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Coming of age in an ant colony: cephalic muscle maturation accompanies behavioral development in *Pheidole dentata*

Mario L. Muscedere · James F. A. Traniello ·
Wulfila Gronenberg

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Abstract Although several neurobiological and genetic correlates of aging and behavioral development have been identified in social insect workers, little is known about how other age-related physiological processes, such as muscle maturation, contribute to task performance. We examined post-eclosion growth of three major muscles of the head capsule in major and minor workers of the ant *Pheidole dentata* using workers of different ages with distinct task repertoires. Mandible closer muscle fibers, which provide bite force and are thus critical for the use of the mandibles for biting and load carrying, fill the posterio-lateral portions of the head capsule in mature, older workers of both subcastes. Mandible closer fibers of newly eclosed workers, in contrast, are significantly thinner in both subcastes and grow during at least the next 6 days in minor workers, suggesting this muscle has reduced functionality for a substantial period of adult life and thus constrains task performance capability. Fibers of the antennal muscles and the pharynx dilator, which control antennal movements and food intake, respectively, also increase significantly in thickness with age. However, these fibers are only slightly thinner in newly eclosed workers and attain their maximum thickness over a shorter time span in minors. The different growth rates of these functionally distinct muscles likely have consequences for how adult *P. dentata* workers, particularly minors, develop their full and diverse task repertoire as they age. Workers may be capable of feeding and interacting socially soon after eclosion, but require a longer period of development to effectively use their mandibles, which enable the efficient performance of tasks ranging from nursing to foraging and defense.

Keywords Ant · Caste evolution · Functional morphology · Mandibles · Muscles · Temporal polyethism

Introduction

Molecular and physiological studies of social hymenopterans demonstrate that worker aging and behavioral maturation, and hence task performance, are accompanied by developmental changes in adults involving brain anatomy and chemistry (Barron et al. 2007; Brown et al. 2004; Gronenberg and Riveros 2009; Jones et al. 2009; Kühn-Bühlmann and Wehner 2006; Riveros and Gronenberg 2010; Schulz and Robinson 2001; Seid et al. 2005, 2008; Seid and Traniello 2005; Stieb et al. 2010), gene expression (Smith et al. 2008), and endocrine profiles (O'Donnell and Jeanne 1993; Robinson 1987; Withers et al. 1995). However, the roles of other structural and physiological developmental events in the ontogeny of worker task competence, while likely important, are not as well established (Robinson 2009). Muscular development in adult insects (Chapman 1998; Finlayson 1975) is poorly understood, although it is taxonomically widespread and could have important effects

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M. L. Muscedere · J. F. A. Traniello
Department of Biology, Boston University,
5 Cummington Street,
Boston, MA 02215, USA

W. Gronenberg
Department of Neuroscience, University of Arizona,
611 Gould-Simpson Science Building,
Tucson, AZ 85721, USA

Present Address:
M. L. Muscedere (✉)
Program in Neuroscience, Boston University,
2 Cummington Street,
Boston, MA 02215, USA
e-mail: mario@bu.edu

on social insect worker task abilities. Most described examples of adult muscle development concern flight muscles, which can continue to grow after the terminal molt (Chapman 1998; de Kort 1990; Finlayson 1975; Marden 2000). In the social insects, adult honey bee workers do not forage until they have experienced two bouts of flight muscle development over several weeks, resulting in dramatic changes in muscle metabolism and biochemistry that significantly increase power output (Roberts and Elekonich 2005; Schippers et al. 2010). Non-flight skeletal muscles can also grow substantially in adult insects; examples include the oviposition muscles of locusts (*Locusta migratoria*), which reach full size and strength only after adult females attain reproductive maturity (Rose 2004), and some skeletal muscles, including mandibular muscles, in the beetle *Ips sexdentatus* (Termier 1970). However, descriptions of the development of muscles apart from those associated with flight in adult insects are rare, and examples of this type of muscle development in social insects are nonexistent.

Ant workers construct nests, forage, care for developing brood, and defend their colonies from predators and competitors. These tasks require the use of the mandibles, which typically have a multi-purpose, shovel-like morphology that reflects their involvement in most colony tasks (Hölldobler and Wilson 1990). While the mandibles open and close only in a single plane, bite speed and force can be altered to achieve very different functional outcomes, from rapid and powerful prey-subduing bites, to slow, precise movements required for nursing small and delicate soft-bodied larvae. Mandibular movements are controlled primarily by the mandible closer (MC) muscles; accordingly, the MCs are particularly large and well-developed in ants, comprising up to two thirds of the total volume of the head capsule and containing at least two distinct fiber types with different contractile properties (Gronenberg et al. 1997; Paul 2001; Paul and Gronenberg 1999). While the MCs dominate the cephalic musculature of ants, many smaller muscles are also present. These include the pharynx dilator (PD) muscles, which facilitate food ingestion by producing suction in the alimentary canal (Paul et al. 2002); the antennal muscles (AM), which produce the rapid and controlled movements of the antennal scape that allow ants to effectively sample their sensory environment (Ehmer and Gronenberg 1997; Paul et al. 2002); and other muscles, including the mandible opener muscles and muscle groups that effect movement of the maxillae, labium, and other inner mouthparts (Paul et al. 2002).

The dimorphic ant *Pheidole dentata* has served as a model for research into the behavioral organization and ecology of age-related worker behavioral development in social insects (Calabi and Traniello 1989a,b; Muscedere et al. 2009; Seid and Traniello 2006; Traniello 2010; Wilson 1976a). Recent work has begun to describe the neurochemical and neuroanatomical correlates of worker behavioral maturation in this species (Seid et al. 2005, 2008; Seid and Traniello 2005). However, the role of other physiological factors, such as muscle development, remains unknown. Colony populations in *P. dentata* are comprised mostly of small minor workers, which are numerically dominant and responsible for most colony activities such as brood rearing, nest construction, and foraging. Minor workers acquire new and more diverse task abilities as they age, adding outside-nest tasks such as foraging to their existing within-nest repertoire (Seid and Traniello 2006). Task efficiency changes with age as well: Muscedere et al. (2009) recently showed that young *P. dentata* minors rarely pick up and pile scattered eggs and microlarvae in standardized laboratory assays, while older workers do so very quickly. When caring for older brood, young minor workers in the same experiments made antennal contact with and licked larvae and pupae with their inner mouthparts, but unlike older workers, rarely manipulated or lifted brood with their mandibles (ML Muscedere, personal observation). Additionally, major workers, which are defensive in function, comprise 10–15% of *P. dentata* colonies and are characterized by allometrically large heads equipped with strong jaws powered by well-developed mandibular muscles (Wilson 1976b). Major workers exhibit a pattern of age-related behavioral maturation apparently similar to that of minors: young majors do not leave the nest to participate in defensive activities (ML Muscedere and JFA Traniello, personal observation).

These observations prompted us to question whether young workers are unable to effectively use their mandibles because their mandibular musculature is not fully developed and to determine whether cephalic muscles develop at differing rates, resulting in constraints on the use of the mandible muscles relative to other cephalic muscles. These considerations are significant to achieving a comprehensive understanding of age-related task performance and testing theories of division of labor (Beshers and Fewell 2001) such as the repertoire expansion model (Seid and Traniello 2006), which posits that neural and physiological development in adult workers underlies their age-related acquisition of new tasks and increased task proficiencies. Therefore, we examined how the sizes and morphologies of developing muscle fibers of the MC, AM, and PD change with age in adult *P. dentata* workers. We focused on minor workers due to their central role in colony labor, but include comparative data on majors to determine if workers in both subcastes undergo similar patterns of muscle maturation.

Materials and methods

Colony maintenance

All workers were sampled from queenright colonies of *P. dentata*, a small, completely dimorphic ant native to the southeast USA. Colonies were collected in Alachua County, Florida and housed in the laboratory in 27×20×11 cm plastic boxes coated with Fluon® (Northern Products, Woonsocket, RI, USA) to prevent escape. Test tubes partially filled with water and plugged with cotton were provided as nests. Colonies and all derived subcolonies were fed 1 M sucrose or 1:1 honey/water solutions and freshly killed insects (fruit flies, bumblebees, and/or mealworms) several times weekly.

Worker sampling and age determination

We first performed a detailed examination of head muscle development in minor workers of four distinct ages from a single colony (hereafter referred to as "colony 1"). Two of these age groups, age class 1 (AC1, 0–2 days post eclosion) and age class 4 (AC4, 16+days post eclosion), were collected directly from the stock colony and distinguished by differences in cuticular coloration, as *P. dentata* minors darken predictably from light yellow to dark brown over approximately the first 16–20 days of adult life (Seid and Traniello 2006; Wilson 1976a). AC1 workers never forage, have a small behavioral repertoire, and work less frequently and efficiently than older nestmates, while mature AC4 workers frequently forage and have large and diverse repertoires that include nursing (Muscedere et al. 2009; Seid and Traniello 2006). AC1 and AC4 workers therefore represent the least and most behaviorally competent age cohorts of minor workers, respectively.

Because age determination of workers of intermediate age (AC2 and AC3) based on cuticular coloration alone is less precise, we generated additional cohorts of minors of known age to examine the time course of muscular development. To do so, we searched the parent colony for the most recently eclosed minors (lightest colored AC1s, less than 1-day old) and established these focal workers in subcolonies containing larvae, major workers (10% of the adult workers in each subcolony), and AC4 minor workers, which we marked by removing the tibia from the right mesothoracic leg. Removing tibiae did not appear to negatively impact behavior, and tibiae were never removed from focal workers; AC4 workers marked in this way were added to provide subcolonies with nurses and foragers adequate to facilitate the normal development of the focal workers. Subcolonies each initially contained 80 adult workers (20–25 focal AC1 minors, 47–52 marked AC4 minors, 8 majors) and 20 small larvae. To obtain minors of known age, we then sampled focal workers from these subcolonies after 3 or 6 days. Focal younger minors could be distinguished from nurse AC4 workers by their lighter cuticular coloration and intact legs. Eight minors per age group (total $N=32$ preparations) were analyzed as described below.

To investigate the generality of the pattern of age-related muscle growth identified in minors from colony 1, we examined AC1 and AC4 minors from other colonies (hereafter "colony 2" and "colony 3"). We sampled three AC1 minors and two AC4 minors from colony 2, along with two AC1 minors and four AC4 minors from colony 3. We also sampled two AC1 majors and four AC4 majors from colony 2, to test whether a similar pattern of age-related muscle development was present in this subcaste (total $N=17$ preparations of workers from additional parent colonies). We applied the same cuticle color criteria used for minor workers to estimate the age of major workers: AC1 majors were uniform light yellow, while AC4 majors were uniform dark brown to black. All workers from colonies 2 and 3 were sampled directly from their parent colonies.

Tissue preparation, imaging, and muscle fiber measurement

To prepare heads for staining, workers were embedded in dental wax, bodies were cut from heads at the pronotum, and heads were cut sagitally lateral to the head midline to facilitate fixation and stain penetration into the head capsule. The remaining antenna (contralateral to the incision) was then cut and prepared heads transferred into 4% paraformaldehyde in phosphate buffered saline (PBS) to fix for 2–5 h at room temperature or overnight at 4°C. Fixed heads were washed twice in PBS for 1 h each, then washed in distilled water for 1 h. Heads were stained in 1% osmium tetroxide in distilled water for 2 h on ice, then 30 min at room temperature. Stained heads were washed three times in distilled water (30 min each), then dehydrated through a series of 50% ethanol (30 min), dimethoxy propane (10 min), and acetone (10 min). Dehydrated heads were embedded in Spurr's resin (at least 6 h each in 50:50 Spurr's/acetone, 90:10 Spurr's/acetone, and 100% Spurr's), polymerized, and then horizontally sectioned at a thickness of 15 μm (colonies 2 and 3) or 20 μm (colony 1).

Sections were viewed with phase contrast on an Olympus BX40 compound microscope, and measurements were taken with a calibrated ocular micrometer. For each preparation, we measured the thickness of five muscle fibers from the AM and PD, and ten muscle fibers (five fast and five slow fibers, see below) from the MC muscle (Figs. 1 and 2). The PD is a group of several muscles with various attachment points within the head capsule and along the pharynx (Paul et al. 2002); we measured fibers from the largest and most conspicuous single PD muscle, which attaches to the ventral wall of the pharynx, passes through the esophageal foramen between the brain and the subesophageal ganglion, and attaches to the posterio-ventral head capsule (Fig. 1). The AM and PD are smaller muscles visible in only a few sections per preparation; fibers from these muscles are also thinner and more closely

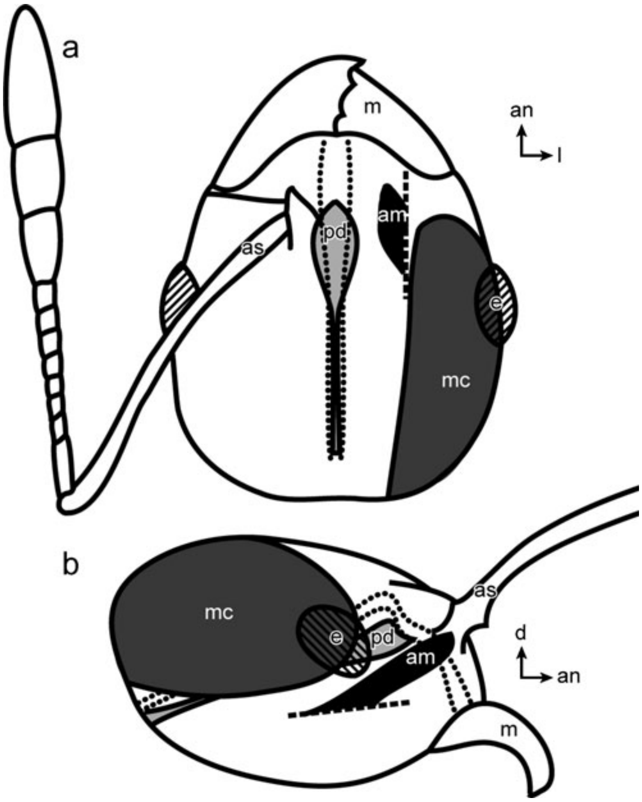

Fig. 1 Schematic **a** frontal and **b** sagittal illustrations of a *P. dentata* minor worker head capsule showing approximate locations of muscles measured in this study. The region of the tentorium that serves as an attachment point for the antennal muscles is indicated by the *dashed line*, and the alimentary canal by the *dotted lines*. The large mandible closer apodemes, to which the mandible closer muscle fibers attach, have been omitted for clarity. *am* antennal muscle, *an* anterior, *as* antennal scape, *d* dorsal, *e* eye, *l* lateral, *m* mandible, *mc* mandible closer muscle, *pd* pharynx dilator muscle

packed than MC fibers, making measurement more difficult. Therefore, for these muscles, we measured the five most easily delineated muscle fibers, regardless of their position within the muscle. For the MC, which has larger fibers that appear in many sections, we standardized our measurements by choosing fibers in the dorso-ventral depth of the eye and restricting our measurements to fibers in the posterio-lateral region of the head capsule (roughly the region delineated by Fig. 3d). We separately measured MC fibers with short sarcomeres and direct attachment to the mandibular apodeme, which contract quickly (“fast” fibers), and MC fibers with longer sarcomeres that attach to the mandibular apodeme via connective fibers, which contract more slowly but are more forceful (“slow” fibers), because these two fiber types contribute differentially to worker bite force and speed (Fig. 3e; Paul 2001; Paul and Gronenberg 2002). For each muscle (or fiber type within the MC), we averaged the five fiber measurements and used these averages as the observations for each preparation. We measured longitudinally sectioned fibers and avoided fibers cut obliquely to standardize our diameter measurements. In preparations in which we could not locate fibers in longitudinal section for a given muscle due to variation in the plane of sectioning, we did not measure fibers for that muscle for that preparation, thus accounting for sample size differences between muscles. Focusing up and down within any individual section ensured we measured the maximum width of each individual muscle fiber. For colony 1 samples, we recorded MC measurements in all preparations (N=32) and AM and PD measurements from all but one preparation each (N=31). Final sample sizes for each muscle from colonies 2 and 3 are given in Table 1.

Results

Muscle fiber thickness and worker age

In minor workers from colony 1, muscle fiber thickness varied significantly among age groups in the AM (Fig. 4a, ANOVA, $F_{3,\ 27}$=4.39, P=0.012) and PD (Fig. 4b, $F_{3,\ 27}$=13.4, P<0.0001) and for both fiber types in the MC (slow fibers, Fig. 4c, $F_{3,\ 28}$=168, P<0.0001; fast fibers, Fig. 4d, $F_{3,\ 28}$=54.8, P<0.0001). The fast and slow MC fibers of mature AC4 minors (mean ± SEM, 21.4±0.3 and 23.7±0.6 μm) were two and three times thicker, respectively, than those of AC1 minors (10.4±0.8 and 8.3±0.5 μm). The slow MC fibers of 6-day-old minors were significantly thinner than those of AC4 minors (Tukey–Kramer post hoc comparisons, Fig. 4c), indicating that MC development continues at least 6 days after eclosion in this subcaste. Slow MC fibers were estimated to attain maximum thickness in minors (as indicated by the mean thickness of these fibers in AC4s) on approximately day 11 of adult life, based on a linear model of growth over the first three time points measured (AC1≈0, 3, and 6 days; regression, $F_{1,\ 22}$=165, P< 0.0001, R^2=0.89, [mean fiber thickness]=8.35+1.42× [days post eclosion]). In contrast, fibers from the AM and PD of AC4s were only 1.25 times thicker than the same fibers in AC1s, and the fibers of 3-day-old (and 6-day-old) minors were not significantly thinner than those of AC4s in either muscle (Tukey–Kramer post hoc comparisons, Fig. 4a, b), indicating these muscles reach maximum thickness in minor workers soon after adult emergence.

In workers from both subcastes in colonies 2 and 3, muscle fibers from all four muscles were thicker on average in AC4s than in AC1s in every instance, supporting the generality of the pattern described above

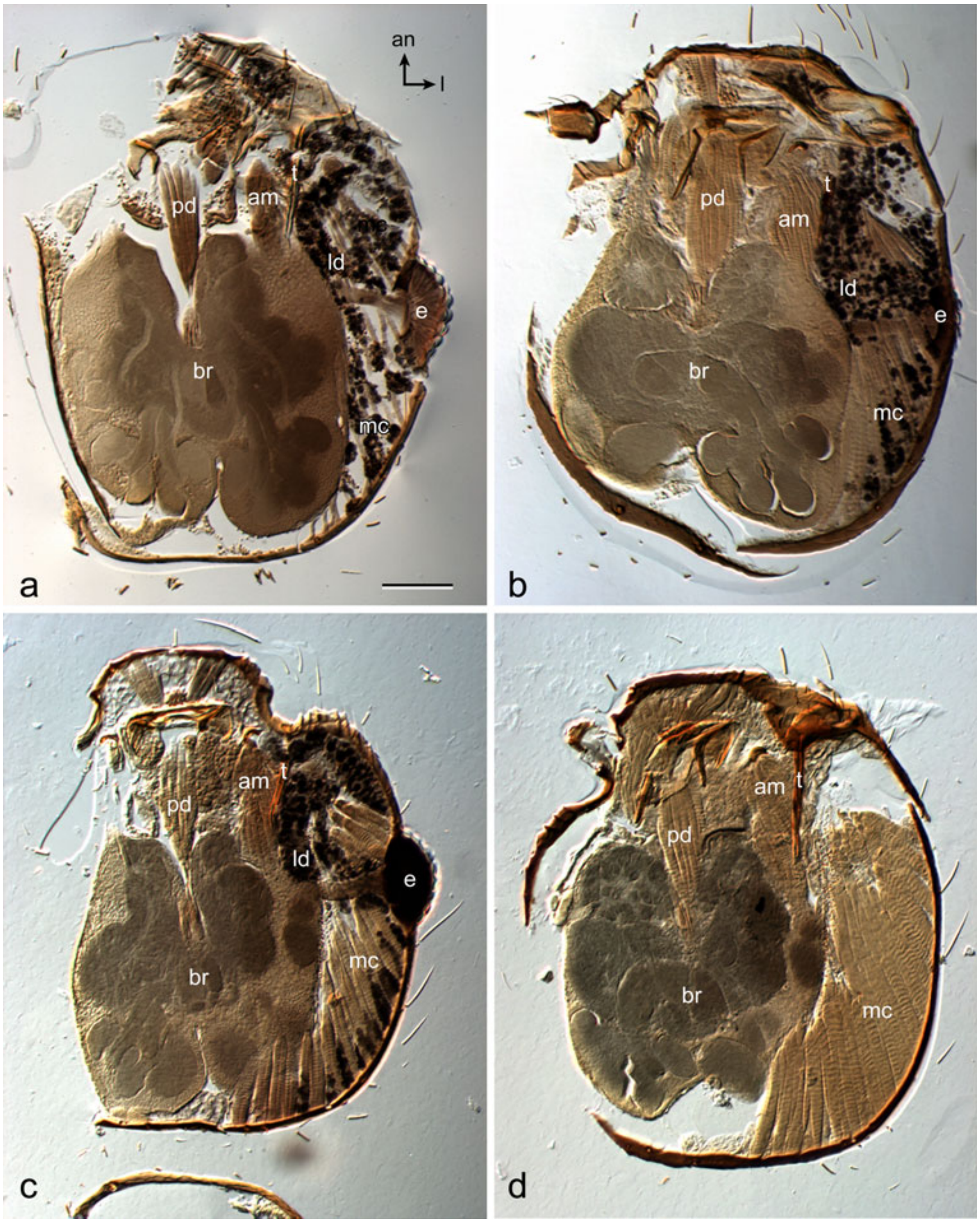

Fig. 2 Internal head anatomy of **a** AC1, **b** 3-day-old, **c** 6-day-old, and **d** AC4 minor workers from colony 1, showing the three muscles under Nomarski interference contrast. *am* antennal muscle, *br* brain, *e* eye, *mc* mandible closer muscle, *pd* pharynx dilator muscle, *t* tentorium, *a* anterior, *l* lateral, *ld* lipid droplets, *p* posterior. *Scale bar*=100 μm

for minor workers from colony 1 (Table 1). Grouping minor workers from colonies 2 and 3 to yield sample sizes amenable to statistical testing, AC4 minors from these colonies had significantly thicker muscle fibers than AC1s in the PD (Mann–Whitney *U* test, $N_1=5$, $N_2=6$, $U=0$, $P=0.004$), slow MC ($N_1=5$, $N_2=6$, $U=0$, $P=0.004$) and fast MC ($N_1=5$, $N_2=6$, $U=0$, $P=0.004$). The distribution of AM fiber thicknesses of AC1 and AC4 workers was likewise non-overlapping: all four AC1 minors measured had AM muscle fibers thinner than the AM fibers of both AC4 minors. Our sample size for this muscle, however, did not yield enough power to indicate that the differences were statistically significant ($N_1=4$, $N_2=2$, $U=0$, $P=0.13$). Like minors, AC4 major workers from colony 2 had markedly thicker cephalic muscle fibers than AC1s (Fig. 5), with the greatest difference evident for the MCs: the AM and PD were approximately 40% and 14% thicker, respectively, in AC4 majors, while slow and fast MC fibers were approximately 115% and 65% thicker in AC4s (Table 1).

Qualitative differences in fiber morphology and fat body distribution within muscles

In *P. dentata* (and hymenopterans in general), all skeletal muscles, including the MC, AM, and PD, are comprised of bundles of tubular muscle fibers (Elder 1975; Gronenberg and Ehmer 1995), which are distinguishable in longitudinal section by their centrally arranged nuclei and regular

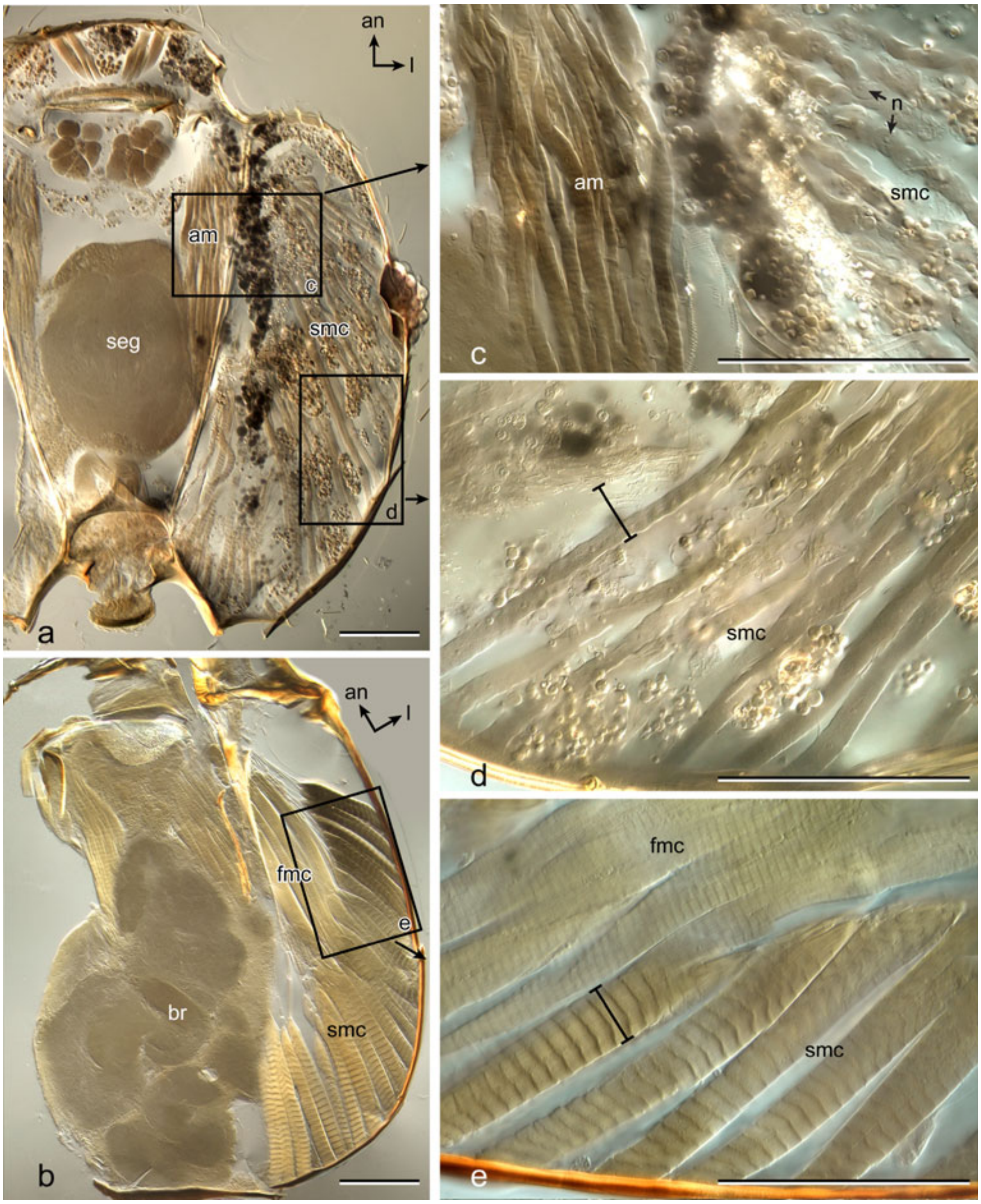

Fig. 3 High magnification comparisons of mandible closer muscles of young (AC1; **a**, **c**, **d**) and old (AC4; **b**, **e**) minor workers from colony 1 under Nomarski interference contrast. *Insets* in **a** and **b** are enlarged in **c** and **d** and in **e**, respectively. Note the striation pattern of the young antennal muscle fibers in **c** and the absence of any striation of young mandible muscle fibers in **c** and **d**. Both mandible closer fiber subtypes (fast and slow) can be seen in the plane of sectioning in **b** and **e**. *I-bars* in **d** and **e** are the same length (25 μm) and illustrate the difference in slow mandible closer fiber thickness between newly eclosed and mature workers. *am* antennal muscle, *an* anterior, *br* brain, *fmc* fast mandible closer fibers, *l* lateral, *n* muscle fiber nuclei, *seg* subesophageal ganglion, *smc* slow mandible closer fibers. *Scale bars*=100 μm

striation pattern. However, in some of our AC1 samples, we observed MC fibers with no discernable striation (Fig. 3c, d). These unstriated fibers had a beaded appearance and non-uniform diameter, with rounded, slightly thicker regions (each containing a single nucleus) connected by thinner bands containing cytoplasm (Fig. 3c). In other AC1 samples (or sometimes within the same preparation), MC fibers were striated and had a uniform diameter, although they were still very thin. All MC fibers were striated in older workers (3 days to AC4). In the AM and PD, fibers were always striated and we could not detect any qualitative differences in fiber appearance between worker age groups.

Age cohorts also differed in the abundance and distribution of adipocytes (fat body cells) in the MC and lateral head capsule. We identified adipocytes by the presence of many well-stained intracellular spherical structures, which are likely lipid droplets (Arrese and Soulages 2010), as osmium tetroxide preferentially stains unsaturated fatty acids. Adipocytes in *P. dentata* workers appeared very similar to those described in the heads of *Monomorium pharaonis* ant queens (type I cells of Jensen and Borgesen (2000)). Also, lipid droplets often appeared to be floating freely in the hemolymph outside of adipocytes. In AC1 preparations, many adipocytes/lipid droplets were present in loose aggregations throughout the hemolymph of the lateral head capsule and were often observed interspersed in the MC muscle between the developing fibers (Figs. 2a, 3a, and 5a). Adipocytes/lipid droplets were less common in 3-day-old (Fig. 2b) and 6-day-old (Fig. 2c) minor worker preparations and were

Table 1 Summary of head muscle measurements (in micrometers) from minor and major workers from colonies 2 and 3

Colony	Antennal muscle		Pharynx dilator		Slow mandible closer		Fast mandible closer	
	AC1	AC4	AC1	AC4	AC1	AC4	AC1	AC4
Minor workers								
Colony 2	10.1 (2, 1.75)	13.8 (1, n/a)	11.1 (3, 0.49)	14.8 (2, 2.10)	10.3 (3, 2.00)	21.5 (2, 2.10)	12.4 (3, 1.68)	24.8 (2, 1.22)
Colony 3	10.3 (2, 0.52)	17.8 (1, n/a)	12.2 (2, 0.52)	16.7 (4, 1.94)	11.0 (2, 0.87)	24.2 (4, 2.40)	15.7 (2, 1.92)	23.2 (4, 1.63)
Combined	10.2 (4, 1.06)	15.8 (2, 2.80)	11.6 (5, 0.75)	16.1 (6, 2.01)	10.6 (5, 1.53)	23.3 (6, 2.51)	13.7 (5, 2.35)	23.8 (6, 1.60)
Major workers								
Colony 2	10.4 (2, 1.40)	14.8 (4, 0.73)	13.0 (2, 0.87)	14.8 (4, 1.54)	12.2 (2, 1.57)	26.2 (4, 2.71)	16.7 (2, 1.22)	27.7 (3, 2.11)

Values are shown as mean (*N*, SD)

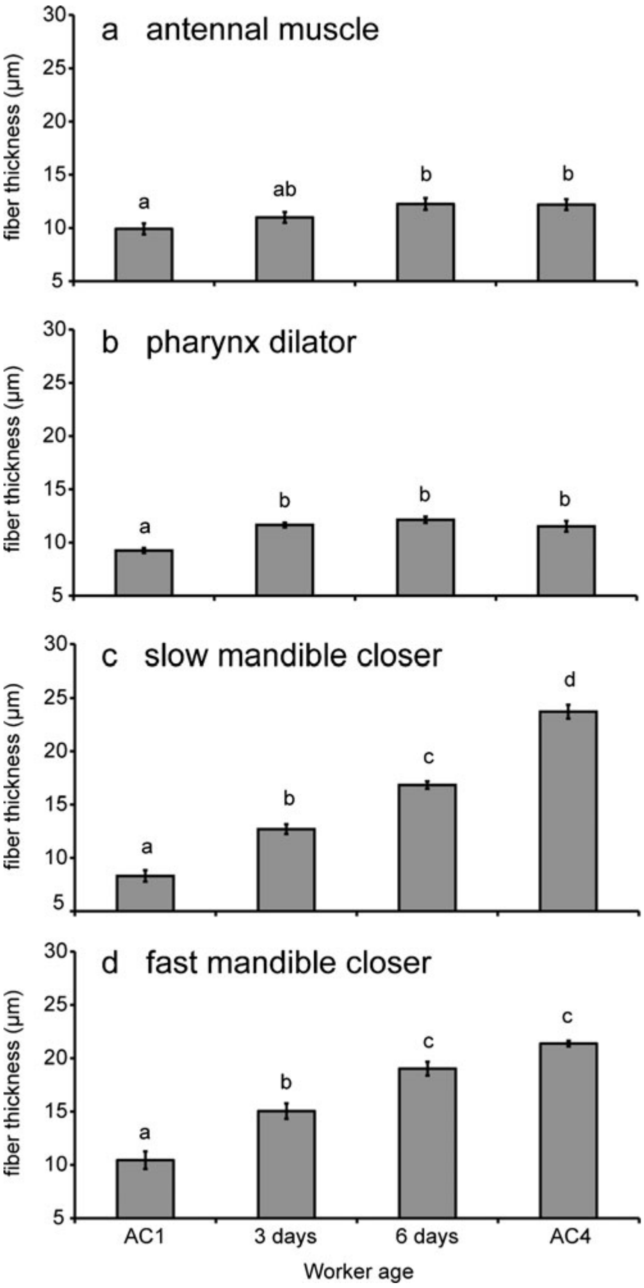

Fig. 4 Mean (± SEM) muscle fiber diameter of colony 1 workers of different ages in **a** antennal muscle, **b** pharynx dilator, **c** slow mandible closer fibers, and **d** fast mandible closer fibers. Levels that do not share *letters* are significantly different (Tukey–Kramer post hoc comparisons)

increasingly concentrated toward the muscle periphery in these age cohorts. In AC4 samples, the posterio-lateral head capsule was usually completely filled with MC comprised of closely packed muscle fibers. Adipocytes/ lipid droplets were rarely present within the muscle of these workers (Figs. 2d, 3b, and 5b), but were sometimes observed in small numbers at the periphery of the muscle and in the hemolymph of the ventral portion of the head

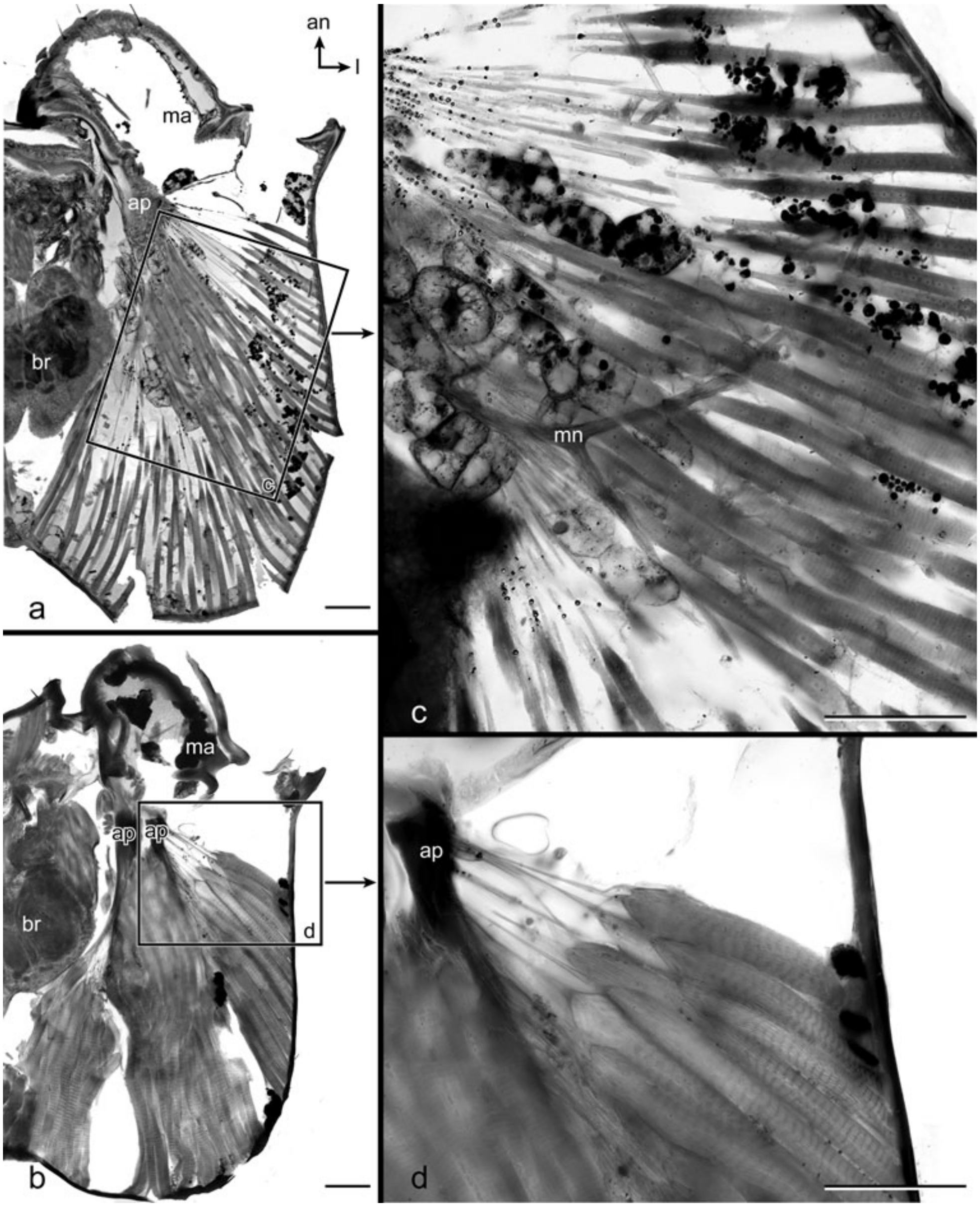

Fig. 5 Mandibular closer muscles in young (AC1; **a**, **c**) and old (AC4; **b**, **d**) major workers from colony 2. *Insets* in **a** and **b** show approximate areas enlarged in **c** and **d**, respectively. Note that in **a**, the cuticle of the head capsule was broken during sectioning. *an* anterior, *ap* mandible closer apodeme, *br* brain, *l* lateral, *ma* mandible, *mn* mandible closer nerve. *Scale bars*=100 µm

capsule underneath the MC. Fibers of the AM and PD were closely packed in all age groups sampled, and we never observed adipocytes or lipid droplets between fibers in these muscles in any age cohort (Fig. 2).

Discussion

We identified a novel and significant anatomical correlate of behavioral development in adult *P. dentata* minor workers: fibers of functionally important head muscles all grew significantly in thickness after adult emergence, with the MC undergoing particularly striking and prolonged development extending beyond the first 6 days of adult life. We also confirmed this pattern in majors, showing that young workers of both subcastes emerge as adults without mature cephalic musculature. To our knowledge, these findings represent the only described case of maturation of non-flight muscles in an adult social insect. Given the importance of these muscle groups for virtually all worker tasks, including feeding (PD), olfactory, gustatory, and tactile probing (AM), and nursing, foraging, and defensive tasks that require mandibular manipulation (MC), our results suggest that muscle growth is a significant contributor to worker behavioral development and the ergonomic efficiency of task performance in *P. dentata*.

The importance of developmental processes in generating division of labor and behavioral maturation in *Pheidole* and other social insect taxa has recently been emphasized (see references cited in the “Introduction”). However, models of division of labor that incorporate developmental change, such as the response threshold model (Beshers and Fewell 2001; Robinson and Page 1989; Robinson et al. 1994), generally focus on how social information and workers' task preferences interact to influence which tasks are performed in a given context. For example, developmental changes in worker brains, such as age- and experience-related neuroanatomical remodeling, aminergic neuromodulation, and gene regulation, have been proposed to affect the sensory responses and task thresholds of aging workers and therefore mediate age-related behavioral development (e.g., Alaux et al. 2009; Barron and Robinson 2005; Jones et al. 2009). Our study emphasizes that comprehensive models of age-related behavior should be extended beyond neural processes that underscore worker sensory abilities and response thresholds to include biomechanical, anatomical, and physiological factors that may indirectly affect worker task performance (Robinson 2009).

All three muscles examined in our study undergo post-eclosion growth, but differences in the rate and magnitude of growth in *P. dentata* minors suggest that workers of this subcaste, which make up the large majority of colonies and perform most colony work, acquire specific behavioral capabilities at different times. Because a muscle's maximum force output is proportional to its cross-sectional area, all else being equal, the great dissimilarity between young and old workers in MC fiber thickness (particularly in forcefully contracting slow fibers) likely translates into substantial differences in maximal bite force. Additionally, the lack of striation as well as the beaded appearance of some AC1 MC fibers suggests that the synthesis and assembly of the contractile apparatus (myofibrillogenesis) is not completed in these fibers at adult emergence. The difference in MC function and maximal bite force between young and old workers is thus likely even greater than what is indicated by differences in fiber thickness alone. Furthermore, the 11-day period we estimate is required for slow MC fibers to reach their mature thickness in minor workers is conservative. Muscle fiber growth likely decelerates over time and approaches an asymptote after which there is little growth until death, while our simple linear model predicts a constant rate of growth at all time points from eclosion up to the time when the muscle fibers have reached their mature size. Therefore, it is probable that minor workers attain full maturity of slow MC fibers when they are older than 11 days. Finally, it is doubtful that the soft, untanned cuticle of newly eclosed workers could resist deformation during forceful mandible closer contractions even if this muscle was fully developed at eclosion.

These observations strongly suggest the existence of developmental constraints on the use of the mandibles and, therefore, constraints on task attendance and the efficiency of task performance, in young adult *P. dentata* workers. Activities that require maximal force output by the MC, such as attacking and pinning prey or predators, likely cannot be effectively performed soon after eclosion by members of either subcaste. AC1 and AC2 workers (up to approximately 7 days post eclosion) are never observed attempting to engage in these tasks in unmanipulated laboratory colonies (Seid and Traniello 2006; ML Muscedere, personal observation). The lack of MC development in newly eclosed workers may also affect the performance of tasks that appear to require less bite force, such as manipulating and carrying brood by minor workers, given the fact that the youngest minors are less likely to perform these tasks (Muscedere et al. 2009). By contrast, the relatively modest and rapid increases in AM and PD fiber thickness in minor workers suggest that these muscles are largely functional at or soon after eclosion. The soft cuticles of young workers are also more likely to be able to withstand the small forces generated by these muscles. Thus, adult workers may acquire the abilities to feed, interact socially, and exchange information through antennal contact before they are able to effectively use their mandibles. We have not examined the motor neurons supplying these muscles, but by analogy with other insect and muscle systems (Bayline et al. 2001; Fernandes and Keshishian 1998, 2005; Hughes and Salinas 1999), it is likely that the delayed development of MC fibers in *P. dentata* correlates with and depends on the elaboration of the mandibular motor neurons. In the termite *Hodotermopsis sjostedti*, for example, the subesophageal ganglion and its constituent mandibular motor neurons are larger in soldiers, which have larger mandibular muscles and powerful biting mandibles, than in conspecific pseudergates ("workers"; Ishikawa et al. 2008). Interestingly, age-related subesophageal ganglion expansion in both subcastes of *P. dentata* is coincident with head muscle maturation (ML Muscedere and JFA Traniello, in review). We would therefore expect mandibular motor neurons in the subesophageal ganglion of *P. dentata* workers to develop more slowly than motor neurons supplying the antennal or the pharyngeal muscles.

Precisely how variation in mandible closer muscle maturity between young and old *P. dentata* workers translates into differences in maximal bite force remains unknown. Biting forces have been directly measured for workers of the carpenter ant *Camponotus rufipes* (maximum forces about 40 mN; Paul and Gronenberg 2002) and the trap-jaw ant *Odontomachus bauri* (up to 69 mN per mandible; Patek et al. 2006), and mandible closer muscle volumes have been estimated for several ant species (ranging from 10 μm^3 in small *Leptothorax sordidulus* to more than 1,700 μm^3 in large bulldog ants, *Myrmecia*; Paul and Gronenberg 1999). It could be possible, in principle, to estimate bite forces of mature *P. dentata* workers based on empirically derived relationships between mandible closer muscle volumes and bite forces. However, to the best of our knowledge, such studies have not been performed except in major workers of *Pheidole obtusospinosa*. In this case, bite forces are linearly correlated with head width, and absolute mandible muscle volume is significantly greater in supermajors than in small majors (M. Huang, in preparation). Additionally, the fact that muscle fiber sarcomeres do not appear fully formed or aligned in young *P. dentata* workers suggests that their muscles generate smaller forces than would be expected based on muscle fiber volume alone. Future experiments involving direct measurements of bite force in workers of different ages, and studies on the minimal forces required for tasks such as prey capture, will therefore be necessary to fully describe the effect of post-eclosion muscle maturation on task capability in *P. dentata*.

The significance, if any, of the changes in adipocyte/lipid droplet distribution in the lateral head capsule to MC development is unclear. In holometabolous insects, larval fat bodies typically dissociate into single cells during metamorphosis (Haunerland and Shirk 1995). Dissociated larval fat cells can undergo cellular remodeling and aggregate to form the adult fat bodies (as in lepidopterans), or cells can undergo programmed death while the adult fat bodies develop independently (as in dipterans). Dissociated larval adipocytes can persist in newly eclosed adults and typically continue to decline in frequency as either of these processes complete (Haunerland and Shirk 1995). The occurrence of these processes in *Pheidole* could explain the observed age-related changes in adipocyte/lipid droplet abundance in a way unrelated to MC development. Alternately, rapid MC development and synthesis of contractile proteins may be facilitated in newly eclosed workers by energy and resources provided by these fat bodies; this hypothesis is plausible given the spatial distribution of adipocytes/lipid droplets in the head capsule (within the developing MC muscle, but not the AM or PD).

Cephalic muscle maturation after eclosion joins a growing list of neurophysiological and neuroanatomical correlates of aging and behavioral development in adult *P. dentata* workers (Seid et al. 2005, 2008; Seid and Traniello 2005) and represents a novel proximate factor associated with the reduced behavioral diversity and lower task efficiency of young workers (Muscedere et al. 2009; Seid and Traniello 2006). Less well understood are the evolutionary factors that affect worker developmental maturity at eclosion. Comparisons between social and solitary hymenopterans, or between solitary and social phenotypes of facultatively social species, may address the question of whether social organization is coupled with differences between insects in development timing and level of maturity at eclosion.

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