adheres to the ventral sides of both the head and the thorax and to the coxa of the forelegs (Dejean and Suzzoni, 1997).

The ponerine species that display licking behavior during collecting liquids (Tab. 5.1), transport and transmit the fluid food as a drop between their mandibles. If workers of these species would deeply hold their glossa into the liquid food in order to suck (cf. Fig. 5.2b), the already collected drop between their mandibles would get into contact with the liquid of the food source. According to adhesive and cohesive forces, the already collected drop would then get lost. Therefore, they lick fluid food during foraging and accumulate it externally, since they do not have a well developed crop and proventriculus (Eisner, 1957) to store the collected food internally. In contrast, workers of *Atta sexdens* and *Camponotus rufipes* possess a crop capable of storing liquids, although in *Atta* it is much smaller (Caetano 1990), and thus suck fluid food (Tab. 5.1).

The ability to regurgitate food from the crop and surrender it to begging nestmates is common among ant species belonging to the phylogenetically more advanced subfamilies such as Formicinae and Myrmicinae (Hölldobler and Wilson, 1990; cf. Tab. 5.1). Some of these species may have lost this capacity secondarily in the course of evolving more specialized food habits, as in harvesting ants of the genus *Pogonomyrmex* (Wilson and Eisner, 1957). When individuals only feed themselves on liquids, they suck it, independently of whether they possess a well developed crop or not, because they do not need to store the liquid for social food exchange. Therefore, the species-specific technique of liquid feeding depends on two parameters: (1) the existence of trophallaxis or a comparable liquid-food transmission, and the consecutive need to temporarily store liquids during foraging; (2) the existence of a well developed crop, and the resulting mode of transporting liquid food.

**Feeding technique and intake rate**

The viscosity of a sucrose solution increases exponentially with increasing sucrose concentration. While the viscosity of a sucrose solution only slightly increases with increasing concentration below 30% (w/w), it starts to increase more and more at higher concentrations, rising steeply between 60% and 70% (Wolf et al., 1984). Accordingly, viscosity becomes important for the mechanics of liquid feeding at about 30% to 40% sucrose concentration. For *Camponotus rufipes*, the fluid intake rate was constant until 30%,

and decreased beyond this concentration with increasing concentration (Fig. 5.4a). The decline of intake rate beyond 30% can thus be explained by the respective increase of viscosity and its mechanical effects on liquids. Such a 'critical' concentration was found in most studies on nectar feeding, and is also predicted by theoretical models based on the Hagen-Poiseuille equation (Núñez, 1966; Kingsolver and Daniel, 1979; Heynemann, 1983; Roubik and Buchmann, 1984; Harder 1986). For *Camponotus mus*, it has recently been shown that fluid intake rates depend on sugar concentration of the solution as well as on colony starvation (Josens et al., 1998; Josens and Roces, 2000). In this ant species, nectar intake rate was also observed to decline with increasing concentration beyond 30% sucrose, but to increase with increasing sucrose concentration in the range between 5% and 30%, which is rather unexpected (Josens et al., 1998). I could not confirm this increase of intake rate between 5% and 30% for the ant species *Camponotus rufipes* (Fig. 5.4a).

During sucking, a continuous flow of the solution over glossa and hypopharynx to the actual mouth of the ant was maintained. Therefore, viscosity directly affected intake rate by influencing fluid movement. This explains the decline of intake rate of *Camponotus* workers beyond 30%, at which viscosity increases markedly (note that between 10% and 30%, viscosity doubles, whereas it is almost ten times higher at 60% compared to 40%; Wolf et al., 1984). But why then did the intake rates of the licking workers of *Pachycondyla villosa* remain constant until 50% sucrose concentration (Fig. 5.4a)?

Intake rates depend on both the licking frequency and the amount of liquid loaded per licking cycle. The decrease of intake rates for *Pachycondyla villosa* between 50% and 70% can be explained by viscosity effects as well. The surface of the ant glossa is equipped with shovel-like structures (see chapter B.6.). During contact with the liquid food source, these shovels are loaded with fluid by capillary forces. At 50% sucrose, the glossa was completely loaded before being retracted, thus reaching the maximum uptake rate (Fig. 5.4a), whereas at higher concentrations and viscosities, the glossa was probably only partly loaded. Accordingly, the glossa did not uptake as much liquid per licking cycle then. Furthermore, the glossa had to work against a higher mechanical resistance due to the higher viscosity of the solution, being reflected in slightly lower licking frequencies at 60% and 70% sucrose concentration (Dasch, 1998). Below 50% sucrose, licking frequency remained constant, independently of sugar concentration (Dasch, 1998). The protraction of the glossa in ants is based on an elastic mechanism, and is consequently a passive movement that cannot be precisely controlled in terms of force and velocity (Paul et al., 2001), which partly explains constant licking frequencies. Bumble bees also maintain a constant lapping frequency

regardless of concentration and viscosity of the offered sucrose solution (Harder, 1986). The constant licking frequency below 50% probably reflects the maximum licking frequency, therefore limiting the maximum uptake rate at lower sucrose concentrations.

Sucking and licking seemed to be differently affected by viscosity. The critical sucrose concentration beyond which the intake rate decreased was 30% for *Camponotus rufipes* and 30%, or lower, for *Atta sexdens* (Fig. 5.4a, b), as expected when considering only viscosity effects. Workers of both species sucked sugar solution at an *ad libitum* food source. During sucking, the fluid flow was directly affected by viscosity. In contrast, intake rates of licking workers of *Pachycondyla villosa* and *Rhytidoponera impressa* remained constant until 50% sucrose (Fig. 5.4a, b). During licking, viscosity affected the loading phase of the glossa, which is adapted to higher viscosities in *Pachycondyla* and *Rhytidoponera* (see chapter B.6.). Therefore, licking is not as susceptible to viscosity effects until 50%, but is limited by maximum licking frequency.

**Feeding habits and intake rate**

The fluid intake rate does not only depend on the species-specific feeding technique (see above), but also reflects adaptations to different ecological niches. Workers of the two species, that frequently feed on liquid food sources (*Camponotus rufipes*, *Pachycondyla villosa*), collected the liquid at much higher intake rates than individuals of the two species not specialized in collecting fluid food (*Atta sexdens*, *Rhytidoponera impressa*-complex). The intake rates for the latter two species were approximately ten times lower (Tab. 5.2; Fig. 5.4a, b). *Rhytidoponera impressa* represents a predatory ant that feeds only rarely on liquid food sources and therefore does not rely on high fluid intake rates to improve foraging performance. Leaf-cutting ants as *Atta sexdens* actually ingest the small amounts of plant sap exposed at the cutting site during leaf-cutting (Littledyke and Cherrett, 1976), and since cutting takes longer than ingesting plant sap, they do not need to drink fast.

The great difference of intake rates between the two groups of species suggests that the ability to collect fluids at high intake rates is important for nectar-feeding species, although many natural food sources produce nectar at lower rates (O´Dowd, 1979; Dreisig, 1988, 2000). In nature, a continuous production rate of nectar or honeydew is not always accompanied by an immediate continuous consumption. Accordingly, droplets of nectar accumulate, representing *ad libitum* food sources to some extent. Particularly for intruders, it should be advantageous to rapidly exploit an unguarded food patch at high rates until the residents defend their territory. Workers of the territorial ant *Camponotus floridanus* visit the

nectaries frequently and in a systematic way, and thereby depress the mean standing crop per nectary (Dreisig, 2000). This reduces the gains of randomly visiting intruders. For that purpose, residents would need fewer individuals if one could collect liquid at high intake rates. Therefore, both strategies of nectar-feeding ants take advantage of high maximum fluid intake rates.

Sugar concentration of natural liquid food sources varies considerably (e.g. extrafloral nectaries of *Ochroma pyramidale*: 64%, O´Dowd, 1979; *Urena lobata*: 18.5%, Dreisig, 2000). Calculating the energy intake rates (mg sucrose per unit time), licking is more advantageous at higher sugar concentrations, whereas sucking provides a higher energy intake rate at lower concentrations (cf. *C. rufipes* and *P. villosa* in Fig. 5.4a). This suggests that nectar-feeding ant species employing licking techniques may be more efficient in exploiting nectar sources of higher concentration. Whether they indeed prefer more concentrated nectar in nature is an issue that remains to be investigated. I conclude that fluid intake rate of ants depends on sugar concentration and viscosity, as well as on feeding technique and on the extent of specialization on collecting liquid food.

## B. THE LABIOMAXILLARY COMPLEX

## 6. Liquid food intake in ants: II. Underlying mechanisms

## Introduction

The variety of insect mouthparts result in feeding organs capable of ingesting solid, particulate, or liquid food in many different ways (Labandeira, 1997). Despite their diversity, fluid-feeding insects use generally two basic techniques of food intake, either licking or sucking, which is reflected in specific mouthpart adaptations, respectively. The mouthparts of sucking species usually form a more or less elongated food canal that functions as a sucking tube (e.g. Lepidoptera: Krenn, 1990; Diptera: Szucsich and Krenn, 2000; Hemiptera: Labandeira and Phillips, 1996; Jervis and Vilhelmsen, 2000). On the other hand, licking behavior correlates with hairy tongues or analogous structures to increase capillary forces for loading the tongue with fluid food (e.g. bees: Snodgrass, 1956; Harder, 1983; Michener and Brooks, 1984; Kingsolver and Daniel, 1995). The two fundamental techniques of liquid food intake correspond to two basic types of anatomical design, at which homologous organs could be differently shaped.

Although workers of some ant species frequently feed on liquid food sources, ant mouthparts are not exclusively adapted to ingest liquids. Their large sturdy mandibles are capable of catching elusive prey, cutting tough leaves, carrying nestmates, or caring for tender brood (Hölldobler and Wilson, 1990; Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul, 2001). Their labiomaxillary complex is suited to ingest solid or particulate as well as fluid food (Gotwald, 1969; Paul et al., 2001). During liquid feeding, ants are able to employ both licking and sucking. But for collecting nectar at a foraging bout, workers of different ant species evidently use always one of them, either licking or sucking (see chapter B.5.). Depending on feeding technique and on the extent of specialization on fluid food sources, different ant species collected liquids at different intake rates. The present study shows that both feeding technique and the extent of specialization on foraging for fluid food are reflected in specific adaptations of the labiomaxillary complex among ants. The aim of this study was to elucidate some of the underlying mechanisms that determine the performance of liquid food intake.

I compared four ant species in terms of glossa surface characteristics and the relative volumes of the muscles that control the process of licking and sucking. Workers of the first two species, *Camponotus rufipes* and *Pachycondyla villosa*, feed on liquids at high intake rates. In contrast, the leaf-cutting ant *Atta sexdens* and the predatory ant *Rhytidoponera impressa* are not specialized on foraging for liquid food and collected liquids at much lower intake rates (see chapter B.5.). For interspecific comparison, I measured the muscle volumes and the glossa surface characteristics of differently sized individuals belonging to the two

polymorphic species (*C. rufipes* and *A. sexdens*), and used the calculated linear regressions for scaling the variables to the worker size of the two non-polymorphic species (*P. villosa* and *R. impressa*), respectively. I was thus able to compare the four species with each other, and correlated relative muscle volumes and glossa surface characteristics with species-specific feeding habits.

## Materials and methods

Measurements were done on workers of the following ant species: *Pachycondyla villosa*, *Rhytidoponera impressa*-complex (Ponerinae), *Atta sexdens* (Myrmicinae), and *Camponotus rufipes* (Formicinae). The ant colonies were kept in plaster-of-Paris nests under a 12h:12h L:D cycle, at 25°C and 50% relative humidity. They were fed chopped cockroaches, crickets or wingless *Drosophila*, and honey-water (30%) or fresh leaves (*Atta sexdens*).

### Scanning electron microscopy

For analysis of the glossa surface, ants were anesthetized with Enfluran (Abbot Ethrane) and, after protraction of the labiomaxillary complex, decapitated. The heads were then fixated in buffered 2.5% glutaraldehyde solution, dehydrated, and put into acetone. Employing critical point drying (Balzers Union, BAL-TEC CPD 030), acetone was exchanged for carbon dioxide and the heads were dried. After sputtering the heads with a 30 nm layer of gold (Balzers Union, BAL-TEC SCD 005), the labiomaxillary complex was viewed using a scanning electron microscope (Zeiss, DSM 962).

### Light microscopy

To produce preparations for volume measurements and to analyze the muscles that are involved in the process of licking and sucking, ants were anesthetized with Enfluran (Abbot Ethrane) and decapitated. After fixation (buffered 4% formaldehyde or 2.5% glutaraldehyde, or ammoniacal ethanol), the heads were stained either with Methylene Blue or with osmium/ethylgallate, or silver-impregnated (according to Gronenberg et al., 1997). The heads were then dehydrated, embedded in Fluka Durcupan, and horizontally, sagittally, or vertically sectioned at 10-15 µm. The histological preparations were used for light microscopy (Zeiss Axiophot).

**Morphometry**

Glossa surface parameters were measured from calibrated scanning-electron-microscopic images. I defined the surface area of the glossa that could be seen in frontal view as the 'frontal surface area of the glossa'. For measurements of this area, the respective images were videorecorded and digitally evaluated with a computer as done for volume measurements (see below). To determine the density of the shovels on the glossa surface, I counted the number of shovels in a given area at least at three different positions of the glossa, ventrally, centrally, and dorsally. I defined the mean number of shovels per unit glossa surface area as 'shovel density of the glossa'. To ascertain the size of the shovels, I measured the maximum and the minimum width of a single shovel and its length. The product of the mean width and the length was defined as 'shovel area' or 'shovel size'.

The schematic drawing of an ant worker head (*Pachycondyla villosa*) was made from light-microscopic images using a calibrated camera lucida attachment to the microscope (Zeiss Axiophot). For volume measurements, light-microscopic images were videorecorded and digitally evaluated with a computer equipped with a video card (Screen Machine, Fast Electronic) and appropriate software (courtesy of Reinhard Wolf). From each microscopic slide, the outlines of the respective structures were traced on the computer screen and the areas computed. Volumes were calculated considering the section thickness. I determined the volumes of the head capsule and of several pharynx and labial muscles, respectively.

For interspecific comparison, I plotted relative muscle volumes and glossa surface characteristics against the corresponding head size of individual workers of the two polymorphic species (*C. rufipes*, *A. sexdens*). Linear regressions revealed scaling equations for the respective parameter and species. Using these equations, I could calculate the parameters for any head size within a particular range. The head sizes of the two non-polymorphic species (*P. villosa*, *R. impressa*) lay within the measured range of head sizes of the two polymorphic species. Morphological parameters could thus be compared across species independently of worker size. All error bars of scaled values represent mean deviations of measured values from the corresponding values calculated by the equations of the linear regressions. I used t-tests for statistics, because I did not have several measured values of a parameter for a given species but one scaled value with its deviation.

## Results

### Licking and sucking

Although ants are able to employ licking as well as sucking, workers of different ant species always use one technique for collecting fluids at *ad libitum* food sources. Workers of *Pachycondyla villosa* and *Rhytidoponera impressa*-complex licked sugar solution, whereas workers of *Camponotus rufipes* and *Atta sexdens* sucked it (see chapter B.5.). The glossa, the distal end of the labium, is the first body part that is involved in both licking and sucking. On its surface, the glossa is equipped with small shovel-like structures that are regularly arranged in parallel rows (Fig. 6.1a-c). These rows of shovels can be found on the glossa surface of workers of all ant species examined. This surface structure enables the adhesion of fluids as well as of solid food particles. Moreover, the shovels enlarge the surface of the glossa considerably and therefore contribute to larger loads of food the glossa can handle.

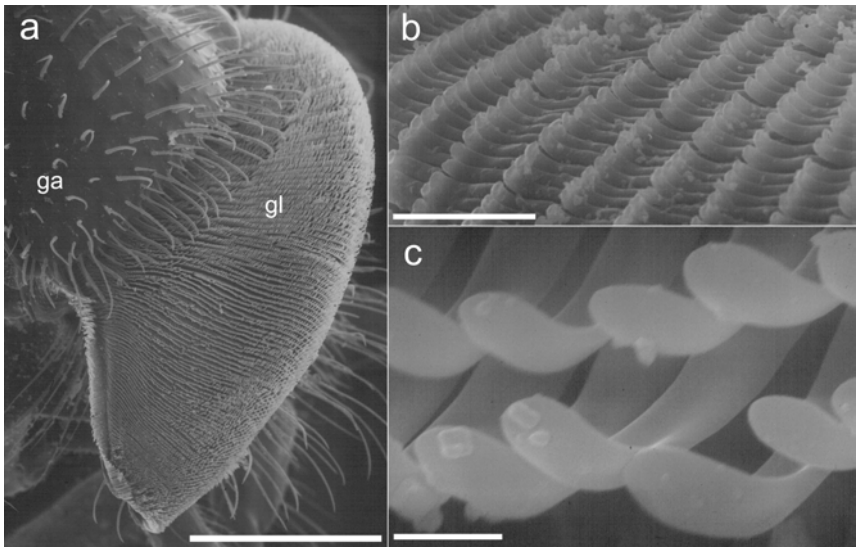



Fig. 6.1: Scanning electron microscopic images of the glossa. (**a**) Lateral view of the glossa and the distal part of one galea (worker of *Camponotus rufipes*; (ga) galea, (gl) glossa; scale bar = 200 µm). (**b**) Oblique view of the glossa surface with parallel rows of cuticular shovels (worker of *Atta sexdens*; scale bar = 10 µm). (**c**) Cuticular shovels on the glossa surface (worker of *Camponotus rufipes*; scale bar = 2 µm).

The whole glossa is retracted upon contraction of the glossa muscle and the paraglossa muscle (8 and 9 in Fig. 6.2). Both muscles work as glossa retractors, whereas the protraction is based on an elastic mechanism. When the glossa muscle and the paraglossa muscle relax, the glossa protracts elastically and automatically (Paul et al., 2001). During licking, the glossa works as an up- and downwards moving shovel. This glossa movement is supported by a synchronous movement of the entire labium, which probably results from alternate contractions of the labial opener and closer (6 and 7 in Fig. 6.2). Accordingly, four different labial muscles contribute to the licking process, the labial opener and closer as well as the glossa and the paraglossa muscle.

For sucking, the labial muscles are not activated, because the glossa functions as a passive duct then and is hold motionless in a protracted position. During sucking, the pharynx dilator muscles (1 in Fig. 6.2) contract periodically, thus producing and maintaining an underpressure within the pharynx (note that Figure 6.2 shows a worker of *P. villosa* licking fluids at *ad libitum* food sources; the basic anatomical design of the involved structures is same among ants independently of the utilized feeding technique). Driven by this underpressure, the fluid is transported over glossa and hypopharynx to the actual mouth of the ant, at which the inner ventral sides of the closed mandibles, the upper side of the labium, and the inner sides of the maxillae usually form the functional sucking tube. Capillary tension ensures a continuous supply of the fluid. The periodic activity of the pharyngeal pump probably caused the slight rhythmic pulsing of the labiomaxillary complex that could be seen during sucking (cf. chapter B.5.). Therefore, the sucking process is mainly performed by the pharynx dilator muscles. In order to enable sucking, the retractor of the buccal tube (5 in Fig. 6.2) controls the proper position of the mouth.

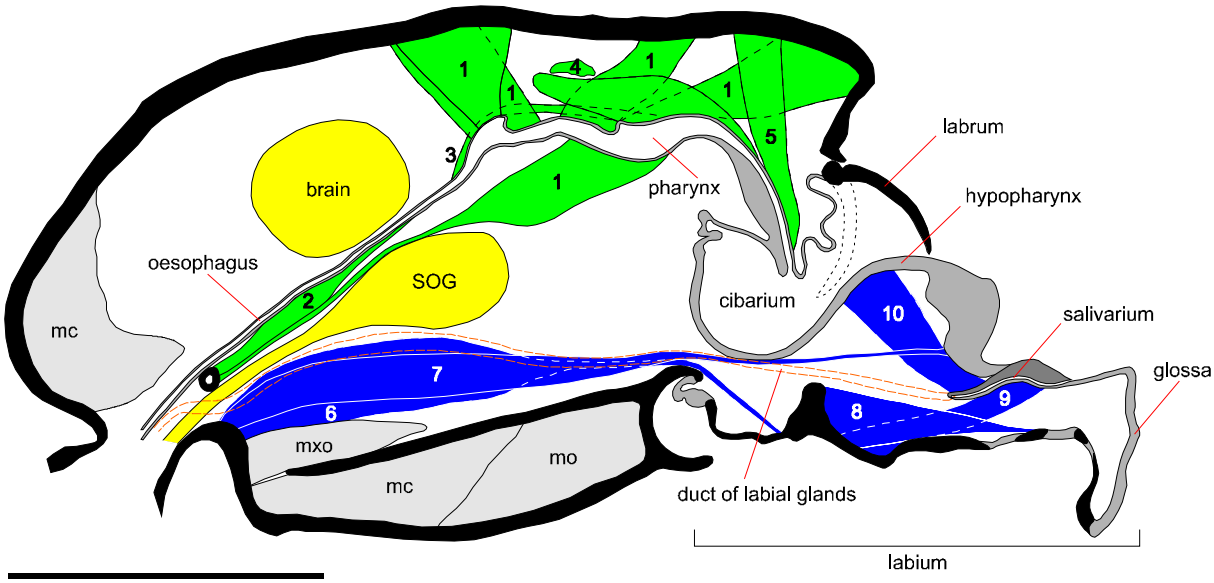



Fig. 6.2: Schematic drawing of a worker head of *Pachycondyla villosa* (several sagittal sections at the midline of two different preparations are layered). Yellow: brain and suboesophageal ganglion (SOG). Green: pharynx muscles; (1) pharynx dilator, (2) pharynx retractor, (3) pharynx longitudinal muscle, (4) pharynx transversal adductor, (5) retractor of buccal tube. Blue: labial muscles; (6) labial opener, (7) labial closer, (8) glossa muscle, (9) paraglossa muscle, (10) hypopharynx muscle. Black: sclerotized head capsule or tentorium. Grey: not or only slightly sclerotized cuticular structures, or mandible and maxillary muscles: (mc) mandible closer, (mo) mandible opener, (mxo) maxillary opener; scale bar = 1 mm.

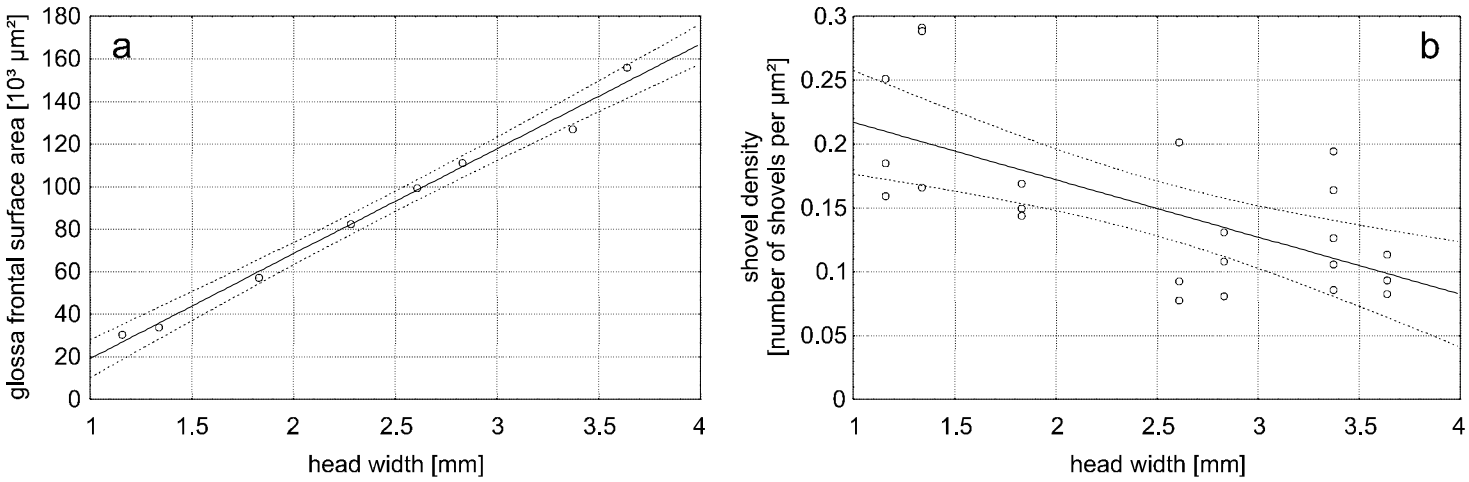



Fig. 6.3: Examples of correlations between glossa surface parameters (ordinate) and head width (abscissa) of workers of *Atta sexdens*. (**a**) Frontal surface area of the glossa [10³ µm²] ($r = 0.9945$; $p < 0.0001$; $N = 8$). (**b**) Shovel density [number of shovels per µm² surface area] ($r = -0.6596$; $p < 0.001$; $n = 23$; $N = 7$).

**Glossa surface**

I measured three glossa surface characteristics (frontal surface area, shovel density, shovel size; cf. Fig. 6.1a-c) of individual workers of the two polymorphic species (*Atta sexdens* and *Camponotus rufipes*). The respective values varied considerably among workers of different size within the same species. The frontal surface area of the glossa increased, whereas the shovel density decreased with increasing head width (Fig. 6.3a, b). The size of the shovels was larger in large individuals, as it was the case for the frontal surface area. These three correlations held for both polymorphic species (Tab. 6.1; slope $b > 0$ for shovel size and frontal surface area, $b < 0$ for shovel density). These findings demonstrate that the size of an ant is crucial for comparing glossa parameters. For interspecific comparison, I therefore used the equations of the linear regressions (Tab. 6.1) for scaling glossa parameters to the respective worker size of the two non-polymorphic species (mean head width of workers of *Rhytidoponera impressa*-complex: 1.29 mm, and of *Pachycondyla villosa*: 2.37 mm; see Table 6.1, right two columns).

The values of the three glossa parameters did not only depend on worker size but also on the species. In *Pachycondyla villosa*, the shovels on the glossa surface were more than three times as large as in *Camponotus rufipes* and *Atta sexdens* (Fig. 6.4a). The opposite was true for the shovel density, which was much higher in the two polymorphic species (Fig. 6.4b). Putting these two parameters together, I defined the 'factor of surface enlargement' as the product of shovel size and shovel density. This factor was largest in *Pachycondyla*

*villosa*, and smallest in *Atta sexdens* (Fig. 6.4d). The third glossa parameter I measured was the frontal surface area. In *Camponotus* and *Atta*, the frontal surface area was equal, whereas it was much larger in *Pachycondyla* (Fig. 6.4c). To have an idea of the amount of food the glossa could load, i.e. the capacity of the glossa, I defined the 'active surface area' of the glossa as the product of shovel size, shovel density, and frontal surface area. Similar to the factor of surface enlargement, the active surface area was largest in *Pachycondyla villosa* (0.44 mm²), medium in *Camponotus rufipes* (0.25 mm²), and smallest in *Atta sexdens* (0.17 mm²; Fig. 6.4e). The values of the active surface area for all the three species were significantly different from each other (t-tests, $p < 0.02$, see legend of Fig. 6.4e for details).

| parameter (= y) | species | a | b | r | p | n | N | y for x = 1.29 mm | y for x = 2.37 mm |
|---|---|---|---|---|---|---|---|---|---|
| shovel size [µm²] | *A. sexdens* | 9.306 | 1.505 | 0.654 | < 0.0001 | 69 | 7 | 11.248 | 12.874 |
| | *C. rufipes* | 17.104 | 1.583 | 0.371 | 0.0121 | 45 | 5 | 19.146 | 20.856 |
| shovel density [per µm²] | *A. sexdens* | 0.262 | -0.045 | -0.660 | 0.0006 | 23 | 7 | 0.204 | 0.156 |
| | *C. rufipes* | 0.285 | -0.065 | -0.615 | 0.0147 | 15 | 5 | 0.201 | 0.130 |
| frontal surface area [10³ µm²] | *A. sexdens* | -30.218 | 49.338 | 0.995 | < 0.0001 | 8 | 8 | 33.427 | 86.712 |
| | *C. rufipes* | -15.822 | 45.935 | 0.942 | 0.0168 | 5 | 5 | 43.434 | 93.043 |

Table 6.1: Correlations between glossa surface parameters (y) and head width (x) of workers for the two polymorphic species *Camponotus rufipes* and *Atta sexdens*. I used the equations of the linear regressions (y = a + b * x) for scaling glossa surface parameters. r = correlation coefficient; p = probability of error; n = number of measured shovels (shovel size), units of glossa surface area (shovel density), glossae (frontal surface area), respectively; N = number of measured individuals; y = glossa surface parameter; x = head width [mm]; x-range of *Camponotus rufipes*: 1.1 - 3.6 mm; x-range of *Atta sexdens*: 1.1 - 4.2 mm.

Scaled to the head width of *Rhytidoponera* workers, the size of the shovels on the glossa surface was same in *Camponotus* and *Rhytidoponera*, but much smaller in *Atta* (Fig. 6.5a). In contrast, the shovel density was equal in all three species, although it tended to be smaller in *Rhytidoponera* (Fig. 6.5b). The resulting factor of surface enlargement was thus largest in *Camponotus*, and smallest in *Atta*. The value for *Rhytidoponera* lay in between and did not differ significantly (Fig. 6.5d). The glossa of workers of *Atta sexdens* featured the smallest frontal surface area, whereas in *Camponotus*, it was as large as in *Rhytidoponera* (Fig. 6.5c). Again, I calculated the active surface area of the glossa, considering all the three glossa parameters that were measured. The active surface area was larger in *Camponotus rufipes* (0.16 mm²) and *Rhytidoponera impressa* (0.13 mm²) than in *Atta sexdens* (0.08 mm²; Fig. 6.5e). The values were significantly different between *Atta* and *Camponotus*, and

between *Atta* and *Rhytidoponera* (t-tests, $p < 0.001$, see legend of Fig. 6.5e for details). Considering the ratio of the active surface areas between *Pachycondyla* and *Camponotus* at a head width of 2.37 mm (cf. Fig. 6.4e), the calculated value for *Pachycondyla villosa* at a fictitious head width of 1.29 mm would approximately be 0.27 mm² and therefore largest among the four species examined. At first sight, the species-specific differences of glossa surface parameters may seem puzzling. But I will later see that they perfectly correlate with species-specific feeding habits.

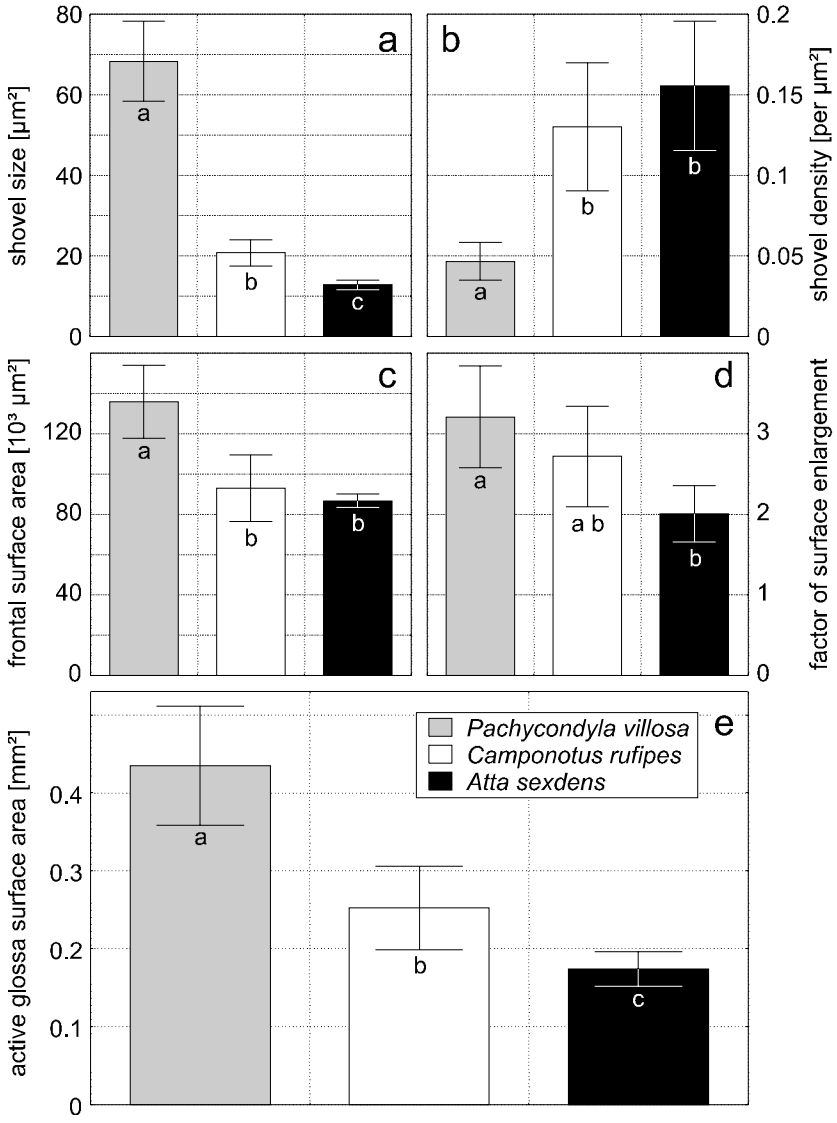



Fig. 6.4: Comparison of glossa parameters (ordinates) between workers of *Pachycondyla villosa* (light grey), *Camponotus rufipes* (white), and *Atta sexdens* (black). For the two polymorphic species, all values are scaled to the head size of workers of *Pachycondyla villosa* (mean head width = 2.37 ± 0.07 mm), and all error bars represent mean standard deviations from the respective values calculated by the equations of the linear regressions (see Tab. 6.1 for n- and N-numbers). Statistically significant differences are expressed by different letters. (**a**) Shovel size [µm²] (mean ± standard deviation, n = 25 shovels, N = 4 individuals for *P. villosa*) (t-tests, *Pachy.*/*Campo.*: $t_7 = 9.265$, $p < 0.0001$; *Pachy.*/*Atta*: $t_9 = 11.232$, $p < 0.0001$; *Campo.*/*Atta*: $t_{10} = 5.256$, $p < 0.001$). (**b**) Shovel density [number of shovels per µm² glossa surface area] (mean ± st. dev., n = 10 area units, N = 4 individuals for *P. villosa*) (t-tests, *Pachy.*/*Campo.*: $t_7 = 4.470$, $p < 0.01$; *Pachy.*/*Atta*: $t_9 = 6.707$, $p < 0.0001$; *Campo.*/*Atta*: $t_{10} = 1.097$, $p > 0.05$). (**c**) Frontal surface area of the glossa [10³ µm²] (mean ± st. dev., N = 4 individuals for *P. villosa*) (t-tests, *Pachy.*/*Campo.*: $t_7 = 3.660$, $p < 0.01$; *Pachy.*/*Atta*: $t_{10} = 5.373$, $p < 0.001$; *Campo.*/*Atta*: $t_{11} = 0.827$, $p > 0.05$). (**d**) Factor of surface enlargement = shovel size * shovel density (t-tests, *Pachy.*/*Campo.*: $t_7 = 1.160$, $p > 0.05$; *Pachy.*/*Atta*: $t_9 = 3.508$, $p < 0.01$; *Campo.*/*Atta*: $t_{10} = 2.297$, $p < 0.05$). (**e**) Active surface area of the glossa [mm²] = shovel size * shovel density * frontal surface area (t-tests, *Pachy.*/*Campo.*: $t_7 = 4.052$, $p < 0.01$; *Pachy.*/*Atta*: $t_9 = 6.679$, $p < 0.0001$; *Campo.*/*Atta*: $t_{10} = 3.079$, $p < 0.02$).

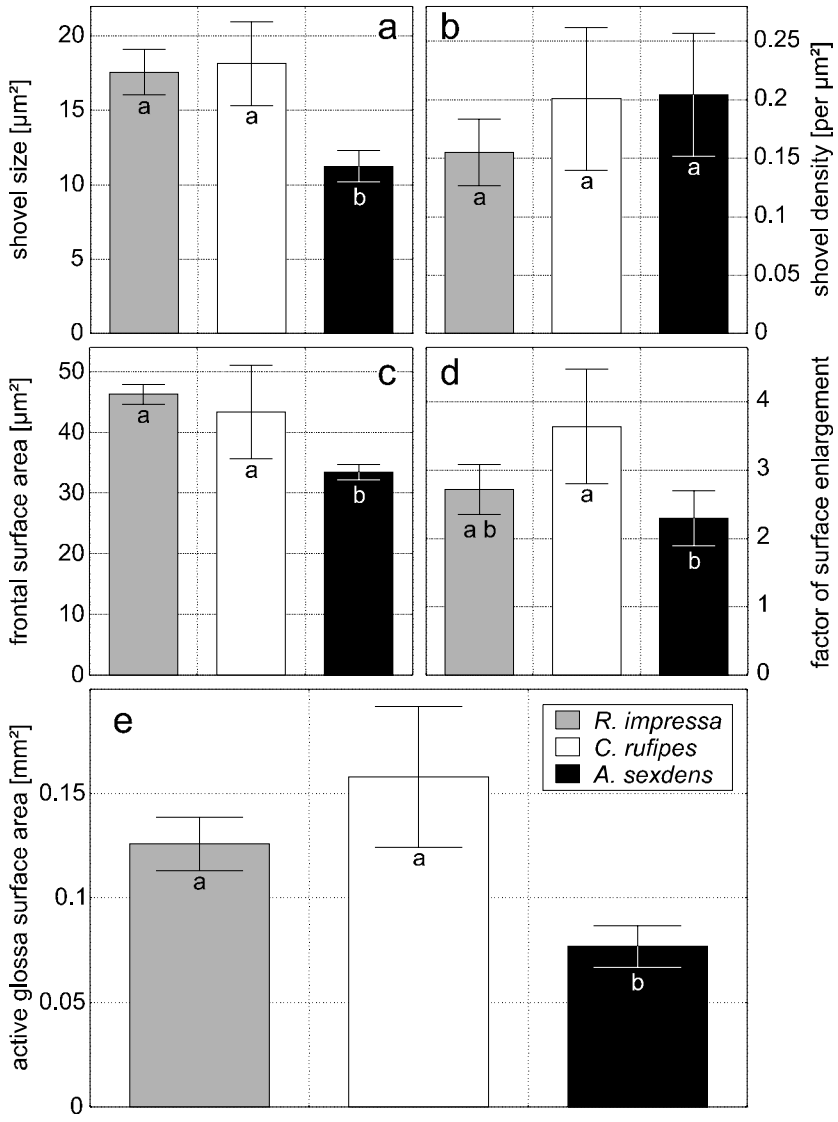



Fig. 6.5: Comparison of glossa parameters (ordinates) between workers of *Rhytidoponera impressa*-complex (dark grey), *Camponotus rufipes* (white), and *Atta sexdens* (black). For the two polymorphic species, all values (± standard deviation, as in Fig. 6.4) are scaled to the head size of workers of *Rhytidoponera impressa*-complex (mean head width = 1.29 ± 0.06 mm; cf. Tab. 6.1). Statistically significant differences are expressed by different letters. (**a**) Shovel size [µm²] (mean ± st. dev., n = 29 shovels, N = 5 individuals for *R. impressa*) (t-tests, *Rhytido./Campo.*: $t_8$ = 0.404, p > 0.05; *Rhytido./Atta*: $t_{10}$ = 8.017, p < 0.0001; *Campo./Atta*: $t_{10}$ = 5.219, p < 0.001). (**b**) Shovel density [number of shovels per µm²] (mean ± st. dev., n = 10 area units, N = 5 individuals for *R. impressa*) (t-tests, *Rhytido./Campo.*: $t_8$ = 1.524, p > 0.05; *Rhytido./Atta*: $t_{10}$ = 2.093, p > 0.05; *Campo./Atta*: $t_{10}$ = 0.102, p > 0.05). (**c**) Frontal surface area [10³ µm²] (mean ± st. dev., N = 5 individuals for *R. impressa*) (t-tests, *Rhytido./Campo.*: $t_8$ = 0.832, p > 0.05; *Rhytido./Atta*: $t_{11}$ = 14.969, p < 0.0001; *Campo./Atta*: $t_{11}$ = 2.834, p < 0.02). (**d**) Factor of surface enlargement (t-tests, *Rhytido./Campo.*: $t_8$ = 2.256, p > 0.05 ; *Rhytido./Atta*: $t_{10}$ = 1.888, p > 0.05; *Campo./Atta*: $t_{10}$ = 3.330, p < 0.01). (**e**) Active surface area of the glossa [mm²] (t-tests, *Rhytido./Campo.*: $t_8$ = 1.989, p > 0.05; *Rhytido./Atta*: $t_{10}$ = 7.151, p < 0.0001; *Campo./Atta*: $t_{10}$ = 5.234, p < 0.001).

**Muscle volumes**

I determined the volumes of the muscles that are involved in the process of licking and sucking in relation to the head capsule volume of the respective worker ant. As the measured glossa characteristics, the relative muscle volumes varied considerably among workers of different size within the same species. In both polymorphic species and over all examined labial and pharynx muscles, the relative muscle volume decreased with increasing head capsule volume (e.g. Fig. 6.6a, b), although the muscle volumes increased in absolute

numbers. This was the case because in larger ants, the mandible closer muscle becomes relatively larger (Paul, 2001). The correlations between relative muscle volume and head capsule volume are shown in Table 6.2 for the two polymorphic species *Atta sexdens* and *Camponotus rufipes*. Plotting the relative muscle volume (y) against the head capsule volume (x), the slope (b) of the respective linear regression was negative for all measured labial and pharynx muscles (logarithmic scale of y- and x-axis; cf. Fig. 6.6a, b). Accordingly, the relative volume of any of the measured muscles was larger in smaller ants. This illustrates again that the size of an ant is important for interspecific comparison. Thus, I used the equations of the linear regressions (Tab. 6.2) for scaling relative muscle volumes to the respective worker size of the two non-polymorphic species (mean head capsule volume of workers of *Rhytidoponera impressa*-complex: 0.86 mm³, and of *Pachycondyla villosa*: 4.01 mm³; see Table 6.2, right two columns).

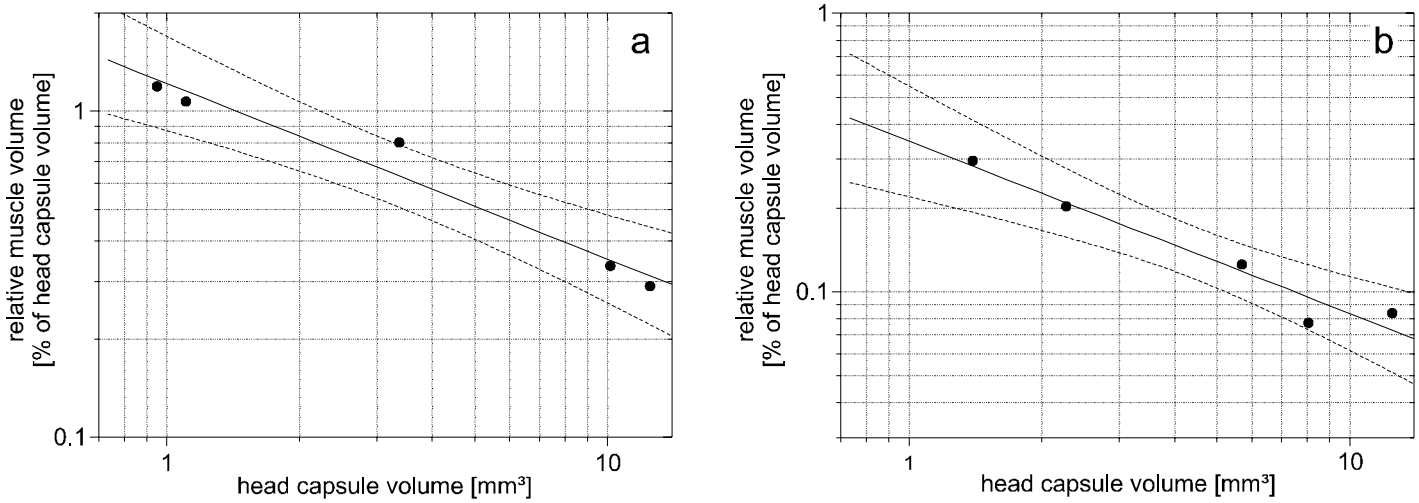



Fig. 6.6: Examples of correlations between relative muscle volumes (ordinate, logarithmic scale) and head capsule volume (abscissa, logarithmic scale) of workers of *Camponotus rufipes*. (**a**) Volume of labial opener in relation to head capsule volume (r = -0.9793; p = 0.0036; N = 5). (**b**) Volume of paraglossa muscle in relation to head capsule volume (r = -0.9733; p = 0.0052; N = 5).

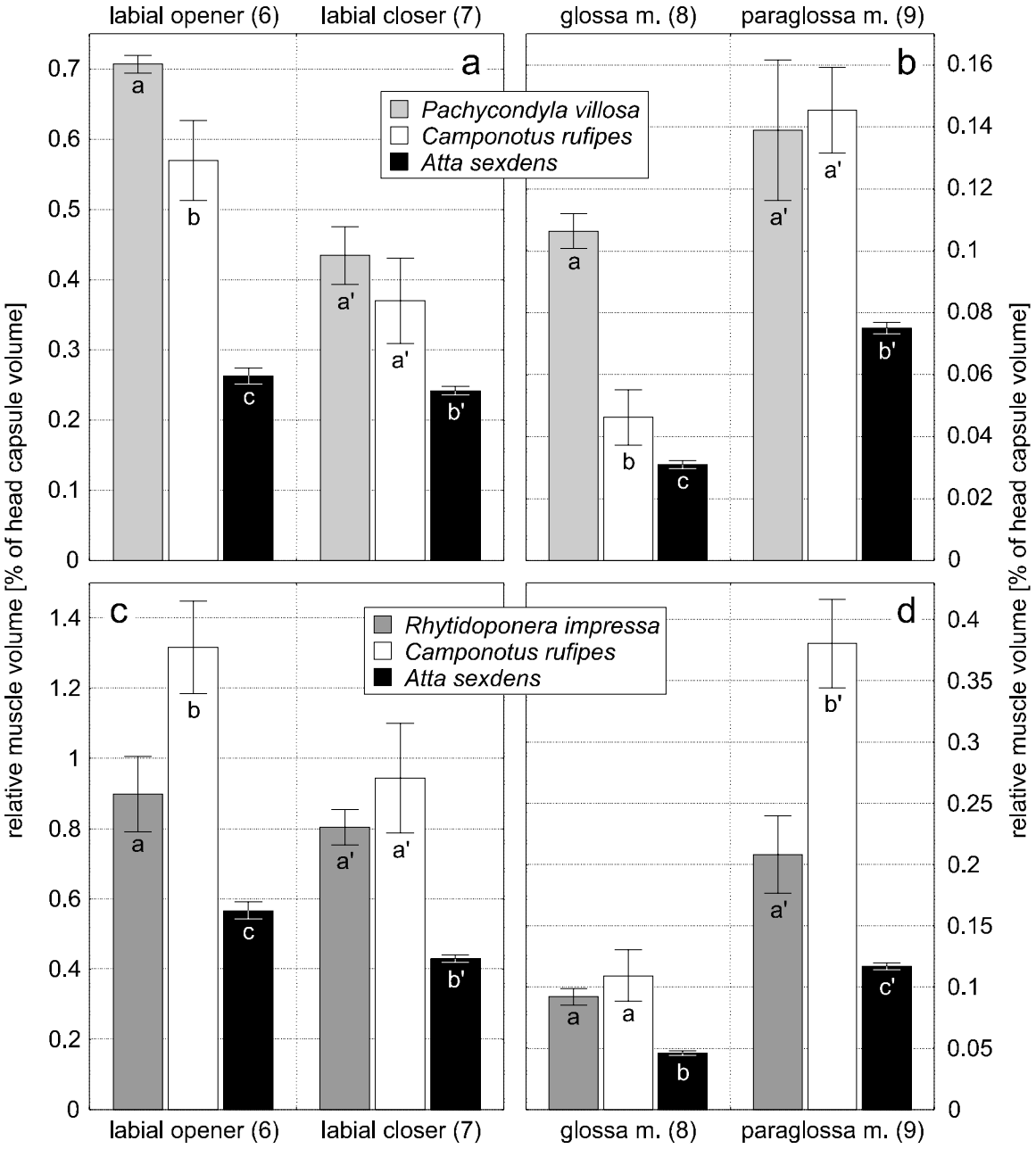
The relative volumes of the various muscles differed among the four species. Scaled to the head size of workers of *Pachycondyla villosa*, the relative volumes of the four labial muscles of *Camponotus rufipes* workers were smaller or equal compared to *Pachycondyla villosa*, but larger than in *Atta sexdens* (Fig. 6.7a, b). All values of relative volumes of labial muscles were significantly different from each other (t-tests, $p < 0.01$, see legend of Fig. 6.7 for details), except for the labial closer and the paraglossa muscle between *Camponotus* and *Pachycondyla*, respectively. I found the greatest difference between *Camponotus* and

*Pachycondyla* for the glossa muscle (Fig. 6.7b, left side), which is probably most important for the process of licking (cf. Paul et al., 2001). At the head capsule volume of workers of *Rhytidoponera*, labial muscle volumes were largest in *Camponotus*, and smallest in *Atta* (Fig. 6.7c, d). Labial muscle volumes of *Rhytidoponera* workers lay in between. All values of labial muscle volumes were again significantly different from each other (t-tests, $p < 0.002$, see legend of Fig. 6.7 for details), except for the labial closer and the glossa muscle between *Camponotus* and *Rhytidoponera*, respectively.

| muscle | species | a | b | r | p | N | y for x = 0.86 mm³ | y for x = 4.01 mm³ |
|---|---|---|---|---|---|---|---|---|
| pharynx dilators (1) | *A. sexdens* | 0.308 | -0.452 | -0.984 | 0.0025 | 5 | 2.173 | 1.085 |
| | *C. rufipes* | 0.482 | -0.467 | -0.948 | 0.0142 | 5 | 3.247 | 1.584 |
| retractor of buccal tube (5) | *A. sexdens* | -1.295 | -0.254 | -0.962 | 0.0088 | 5 | 0.053 | 0.036 |
| | *C. rufipes* | -0.994 | -0.556 | -0.907 | 0.0336 | 5 | 0.110 | 0.047 |
| labial opener (6) | *A. sexdens* | -0.279 | -0.499 | -0.996 | 0.0037 | 4 | 0.566 | 0.263 |
| | *C. rufipes* | 0.085 | -0.538 | -0.979 | 0.0036 | 5 | 1.316 | 0.575 |
| labial closer (7) | *A. sexdens* | -0.391 | -0.372 | -0.998 | 0.0022 | 4 | 0.429 | 0.242 |
| | *C. rufipes* | -0.064 | -0.602 | -0.956 | 0.0110 | 5 | 0.944 | 0.374 |
| glossa muscle (8) | *A. sexdens* | -1.354 | -0.255 | -0.984 | 0.0024 | 5 | 0.046 | 0.031 |
| | *C. rufipes* | -0.997 | -0.554 | -0.892 | 0.0418 | 5 | 0.109 | 0.047 |
| paraglossa muscle (9) | *A. sexdens* | -0.951 | -0.288 | -0.994 | 0.0006 | 5 | 0.117 | 0.075 |
| | *C. rufipes* | -0.459 | -0.618 | -0.973 | 0.0052 | 5 | 0.380 | 0.147 |

Table 6.2: Correlations between relative muscle volumes (y) and head capsule volume (x) of workers for the two polymorphic species *Camponotus rufipes* and *Atta sexdens*. I used the equations of the linear regressions [$\lg(y) = a + b * \lg(x)$] for scaling relative muscle volumes. r = correlation coefficient; p = probability of error; N = number of measured individuals; y = muscle volume [% of head capsule volume]; x = head capsule volume [mm³].

Fig. 6.7 (opposite above): Comparison of relative volumes (ordinates) of labial muscles between ▶ workers of *Pachycondyla villosa* (light grey), *Rhytidoponera impressa*-complex (dark grey), *Camponotus rufipes* (white), and *Atta sexdens* (black). For the two polymorphic species, all values are scaled to the head size of workers of *Pachycondyla villosa* (mean head capsule volume = 4.01 ± 0.08 mm³; **a**, **b**), and of *Rhytidoponera impressa*-complex (mean head capsule volume = 0.86 ± 0.02 mm³; **c**, **d**). All error bars for the two polymorphic species represent mean standard deviations from the respective values calculated by the equations of the linear regressions (see Tab. 6.2 for N-numbers). For the two non-polymorphic species, values are means ± standard deviation (N = 4 individuals, respectively). (**a**, **c**) Labial opener (6 in Fig. 6.2) and labial closer (7). (**b**, **d**) Glossa muscle (8) and paraglossa muscle (9). Statistically significant differences are expressed by different letters. (**a**) t-tests, labial opener: *Pachy.*/*Campo.*: $t_7 = 5.248$, $p < 0.002$; *Pachy.*/*Atta*: $t_6 = 52.862$, $p < 0.0001$; *Campo.*/*Atta*: $t_7 = 11.785$, $p < 0.0001$; labial closer: *Pachy.*/*Campo.*: $t_7 = 1.880$, $p > 0.05$;




*Pachy./Atta*: $t_6 = 9.240$, $p < 0.0001$; *Campo./Atta*: $t_7 = 4.649$, $p < 0.01$; (**b**) t-tests, glossa muscle: *Pachy./Campo.*: $t_7 = 12.353$, $p < 0.0001$; *Pachy./Atta*: $t_7 = 26.113$, $p < 0.0001$; *Campo./Atta*: $t_8 = 3.777$, $p < 0.01$; paraglossa muscle: *Pachy./Campo.*: $t_7 = 0.501$, $p > 0.05$; *Pachy./Atta*: $t_7 = 5.634$, $p < 0.001$; *Campo./Atta*: $t_8 = 11.359$, $p < 0.0001$; (**c**) t-tests, labial opener: *Rhytido./Campo.*: $t_7 = 5.249$, $p < 0.002$; *Rhytido./Atta*: $t_6 = 6.023$, $p < 0.001$; *Campo./Atta*: $t_7 = 12.508$, $p < 0.0001$; labial closer: *Rhytido./Campo.*: $t_7 = 1.881$, $p > 0.005$; *Rhytido./Atta*: $t_6 = 14.737$, $p < 0.0001$; *Campo./Atta*: $t_7 = 7.356$, $p < 0.001$; (**d**) t-tests, glossa muscle: *Rhytido./Campo.*: $t_7 = 1.737$, $p > 0.05$; *Rhytido./Atta*: $t_7 = 13.151$, $p < 0.0001$; *Campo./Atta*: $t_8 = 6.715$, $p < 0.001$; paraglossa muscle: *Rhytido./Campo.*: $t_7 = 7.635$, $p < 0.001$; *Rhytido./Atta*: $t_7 = 5.744$, $p < 0.001$; *Campo./Atta*: $t_8 = 16.360$, $p < 0.0001$.

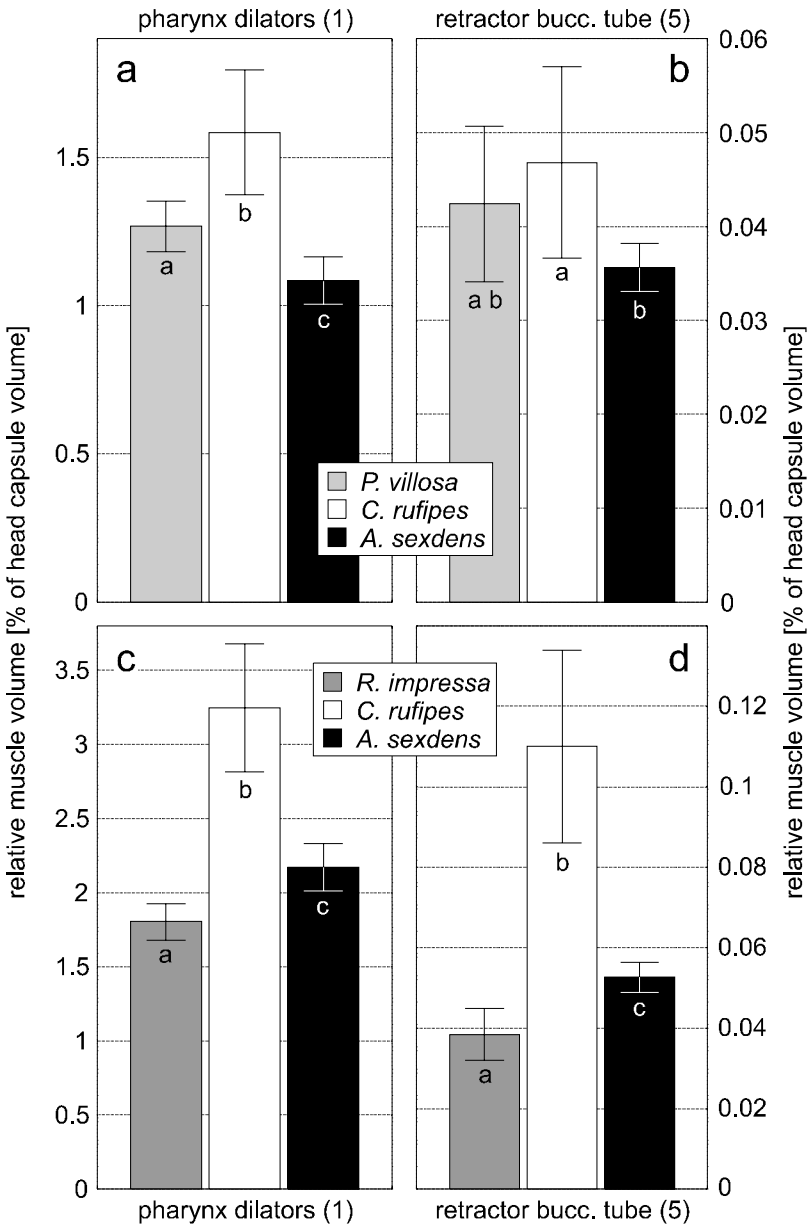



Fig. 6.8: Comparison of relative volumes (ordinates) of pharynx muscles between workers of *Pachycondyla villosa* (light grey), *Rhytidoponera impressa*-complex (dark grey), *Camponotus rufipes* (white), and *Atta sexdens* (black). For the two polymorphic species, all values (± standard deviation, as in Fig. 6.7; see Tab. 6.2 for N-numbers) are scaled to the head size of workers of *Pachycondyla villosa* (mean head capsule volume = 4.01 ± 0.08 mm³; **a**, **b**), and of *Rhytidoponera impressa*-complex (mean head capsule volume = 0.86 ± 0.02 mm³; **c**, **d**). For the two non-polymorphic species, values are means ± standard deviation (N = 4 individuals, respectively). (**a**, **c**) Pharynx dilators (1 in Fig. 6.2). (**b**, **d**) Retractor of the buccal tube (5). Statistically significant differences are expressed by different letters. (**a**) t-tests, *Pachy.*/*Campo.*: $t_7 = 3.068$, $p < 0.02$; *Pachy.*/*Atta*: $t_7 = 3.294$, $p < 0.02$; *Campo.*/*Atta*: $t_8 = 4.970$, $p < 0.002$; (**b**) t-tests, *Pachy.*/*Campo.*: $t_7 = 0.719$, $p > 0.05$; *Pachy.*/*Atta*: $t_7 = 1.579$, $p > 0.05$; *Campo.*/*Atta*: $t_8 = 2.387$, $p < 0.05$; (**c**) t-tests, *Rhytido.*/*Campo.*: $t_7 = 7.137$, $p < 0.001$; *Rhytido.*/*Atta*: $t_7 = 3.892$, $p < 0.01$; *Campo.*/*Atta*: $t_8 = 5.232$, $p < 0.01$; (**d**) t-tests, *Rhytido.*/*Campo.*: $t_7 = 6.404$, $p < 0.001$; *Rhytido.*/*Atta*: $t_7 = 3.883$, $p < 0.01$; *Campo.*/*Atta*: $t_8 = 5.301$, $p < 0.001$.

Looking at the pharynx muscles that control sucking, volume ratios among species were unlike in comparison to the labial muscles that master licking. Scaled to the head size of *Pachycondyla villosa*, the volume of the pharynx dilators as well as of the retractor of the buccal tube was largest in *Camponotus rufipes*, and smallest in *Atta sexdens* (Fig. 6.8a, b). The values differed significantly from each other (t-tests, $p < 0.05$, see legend of Fig. 6.8 for details), except for the retractor of the buccal tube between *Pachycondyla* and *Camponotus*,

and between *Pachycondyla* and *Atta*, respectively. At the head capsule volume of workers of *Rhytidoponera*, workers of *Camponotus rufipes* had considerably larger pharynx muscles than individuals of the other two species (Fig. 6.8c, d). In *Rhytidoponera*, pharynx muscles were smallest. All values of pharynx muscle volumes, scaled to the head size of *Rhytidoponera*, were significantly different from each other (t-test, $p < 0.01$, see legend of Fig. 6.8 for details). To summarize, workers of *Pachycondyla villosa* possessed the most voluminous labial muscles, whereas the pharynx muscles were largest in workers of *Camponotus rufipes*. In *Atta sexdens*, labial muscles were smallest, on the other hand, workers of *Rhytidoponera impressa*-complex had the smallest pharynx muscles.

## Discussion

**Glossa surface**

I found the active glossa surface area to be largest in *Pachycondyla villosa*. It had a similar size in *Rhytidoponera impressa*-complex and *Camponotus rufipes*. And it was smallest in *Atta sexdens* (Fig. 6.4e, 5e). For collecting nectar during a foraging bout, the two ponerine species always licked the offered sugar solution and accumulated it as a drop between their mandibles, whereas the two polymorphic species always sucked it and stored the liquid food within their crop (see chapter B.5.). In order to increase fluid uptake rate during licking, the glossa should be capable of loading as much fluid as possible per single lick. The active surface area of the glossa is thus crucial for maximizing uptake rates during licking, because licking frequency cannot be raised but remains almost constant over the whole range of sucrose concentration (Dasch, 1998; see chapter B.5.; cf. bumblebees: Harder, 1986). In contrast, a large active surface area of the glossa is less important for sucking, since the glossa works only as a passive duct and is deeply hold into the drop of sugar solution while it ensures a continuous flow of fluid (see chapter B.5.). Accordingly, workers of species that lick fluids (*Pachycondyla villosa*, *Rhytidoponera impressa*-complex) depend on and therefore feature large active surface areas of the glossa, whereas workers of those species that suck liquids (*Camponotus rufipes*, *Atta sexdens*) possess smaller active surface areas (Fig. 6.4e, 5e). But why then was the active surface area of the glossa equal in *Rhytidoponera* and *Camponotus*?

A second factor which influences the size of the glossa, is the extent of specialization on collecting liquid food. Workers of *Pachycondyla villosa* and *Camponotus rufipes* are ubiquitous visitors of fluid food sources, reflected by high fluid intake rates (see chapter B.5.). *Rhytidoponera impressa*-complex represents a predatory species, and *Atta sexdens* is a

leaf-cutting ant. Workers of the last two species are not specialized on collecting fluids. So although *Rhytidoponera* workers licked sugar solution, *Camponotus* workers that sucked fluids tended to feature larger active surface areas of the glossa (Fig. 6.5e). They rely much more on high fluid intake rates, and sometimes lick liquids as well, if not a large drop of an *ad libitum* food source is presented (see chapter B.5.). Both the used feeding technique during foraging and the extent of specialization on collecting liquid food therefore determine the size of the active surface area of the glossa.

Considering the fluid intake rates, I found licking to be more advantageous at higher sugar concentrations, whereas sucking provided higher intake rates at lower concentrations (see chapter B.5.). This correlates with shovel size and shovel density of the glossa. Workers of *Pachycondyla* had much larger shovels but a lower shovel density than *Camponotus* workers (Fig. 6.4a, b). Highly concentrated sugar solutions feature high viscosities, corresponding to larger droplets of fluid when splashed. Larger droplets better fit to larger shovels arranged at larger distances between each other, as found in *Pachycondyla villosa*. Thus, large shovels and a low shovel density could reflect an adaptation to high concentrations and viscosities of fluid food. On the other hand, smaller shovels organized at high densities like in *Camponotus rufipes* (Fig. 6.4a, b) seem to better work at lowly concentrated solutions, which were ingested fastest by workers of this species (see chapter B.5.). Furthermore, it is conceivable that small shovels reduce frictional forces of the fluid flow during sucking. In addition to the feeding technique, the extent of specialization on collecting liquid food correlates with shovel size and density as well, which would explain that workers of *Rhytidoponera* and *Camponotus* have the same shovel size (Fig. 6.5a), analogously to the active surface area of the glossa (see above).

The size and the surface structure of the tongue does not only determine the performance of liquid feeding in ants, but also in other nectar-feeding animals such as hummingbirds (Ewald and Williams, 1982; Kingsolver and Daniel, 1983; Tamm and Gass, 1986), bats (Greenbaum and Phillips, 1974; Phillips et al., 1977; Griffiths, 1982), and bees. Among bumblebees, proboscis length was positively correlated with feeding rates (time required to visit a given flower) on flowers with long corolla tubes (Harder, 1982). Conversely, bumblebees with a shorter proboscis did a better job when feeding on short-corolla flowers (Inouye, 1980). In some tropical euglossine bee species, the extremely long glossa (3-4 cm) is considerably less hairy, both in hair density and hair length, than in bumblebees and honeybees (Michener and Brooks, 1984). Reduced hairiness in these euglossines may be important in reducing the resistance to nectar flow during unloading the

long proboscis (Kingsolver and Daniel, 1995). Therefore, tongue morphology is generally substantial to understand the physiological performance of liquid feeding.

**Muscle volumes**

In comparison to the mandible closer (Gronenberg et al., 1997; Paul and Gronenberg, 1999; Paul, 2001), muscle morphology suggests that the muscles of the labiomaxillary complex of ants are less specialized for specific tasks, and feature rather slow than fast muscle characteristics (Paul et al., 2001). Considering the relative volumes of labial and pharynx muscles, I found significant differences among the four examined ant species, which probably reflect adaptations to species-specific feeding habits.

The four currently studied ant species differ in terms of used technique of liquid feeding during foraging and the extent of specialization on collecting fluid food. Workers of *Pachycondyla villosa* possessed the most voluminous labial muscles (Fig. 6.7a, b), corresponding to licking behavior (see chapter B.5.). The labial muscles (6, 7, 8, 9 in Fig. 6.2) control the licking process. Hence, they become more important for ants that lick fluid food, reflected by larger relative muscle volumes. Workers of *Pachycondyla villosa* as well as of *Camponotus rufipes* frequently collect liquids. Since licking workers of *Rhytidoponera* are not specialized on collecting fluid food, they featured smaller labial muscles than, or approximately equal labial muscle volumes as in *Camponotus* workers (Fig. 6.7c, d), although the latter sucked liquids at *ad libitum* food sources, at which labial muscles are inactive. The leaf-cutting ant *Atta sexdens* is neither specialized on collecting liquid food nor licked sugar solution at *ad libitum* food sources. Therefore, workers of this species had the relatively smallest labial muscles that were found (Fig. 6.7a-d).

Conversely, workers of species that sucked the offered sugar solution were characterized by relatively larger pharynx muscles (*Camponotus rufipes*, *Atta sexdens*; Fig. 6.8a-d). The pharynx muscles, particularly the pharynx dilators (1 in Fig. 6.2), are most important for the process of sucking. These muscles occupied thus relatively more head capsule volume in workers of species, that depend on a powerful sucking apparatus. Workers of *Pachycondyla* and *Rhytidoponera* also ingested liquid food by sucking, but only for their individual needs. For collecting fluids during foraging due to the necessities of the whole colony, they licked liquid food and accumulated it externally as a drop between their mandibles (see chapter B.5.). Therefore they did not use the pharyngeal sucking pump when foraging, but only during individual ingestion. Hence, workers of these species featured smaller pharynx muscles (Fig. 6.8a-d). As for the labial muscles, the extent of specialization

on collecting liquid food seems to affect muscle volumes more markedly than the respective feeding technique, which would explain that licking workers of *Pachycondyla* had larger pharynx muscles than sucking ones of *Atta* (Fig. 6.8a, b).

The differences of relative muscle volumes among species suit to the species-specific fluid intake rates measured (cf. chapter B.5.). Large labial and pharynx muscles correspond to high intake rates, whereas small muscle volumes correlate with low intake rates. This suggests that the volume of the involved muscles is another determinant, in addition to glossa surface characteristics, that influences intake rates and therefore the accomplishment of feeding.

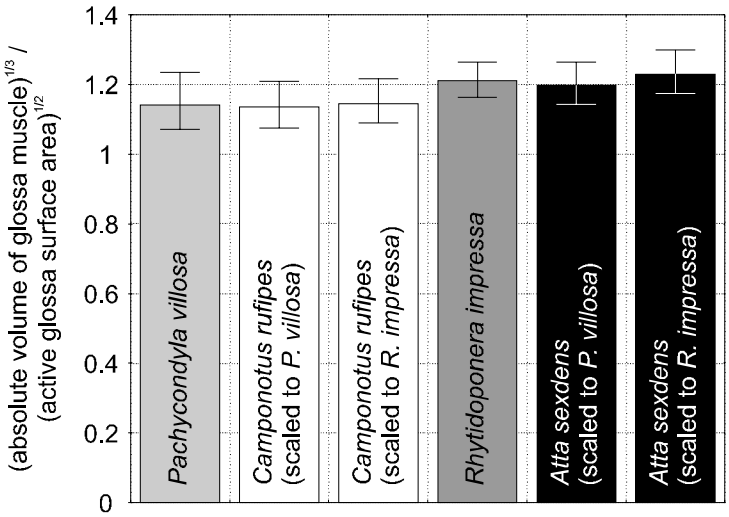



Fig. 6.9: The ratio of glossa muscle volume and glossa surface area is constant among the four examined ant species [(absolute volume of the glossa muscle)$^{1/3}$ / (active surface area of the glossa)$^{1/2}$]. For the two polymorphic species, this ratio is calculated for two different head sizes.

**How much muscle per unit surface area?**

Comparing glossa surface characteristics with relative muscle volumes, it seems that they have an impact on each other. A large active surface area of the glossa is associated with large labial muscles (e.g. *Pachycondyla villosa*), particularly with a relatively voluminous glossa muscle (cf. Fig. 6.7b), which is probably the most important muscle for the licking process (cf. Fig. 6.2; Paul et al. 2001). I thus correlated muscle volume with glossa surface area and asked, how much glossa muscle is necessary per unit surface area?

To answer that question, I divided the absolute volume of the glossa muscle by the corresponding active surface area of the glossa for each species, respectively. In order to consider the difference between volume and surface, I took either the cubic or the square root before. The results of this calculation are shown in Figure 6.9. Interestingly, this ratio is constant over all species examined, independently of utilized feeding technique, extent of

specialization on collecting liquid food, and size of the ant. This demonstrates that a specific volume of glossa muscle corresponds to a specific active surface area of the glossa. Hence, the single components of the whole system seem to be closely adjusted to each other according to a general rule, while the entire system is adapted to distinct requirements in compliance with the respective ecological niche.

To conclude, fluid intake rates as well as glossa surface characteristics and relative volumes of labial and pharynx muscles correlate with both the utilized technique of liquid feeding and the extent of specialization on collecting fluid food. I therefore think that differences among species in terms of glossa surface characteristics and relative muscle volumes reflect adaptations to species-specific feeding habits and determine the performance of liquid feeding.

# THE MOUTHPARTS OF ANTS -

## Summary

**[A.1.]** Ant mandible movements cover a wide range of forces, velocities and precision. The key to the versatility of mandible functions is the mandible closer muscle. In ants, this muscle is generally composed of distinct muscle fiber types that differ in morphology and contractile properties. Fast contracting fibers have short sarcomeres (2-3 µm) and attach directly to the closer apodeme, that conveys the muscle power to the mandible joint. Slow but forceful contracting fibers have long sarcomeres (5-6 µm) and attach to the apodeme either directly or via thin thread-like filaments. Volume proportions of the fiber types are species-specific and correlate with feeding habits. Ants with fast-moving mandibles have a high proportion of fast fibers, whereas workers of species performing forceful mandible movements possess more slow but forceful fibers. Trap-jaw ants feature highly specialized catapult mechanisms. Their mandible closing is known as one of the fastest movements in the animal kingdom.

**[A.2.]** Comparison of different ant species suggests two basic principles underlying the design of the mandible closer muscle: (1) Ants that depend on fast mandible strikes, such as predators, generally feature long heads which contain long fast muscle fibers directly attaching to the apodeme at steep angles. Their muscles comprise only a few filament-attached fibers and they maximize speed of action at the expense of force output. (2) Ants performing particularly forceful mandible movements, such as seed-cracking or leaf-cutting, rely on many short parallel muscle fibers accommodated within a broad head capsule. Their slower muscles incorporate a large proportion of filament-attached fibers. Two biomechanical models explain how the attachment angles are optimized with respect to force and velocity output and how filament-attached fibers help to generate the largest force output from the available head capsule volume.

**[A.3.]** The entire mandible closer muscle is controlled by 10-12 motor neurons, 4-5 of which exclusively supply fast muscle fibers. Slow muscle fibers comprise a posterior and an antero-lateral group, each of which is controlled by 1-2 motor neurons. In addition, 3-4 motor neurons control all muscle fibers together. Simultaneous recordings of muscle activity and mandible movement reveal that fast movements require rapid contractions of fast muscle fibers. Slow and accurate movements result from the activation of slow muscle fibers. Forceful movements are generated by simultaneous co-activation of all muscle fiber types. For fine control, distinct fiber bundles can be activated independently of each other. Retrograde tracing shows that most dendritic arborizations of the different sets of motor neurons share the same neuropil in the suboesophageal ganglion. In addition, fast motor neurons and neurons supplying the lateral group of slow closer muscle fibers each invade

specific parts of the neuropil that is not shared by the other motor neuron groups. Some bilateral overlap between the dendrites of left and right motor neurons exists, particularly in fast motor neurons. The results explain how a single muscle is able to control the different movement parameters required for the proper function of ant mandibles.

**[B.4.]** The labiomaxillary complex of ants is essential for food intake. I investigated the anatomical design of the labiomaxillary complex in various ant species focusing on movement mechanisms. Six labial and six maxillary muscles with different functions control the several joints and ensure the appropriate performance of the labiomaxillary complex. According to measurements of sarcomere lengths, muscle fiber lengths and diameters, the labial and maxillary muscles feature rather slow than fast muscle characteristics, and do not seem to be specialized for specific tasks. Since glossa protractor muscles are absent, the protraction of the glossa, the distal end of the labium, is a non muscular movement. By histological measurements of hemolymph volumes a pressure-driven mechanism could be excluded. Additional experiments showed that, upon relaxation of the glossa retractor muscles, the glossa protracts elastically. This elastic mechanism possibly has an impact on food intake rates. In contrast to many other elastic mechanisms among arthropods, glossa protraction in ants is based on a mechanism where elasticity works as an actual antagonist to muscles. I compared the design of the labiomaxillary complex of ants with that of the honey bee, and suggest an elastic mechanism for glossa protraction in honey bees as well.

**[B.5.]** Ants employ two different techniques for liquid food intake, in which the glossa works either as a passive duct (sucking), or as an up- and downwards moving shovel (licking). For collecting fluids at *ad libitum* food sources, workers of a given species always use only one of both techniques. The species-specific feeding technique depends on the existence of a well developed crop and on the resulting mode of transporting the fluid food. Workers of ponerine species licked fluid food during foraging and transported the fluid as a drop between their mandibles, whereas workers of species belonging to phylogenetically more advanced subfamilies that possess a crop capable of storing liquids sucked the fluid food. In order to evaluate the performance of collecting liquids during foraging, I measured fluid intake rates of four ant species adapted to different ecological niches. For that purpose, ant workers were presented with pure sucrose solutions of a wide range of concentrations under controlled feeding conditions in the laboratory. The results demonstrate that fluid intake rate depends on sugar concentration and the associated fluid viscosity, on the species-specific feeding technique, and on the extent of specialization on collecting liquid food. Workers of the two nectar-feeding ant species (*Camponotus rufipes* and *Pachycondyla*

*villosa*) collected fluids at high intake rates, while workers of the leaf-cutting ant *Atta sexdens* and the predatory ant *Rhytidoponera impressa*-complex did so at low fluid intake rates. Calculating the energy intake rates (mg sucrose per unit time), licking is more advantageous at higher sugar concentrations, whereas sucking provides a higher energy intake rate at lower concentrations.

**[B.6.]** In ants, the glossa is the first body part that is involved in liquid food intake. I compared four ant species (*P. villosa*, *R. impressa*-complex, *C. rufipes*, *A. sexdens*) in terms of glossa surface characteristics and relative volumes of the muscles that control licking and sucking. In all examined ant species, the glossa surface is equipped with small shovel-like structures that are regularly arranged in parallel rows. I measured shovel size, shovel density, and frontal surface area of the glossa and calculated the 'active glossa surface area' for each species as the product of the three measured parameters. Workers of species that licked fluids at *ad libitum* food sources possessed larger active surface areas of the glossa as well as larger muscles that control licking than workers of species that sucked liquids. On the other hand, the latter featured larger muscles that are responsible for the sucking process. In addition to the feeding technique, the extent of specialization on collecting liquid food during foraging correlated with glossa surface characteristics and relative muscle volumes. Both probably reflect adaptations to the species-specific ecological niche and determine the physiological performance of liquid feeding. Despite species-specific differences, the ratio of the absolute volume of the glossa muscle and the corresponding active glossa surface area was constant among workers of all four species, demonstrating that single components of the whole system are closely adjusted to each other according to a general rule.

# DIE MUNDWERKZEUGE DER AMEISEN -

## Zusammenfassung

**[A.1.]** Ameisenmandibeln führen eine Vielzahl verschiedener Bewegungen bezüglich Kraft, Geschwindigkeit und Präzision aus, bei denen die Mandibeln auf unterschiedlichste Art und Weise genutzt werden. Der Schlüssel zu dieser Vielfalt ist der Mandibelschließmuskel. In Ameisen besteht dieser Muskel aus verschiedenen Muskelfasertypen, die sich sowohl morphologisch als auch anhand ihrer kontraktilen Eigenschaften unterscheiden. Schnell kontrahierende Fasern haben kurze Sarkomere (2-3 µm) und greifen stets direkt am Schließerapodem an, welches die Muskelkraft auf das Mandibelgelenk überträgt. Langsam aber kraftvoll kontrahierende Fasern besitzen lange Sarkomere (5-6 µm) und setzen entweder direkt oder mittels dünnen fadenförmigen Filamenten am Apodem an. Die Anteile der Fasertypen am Gesamtvolumen des Mandibelschließers sind artspezifisch und korrelieren mit der für die Art typischen Lebensweise. Ameisen, deren Mandibeln sehr schnelle Bewegungen ausführen können, besitzen einen hohen Anteil an schnellen Muskelfasern, während der Mandibelschließer von Ameisen mit kraftvollen Mandibelbewegungen mehr kraftvolle Fasern aufweist. Schnappfallenkiefer-Ameisen verfügen über einen hoch spezialisierten Katapultmechanismus. Das Schließen ihrer Mandibeln ist als eine der schnellsten Bewegungen des Tierreichs bekannt.

**[A.2.]** Ein zwischenartlicher Vergleich legt zwei grundlegende Prinzipien des Aufbaus des Mandibelschließers nahe: (1) Ameisen, die auf sich besonders schnell schließende Mandibeln angewiesen sind, wie jägerische Arten, sind durch langgestreckte Köpfe gekennzeichnet, die lange schnelle Muskelfasern beinhalten, welche direkt am Apodem in spitzen Winkeln angreifen. Ihre Muskeln bestehen aus nur wenigen Filamentfasern. Sie maximieren Geschwindigkeit auf Kosten der Kraft. (2) Ameisen, die mit ihren Mandibeln sehr kraftvolle Tätigkeiten bewerkstelligen, wie das Knacken von Körnern oder das Schneiden von Blättern, benötigen viele, eher kurze Muskelfasern, die in einer breiten Kopfkapsel untergebracht sind. Ihre langsameren aber kräftigeren Muskeln enthalten viele Filamentfasern. Zwei biomechanische Modelle erklären, wie die Angriffswinkel der Muskelfasern am Apodem im Hinblick auf Kraft und Geschwindigkeit optimiert werden und wie Filamentfasern dazu beitragen, aus dem vorhandenen Kopfkapselvolumen die größtmögliche Kraft zu entwickeln.

**[A.3.]** Der gesamte Mandibelschließmuskel wird von 10-12 Motoneuronen gesteuert, wovon 4-5 ausschließlich schnelle Muskelfasern innervieren. Langsame Muskelfasern umfassen eine posteriore und eine antero-laterale Gruppe, die jeweils von 1-2 Motoneuronen angesteuert werden. Außerdem innervieren 3-4 Motoneuronen alle Muskelfasern zusammen. Gleichzeitige Aufnahmen von Muskelaktivität und Mandibelbewegung ergeben, daß schnelle Bewegungen rasche Kontraktionen schneller Muskelfasern bedürfen. Langsame und präzise Bewegungen resultieren aus der Aktivierung langsamer Muskelfasern. Kraftvolle Bewegungen werden durch gleichzeitige Aktivierung aller Muskelfasertypen erzeugt. Für die Feinabstimmung können unterschiedliche Muskelfaserbündel unabhängig voneinander aktiviert werden. Retrograde Färbungsexperimente zeigen, daß die meisten dendritischen

Verästelungen der verschiedenen Gruppen von Motoneuronen im gleichen Neuropil im Unterschlundganglion verlaufen. Darüberhinaus dringen schnelle Motoneuronen und Neuronen, die die laterale Gruppe von langsamen Muskelfasern versorgen, jeweils in spezifische Bereiche des Neuropils ein, welche nicht mit den anderen Gruppen von Motoneuronen geteilt werden. Zwischen den Dendriten der linken und rechten Motoneuronen tritt teilweise bilaterale Überlappung auf, besonders bei den schnellen Motoneuronen. Die Ergebnisse verdeutlichen, wie ein einzelner Muskel eine solche Vielfalt an Bewegungen generiert, um den Ansprüchen des reichhaltigen Funktionsspektrums der Ameisenmandibeln gerecht zu werden.

**[B.4.]** Der Labiomaxillar-Komplex ist für die Nahrungsaufnahme der Ameisen essentiell. Ich untersuchte den Bauplan und die Bewegungsmechanismen des Labiomaxillar-Komplexes bei verschiedenen Ameisenarten. Sechs Labial- und sechs Maxillarmuskeln mit jeweils unterschiedlichen Funktionen steuern die verschiedenen Gelenke. Messungen von Sarkomerlängen, Muskelfaserlängen und -durchmesser zufolge weisen sowohl Labial- als auch Maxillarmuskeln eher langsame als schnelle Kontraktionseigenschaften auf und scheinen nicht auf bestimmte Tätigkeiten spezialisiert zu sein. Da Glossa-Streckmuskeln fehlen, beruht das Herausklappen der Glossa, dem distalen Ende des Labiums, nicht auf einer durch Muskelkontraktion hervorgerufenen Bewegung. Anhand histologischer Messungen von Hämolymphvolumina konnte ein auf erhöhtem Druck basierender Mechanismus ausgeschlossen werden. Weiterführende Experimente zeigten, daß bei Relaxation der Glossa-Rückziehmuskeln die Glossa aufgrund von Elastizität ausklappt. Dieser elastische Mechanismus beeinflußt möglicherweise Nahrungsaufnahmeraten. Im Gegensatz zu vielen anderen elastischen Mechanismen bei Arthropoden liegt dem Herausklappen der Glossa ein Mechanismus zugrunde, bei dem Elastizität als wirklicher Antagonist zu Muskeln wirkt. Ein Vergleich des Bauplans des Labiomaxillar-Komplexes von Ameisen mit dem der Honigbiene legt nahe, daß die Glossa der Honigbiene ebenfalls mittels Elastizität in die exponierte Position gebracht wird.

**[B.5.]** Um flüssige Nahrung aufzunehmen, nutzen Ameisen ihre Glossa entweder als passiven Kanal (Saugen) oder als eine sich auf- und abwärts bewegende Schaufel (Lecken). Während des Sammelns von Flüssigkeiten an *ad libitum* Futterquellen bedienen sich Arbeiterinnen einer Art stets nur einer von beiden Aufnahmetechniken. Die jeweils artspezifische Nahrungsaufnahmetechnik hängt von dem Vorhandensein eines gut ausgeprägten Kropfes sowie der daraus resultierenden Weise des Nahrungstransportes ab. Ponerinen leckten flüssige Nahrung und transportierten sie als Tropfen zwischen ihren Mandibeln, während Arbeiterinnen von Arten, die phylogenetisch weiter abgeleiteten Unterfamilien angehören und einen zur Zwischenspeicherung beträchtlicher Mengen Flüssigkeit befähigten Kropf besitzen, die Flüssigkeit aufsaugten. Um die Leistung der Aufnahme flüssiger Nahrung zu beurteilen, bestimmte ich die Aufnahmeraten (Flüssigkeitsmenge pro Zeiteinheit) vier verschiedener Ameisenarten, die an unterschiedliche ökologische Nischen

angepaßt sind. Hierfür setzte ich den Arbeiterinnen Saccharose-Lösungen diverser Konzentrationen unter kontrollierten Laborbedingungen vor. Die Ergebnisse zeigen, daß die Aufnahmerate von der Zuckerkonzentration und der damit verbundenen Viskosität abhängt, außerdem von der artspezifischen Aufnahmetechnik und dem Grad der Anpassung an das Sammeln flüssiger Nahrung. Arbeiterinnen der beiden nektarsammelnden Ameisenarten (*Camponotus rufipes*, *Pachycondyla villosa*) erzielten hohe Aufnahmeraten, wohingegen die Blattschneiderameise *Atta sexdens* und Arbeiterinnen der jägerischen Art *Rhytidoponera impressa*-Komplex niedrigere Aufnahmeraten erreichten. Berechnet man die Energie-Aufnahmerate (mg Saccharose pro Zeiteinheit), so ist Lecken bei höheren Zuckerkonzentrationen vorteilhafter, während Saugen bei niedrigeren Konzentrationen eine höhere Energie-Aufnahmerate erbringt.

**[B.6.]** An der Aufnahme flüssiger Nahrung ist bei Ameisen die Glossa als erstes Körperteil beteiligt. Ich verglich vier verschiedene Ameisenarten (*P. villosa*, *R. impressa*-Komplex, *C. rufipes*, *A. sexdens*) bezüglich der Charakteristika der Glossaoberfläche sowie der relativen Volumina der beim Lecken und Saugen aktiven Muskeln. Die Glossaoberfläche aller vier Arten ist mit schaufelartigen Strukturen bestückt, die gleichmäßig in parallelen Reihen angeordnet sind. Ich bestimmte jeweils die Schaufelgröße, die Schaufeldichte und die frontale Oberfläche der Glossa und berechnete daraus als Produkt dieser drei Parameter die „aktive Glossaoberfläche“ für jede der vier Arten. Arbeiterinnen jener Arten, die Flüssigkeiten an einer *ad libitum* Nahrungsquelle aufleckten, weisen eine größere aktive Glossaoberfläche auf und besitzen größere Muskeln, die für den Leckvorgang verantwortlich sind, als Ameisen, die flüssige Nahrung aufsaugten. Andererseits sind letztere durch größere Muskeln charakterisiert, die den Saugvorgang steuern. Zusätzlich zur Nahrungsaufnahmetechnik korreliert der Grad der Anpassung an das Sammeln flüssiger Nahrung mit den Eigenschaften der Glossaoberfläche und den relativen Muskelvolumina. Beide spiegeln wahrscheinlich Anpassungen an die artspezifische ökologische Nische wieder und bestimmen die physiologischen Leistungen der Aufnahme flüssiger Nahrung. Trotz artspezifischer Unterschiede ist das Verhältnis zwischen Absolutvolumen des Glossamuskels und der jeweiligen aktiven Glossaoberfläche bei Arbeiterinnen aller vier Arten konstant. Dies zeigt, daß einzelne Komponenten eines Systems entsprechend einer allgemeineren Regel fein aufeinander abgestimmt sind.

# THE MOUTHPARTS OF ANTS -

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# APPENDIX

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| 1978 – 1982 | Grundschule in Nürnberg |
| 1982 – 1991 | Pirckheimer-Gymnasium in Nürnberg |
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**Studium:**

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| Okt. 1991 – März 1998 | Biologie und Chemie (Lehramt an Gymnasien) an der Universität Würzburg |
| Oktober 1993 | Zwischenprüfung Biologie (Botanik, Zoologie) |
| April 1994 | Zwischenprüfung Chemie (Anorg., Org., Physikal. Chemie) |
| 1996 | Staatsexamensarbeit in Biologie |
| Herbst 1997 | Staatsexamen Biologie, Chemie und Erziehungswissenschaften (Lehramt an Gymnasien) |

**Promotionsstudium:**

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| seit April 1998 | Thema: “The Mouthparts of Ants”, am Lehrstuhl für Verhaltensphysiologie und Soziobiologie der Univ. Würzburg; Stipendiat des DFG-Graduiertenkollegs; DFG-Stelle, BATIIa/2 |
| Nov. 1999 – April 2000 | Forschungsaufenthalt in Tucson (USA), Arizona Research Laboratories, Division of Neurobiology; Stipendiat der University of Arizona; DAAD-Stipendium |

Würzburg, 15.06.2001,

## Publications

**Full papers:**

1 Gronenberg W, **Paul J**, Just S, Hölldobler B (1997): Mandible muscle fibers in ants: Fast or powerful. *Cell & Tissue Research* 289: 347-361.

2 **Paul J**, Gronenberg W (1999): Optimizing force and velocity: Mandible muscle fiber attachments in ants. *Journal of Experimental Biology* 202: 797-808.

3 **Paul J** (2001): Review: Mandible movements in ants. *Comparative Biochemistry and Physiology* (in press).

4 **Paul J**, Roces F, Hölldobler B (2001): How do ants put out their tongue? *Journal of Morphology* (accepted).

5 **Paul J**, Roces F (2001a): Liquid food intake in ants: I. Feeding techniques and intake rates. *Journal of Experimental Biology* (submitted).

6 **Paul J**, Roces F (2001b): Liquid food intake in ants: II. Underlying mechanisms. *Journal of Experimental Biology* (submitted).

**Article in preparation:**

7 **Paul J**, Gronenberg W (2001): Motor control of the mandible closer muscle in ants.

**Conference abstracts:**

8 **Paul J**, Just S, Gronenberg W (1996): Functional morphology of tubular fibers in ant mandibular muscles: A comparative approach. *24th Göttingen Neurobiology Conference*. Stuttgart, New York: Thieme. Volume II: 100.

9 **Paul J**, Roces F (1999): Labial muscle volumes in ants correlate with feeding habits. *27th Göttingen Neurobiology Conference*. Stuttgart, New York: Thieme. Volume II: 225.

10 **Paul J**, Roces F (2000a): Mouthpart movements in ants: biomechanics, muscle fibers, and motor control. *21st Congress of the European Society for Comparative Physiology and Biochemistry* 126: 117.

11 **Paul J**, Roces F (2000b): Feeding behavior and the control of mouthparts in ants. *93rd Meeting of the German Zoological Society. Zoology* 103: 32.

**Staatsexamensarbeit:**

12 **Paul J** (1996): Vergleichende Funktionsmorphologie von Mandibelschließmuskeln bei Ameisen. Staatsexamensarbeit, Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Universität Würzburg.

## Erklärung

Hiermit erkläre ich, die vorliegende Arbeit in allen Teilen selbständig und nur mit den angegebenen Hilfsmitteln und Quellen angefertigt zu haben.

Diese Dissertation hat weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.

Außerdem erkläre ich hiermit, früher weder akademische Grade erworben zu haben, noch habe ich versucht solche zu erlangen.

Würzburg, 15.06.2001,