The loss of flight in ant workers enabled an evolutionary redesign of the thorax for ground labour

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Research

Keywords: Formicidae, Mutillidae, queen, Micro-CT, Muscles, Endoskeleton, Central Place Foraging

DOI: https://doi.org/10.21203/rs.3.rs-37796/v1

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Abstract

Background Explanations for the ecological dominance of ants generally focus on the benefits of division of labour and cooperation during foraging. However, the principal innovation of ants relative to their wasp ancestors was the evolution of a new phenotype: a wingless worker caste optimized for ground labour. Ant workers are famous for their ability to lift and carry heavy loads, but we know surprisingly little about the morphological basis of their strength. Here we examine the consequences of the universal loss of flight in ant workers on skeletomuscular adaptations in the thorax for enhanced foraging on six legs.

Results Using X-ray microcomputed tomography and 3D segmentation, we compared winged queens and wingless workers in Euponera sikorae (subfamily Ponerinae) and Cataglyphis savignyi (subfamily Formicinae). Workers are characterized by five major changes to their thorax: i) fusion of the articulated flight thorax (queens) into a rigid box optimized to support the muscles that operate the head, legs and abdomen, ii) redesign of internal cuticular structures for better bracing and muscle attachment, iii) substantial enlargement of the neck muscles for suspending and moving the head, iv) lengthening of the external trochanter muscles, predominant for the leg actions that lift the body off the ground, v) modified angle of the petiole muscles that are key for flexion of the abdomen. We measured volumes and pennation angles for a few key muscles to assess their increased efficacy. Our comparisons of additional workers across five genera in subfamilies Dorylinae and Myrmicinae show these modifications in the wingless thorax to be consistent. In contrast, a mutillid wasp showed a different pattern of muscle adaptations as a result of the lack of wing muscles.

Conclusions Rather than simply a subtraction of costly flight muscles, we propose the ant worker thorax evolved into a power core underlying stronger mandibles, legs, and sting. This contrasts with solitary flightless insects where the lack of central place foraging generated distinct selective pressures for rearranging the thorax. Stronger emphasis is needed on innovations of social insects as individuals at the phenotypic level to further our understanding of the evolution of social behaviours.

Background

Ants are among the most abundant terrestrial animal groups on Earth, and the benefits of division of labour within colonies is thought to be the central factor in their ecological success. Ants stand out from social bees and wasps because their workers are universally flightless. This striking morphological differentiation between castes avoids trade-offs and optimizes for ground abilities. Ant reproductives (queens and males) need to fly when they are young, but workers are freed from the need to find mates and disperse. Instead, ant workers are famous for their abilities to lift and carry objects [1–5], suggesting adaptations for enhanced foraging on six legs. The morphology underlying those enhanced abilities, however, remains unclear.

All social insects are central place foragers—food is brought back to the nest instead of being eaten on the spot—and the physical ability to retrieve comparatively large amounts of food is paramount [6]. While the workers of social bees and wasps bring back their food by flight, ant workers transport food by walking. Importantly, ant workers are strong enough to lift and carry aloft prey and other food items,
unlike various solitary predatory wasps that drag prey when too large to retrieve by flight. Foraging efficiency is also impacted by speed of walking, while running is essential to escape from predators (in contrast, wasps and bees escape by flight). Moreover, the abdomen can serve to hold liquid food, while deftness of its tip is essential in predation and defense to sting or spray formic acid. (The sting has its own set of muscles for extrusion and retraction).

The thorax (known as ‘mesosoma’ in Apocritan hymenopterans because it includes the usual three segments fused with the first true abdominal segment, called propodeum [7–9]) bears the wings and legs and supports the head and abdomen, hence it is subjected to often-conflicting mechanical constraints. Keller et al. (2014) [10] identified a shift in the relative size of the external dorsal plates (where muscle fibres attach) in the anterior thorax as a critical difference between winged queens and flightless ant workers. Reduction of the mesonotum (dorsal plate of the second thoracic segment, T2) and concomitant expansion of the pronotum (dorsal plate of T1) in workers reflect a trade-off: elimination of flight-related structures allows an increase of head muscles in the prothorax. However, the posterior external plates vary little between queens and workers, and it remained unclear if there is also remodelling of the endoskeleton and muscles transmitting force to the legs and abdomen. Among the Hymenoptera, only ant workers and a few solitary parasitoids (e.g. females of Chyphotidae, Mutillidae, and Tiphiiidae) are wingless. The anatomy of the wingless thorax was investigated early on by Lubbock (1881) and Janet (1907) [7,11], described externally by Tulloch (1935) and Reid (1941) [9,12], and recently described using 3D reconstruction [13]. Despite this accumulation of data over more than a century, the morphological consequences of wing loss have hardly been investigated.

Winglessness in ant workers has been considered a reduction to facilitate mobility in cramped environments [6], without examining consequences on thorax architecture. We hypothesized that the evolution of a worker caste freed from the anatomical and functional constraints of flight is a critical innovation that allowed the remodelling of the thorax for ground labour. Using X-ray microcomputed tomography (micro-CT), we examined a phylogenetically broad sample of ant genera (Table 1), and performed detailed segmentation to compare conspecific workers and winged queens in two distantly related exemplar genera, Euponera (subfamily Ponerinae) and Cataglyphis (Formicinae). We show significant fusion of external plates and modifications of endoskeleton, neck, leg, and petiole muscles. Furthermore, we discuss that ant workers have a distinct thorax relative to other wingless insects (including solitary wingless hymenopterans), and explain this as a function of sociality and living in perennial nests: central place foraging selects for optimal gathering of food.

**Methods**

**Taxon selection**

We focused on two species with workers of similar body sizes, belonging to distantly related ant subfamilies. Euponera sikorae is a generalized species in the subfamily Ponerinae, and a good example of the lowest queen-worker dimorphism in ants, likely to represent the ancestral condition [14]: the thorax...
of workers remains generally stout, with parallel sides and an uninterrupted dorsum. *Cataglyphis savignyi* is an exemplar of the large subfamily Formicinae, and illustrates a pronounced level of queen-worker dimorphism [15]: the thorax of workers is slender, especially in its middle (Additional file 8). Head width of workers varies from 1-3 mm (N. Lecocq De Pletincx pers. comm.), and we scanned an individual similar in body size to the queen.

The most recent common ancestor of *Cataglyphis* and *Euponera* is a deep node in the ant phylogeny [16]. Thus, it is likely that any similarity in thorax design between these two taxa is shared by most ants. Our primary analysis concentrates on two species because μCT analysis is labour-intensive. However, we have examined scans of workers from a number of genera across the ant phylogeny (Table 1) to verify that the changes we describe are general features of the ant thorax. We also examined the thorax of a solitary Mutillid wasp, representing an independent loss of flight within the Hymenoptera.

*Euponera sikorae* was collected in Madagascar (Zahamena NP); *Cataglyphis savignyi* in Israel (Arad Park); subfamily Mutillinae, tribe Smicromyrmini or Trogaspidiini in Uganda (Kanyawara Biological Station, Kibale).

**Specimen preparation and micro-CT scanning**

Micro-CT scans were carried out at OIST using a Zeiss Xradia 510 Versa 3D X-ray microscope. Ant specimens were stored in 90% ethanol, then stained in a 2M iodine solution for a minimum of 24 hours and transferred into pipette tips filled with 99% ethanol before scanning. Scan settings such as current, voltage, and exposure were adjusted for each specimen to yield maximum scan quality. Full 360 degree rotation resulted in 1601 projections. Scans had resolutions of $993 \times 1013 \times 988$ pixels (vertical stitching increased this to $3159 \times 3514 \times 988$ pixels for *Euponera* and *Cataglyphis* queens, respectively). Post-imaging 3D reconstruction was performed using the Zeiss Scout-and-Scan Control System Reconstructor software (version 11.2) and output files were saved in DICOM format.

**Cuticle thickness measurements**

Scan files were loaded into Drishti 2.6.5 [17] to generate 3D models. Two ‘Clipping Planes’ were positioned at a 90° angle across pronotum and propodeum to get the most accurate measurements of cuticle thickness. For every plane, a ‘Viewport’ was added to visualize the section, and ten measurements were made on each section (clockwise, numbered from 1 to 10) using the ‘Path’ function.

**Segmentation**

We assessed homologies of muscles and sclerites relative to *Apis mellifera* [18] in which both workers and queens are winged. Muscle nomenclature follows Friedrich & Beutel 2008 and Liu et al. 2019 [13,19]: for example, Idvm5 denotes a dorsoventral muscle in T1 inserting on the propleura (ventral plate), while Ilscm6 is a sterno-coxal muscle in T2 inserting on the trochanter.
We combined Amira 6.3.0 and ITK-SNAP 3.6.0 [20], with manual segmentation every five to ten slices followed by either the ‘Interpolation’ tool in both softwares or the ‘region competition’ algorithm for semi-automatic segmentation in ITK-SNAP. Subsequently, we manually cleaned the edges of automatically segmented structures, in particular between neighbouring muscles that are difficult to recognize as separate structures by the algorithm.

Muscle volume measurements

Muscle segmentations were exported as mesh files (.stl) from ITK-SNAP into the software Blender 2.81 (www.blender.org). Spaces between fibres result from shrinkage during ethanol preservation, and we integrated over entire muscle segmentations. After scaling using voxel size, we fitted a 3D object (usually an Icosphere) to each muscle mesh. We achieved good fit using 1) the “subsurface” modifier on the 3D object to create more polygons and 2) the “shrinkwrap” modifier to fit this 3D object around the muscle mesh. We measured the volume of this 3D object and used it as the physiological muscle volume. In case of a poor fit, we split the muscle mesh into two meshes of simpler shapes and fitted 3D objects separately. Volumes were then normalized (Table 1) using the volume of the inner thorax which was segmented with Amira by filling the space in between thorax cuticle and closing cuticle openings (i.e. head, leg and petiole insertions).

Fibre tracing and angle measurement

Vertebrate muscles have mostly parallel fibres between points of attachment (e.g. bone extremities), thus measuring physiological cross-sectional area (PCSA) is meaningful to infer muscle force. In contrast, insect locomotory muscles can ‘spread out’ as they increase in size, i.e. additional fibres attach to a greater area of the endo- and exoskeleton, then converge to a tendon. Hence, pennation angles are more relevant to distinguish between efficient and less efficient fibres. We used segmentations as a basis to compare pennation of petiole muscles for Euponera queen and worker. We traced individual fibres with Amira XTracing 2019.2 and used Blender 2.81 to calculate the angle between each fibre and the direction of force production of the respective muscle (Katzke et al. in prep.). In this approach, the coordinate system defined by the micro-CT image stack is utilized to treat orientations of anatomical features as vectors.

Results

While ant queens retain the ancestral thorax morphology of flying Hymenoptera, we identified five major deviations of the worker thorax involving the exoskeleton, endoskeleton, neck muscles, leg muscles, and petiole muscles. Although our discussion focuses on Cataglyphis and Euponera, these modifications are present across all the ant workers examined (Table 1). Together, they represent the evolution of an enhanced power core for more effective foraging on six legs.

Fusion of the flexible articulated thorax into a rigid thoracic box
The thorax of a winged insect is essentially a flying engine with powerful wing muscles that vibrate articulated external plates called *sclerites*. As in other winged Hymenoptera, ant queens have huge indirect wing muscles (41% and 52% of thorax volume in *Euponera* and *Cataglyphis*, respectively) that fill the prominent mesothorax (T2) but also adjacent segments (Figure 1C and Figure S1). This is because the longitudinal muscles are attached to flexible cuticular invaginations (*phragmata*) of the mesonotum that extend into both the prothorax (T1) towards the front and the propodeum (IA) towards the back (Additional file 1; the metathorax, T3, is much reduced dorsally and only serves for insertion of the hindwings [8]). Hence, much of the relatively small cavity of the prothorax of ant queens is occupied by wing muscles, with the neck muscles squeezed against the exoskeletal outer wall. Flapping of the wings is achieved not by direct muscle action, but by vibration of the entire thoracic box caused by the indirect wing muscles that deform the dorsal plates to which the wings are attached [21]. Flexibility of the thorax and free articulation of the dorsal plates (Figure 1A) are thus essential for flying, and winged ant queens conform to the general groundplan of Hymenoptera.

Ant workers lack wing muscles and the corresponding phragmata, and the plates of T2, T3 and IA are fused into a single rigid structure forming the posterior two-thirds of the thorax (Figure 1B). The first third is the prothorax (T1) housing all the muscles that move the head, and it is greatly enlarged dorsally [10].

Thickness of *Euponera* cuticle is about three times that of *Cataglyphis*, in both queens and workers (Additional file 2). Thickness varies according to dorsal or lateral position of measurements within one segment, and variability is higher within one individual than between castes. Mean thickness is similar for workers and queens except for the propodeum of *Cataglyphis*.

**Redesign of the endoskeleton for optimized bracing and muscle attachment**

Inside the thoracic cavity, three invaginations of the *sternae* (ventral plates) rise up in front of each pair of legs, forming a row of rigid forked pillars. These *furcae* function as internal attachments for muscles that run between segments (head-to-prothorax, prothorax-to-mesothorax, metathorax-to-petiole), and muscles that move the legs (Figure 2). In queens, the main stem of the furcae rise not more than one-third of the thorax's height before bifurcating towards the sides. This configuration results from the constraint of the huge longitudinal wing muscles that occupy the top two-thirds of the thoracic cavity (Figure 1C, Additional file 1). In workers, the loss of wing muscles freed space within this cavity, allowing changes in size and shape of all three furcae.

The furcae of T2 and T3 are ancestrally fused into a single structure (Figure 2, Additional file 3). The T2 furca bears a narrow transverse bridge that connects the distal points of its forked arms. In winged Hymenoptera, these lateral arms connect with the walls of the upper thorax via a short muscle, providing the necessary flexibility for flight. In ant workers this muscular connection is lost, and we found that the T2+3 furcal arms are fused with the walls of the thorax, forming an internal bracing structure that possibly increases the overall rigidity of the thoracic box.
In contrast with the short and fused T2+3 furcae, the profurca (T1) is larger and more elaborate across Hymenoptera. Both the median stem and the forked lateral arms are stout and support a sizeable bridge, shaped like a thin horizontal platform (Additional file 4). This flexible platform provides attachment surfaces for three neck muscle pairs on its anterodorsal side (muscles IVM9, IVM1, and IVM3) and a key leg muscle on its posteroventral side (see below). In flying queens, this platform is horizontal to allow the longitudinal wing muscles and oesophagus to run dorsally (discussed in Keller et al. 2014 [10]). Consequently, the neck muscles attach to the platform at a low angle. However, the lack of wing muscles in ant workers removes this constraint, and the platform takes a more vertical orientation resulting in a greater attachment surface and more favourable angles of origin for the large muscles that insert directly on the back of the head and support it (see below).

Unlike the T2+3 furcae, the profurca is not fused with the thorax walls, instead its median stem articulates freely in front of the foreleg insertions while its lateral arms connect to the thorax walls with membranes and a short muscle. The neck muscles act antagonistically to both foreleg muscles (see below) and the intersegmental muscles (IVM7) that connect the profurca with T2+3 furcae (Figure 2), transmitting stress from the head to all legs. Similarly, posture is maintained at the back of the thorax with intersegmental muscles (IIIIVM2, IIIIVM7) connecting the T2+3 furcae to the petiole, transmitting stress from the abdomen to the thoracic box.

Reorganization of the neck muscles
The neck articulation in Hymenoptera is composed of four skeletal elements (Additional file 5): the postocciput, a short cup-shaped extension of the back of the head; a pair of triangular propleura that are ventrally situated in T1; and the profurca which sits inside T1. The head’s postocciput has a strong articulation with the thorax via a stiff neck membrane [22] as well as the anterior apodemes of the propleura [18]. The propleura are never fused to one another and these large plates can shift antagonistically for sideways and rotational movement of the head [23].

No muscles inside the head are involved in the movement of the head relative to the thorax. Rather, all neck muscles are prothoracic, moving the head either directly by inserting on the postocciput or indirectly by inserting on the propleura. In both queens and workers, five pairs of direct muscles lift the head up-and-down (Figure 1 and Additional file 4). The lack of the anterior phragma in workers (associated with loss of wing muscles) affects especially muscle pair IVM1. In queens, IVM1 originates at the sides of their pliable phragma (Figure S5), while in workers IVM1 originates further back at the anterior margin of the roof of T2 (part of the rigid thoracic box; Figure 2). Consequently, the better anchoring of muscle IVM1, its extra length and lower angle of insertion to the postocciput (Additional file 6) give greater support and strength to the worker head in comparison to that of winged queens. The direct muscles ITPM2 originate at the base of propleura, hence their geometry differs little between queens and workers, but ITPM2 is relatively larger in workers for both species (Figure 4, purple). In contrast, muscle pairs IVM9 and IVM3 are considerably affected by the queen-worker difference in the angle of the profurca platform (Additional file 4). In queens, as in wasps and bees, this platform is completely
horizontal, and thus, these muscles have a very shallow angle to their origin. Contrary to queens, the upright inclination of the platform in workers allows a more favourable perpendicular angle of origin and a greater attachment surface for the equivalent muscles.

Two indirect muscle pairs are mostly responsible for sideways and rotational head movement by inserting on the anterior ends of the propleura. Ivlm1 originates on the profurca and thus benefits from the more favourable inclination of its platform in workers. Idvm5 shows the most spectacular difference between queens and workers (Fig. S4). This large muscle pair originates at the pronotum (dorsal plate of T1) which is always considerably larger across worker ants. In addition to the increased area of attachment, the absence of wing muscles allows this muscle to support the propleura from a diagonal rather than a vertical angle. Both Idlm5 and Ivlm1 are relatively larger in workers for both species (Figure 4, red and dark red lines).

One crucial leg muscle differs between workers and flying queens

Back and forth movements of insect legs are controlled by a series of short muscles that originate within the thorax and insert around the opening of the coxae, the basalmost segment of each leg that articulates with the body. Leg flexion and extension, on the other hand, are controlled by muscles that reside entirely within the segments of the legs [18]. A notable exception is the external trochanter muscles. One per leg (Iscm6, IIscm6, IIIscm6), these muscles originate in the thorax but end in a long tendon that crosses the coxae and inserts on the trochanter (second leg segment) of all legs (Figure 3). Contraction of the external trochanter muscles causes depression of the leg, lifting the body and carrying the ant’s weight plus any load. Because these muscles are housed outside the legs, with only their tendons crossing the coxae, their action is independent from the rotational motion of the coxae, and force is transmitted efficiently from the thorax to the legs no matter if walking or standing.

While the muscles of the coxae and those internal to the legs do not differ much between the castes, our segmentations show that the external trochanter muscles are very distinct in length, geometry, and place of origin between queens and workers (Figure 3, Additional file 7). In queens, the external trochanter muscles are shorter because they originate on the furcal arms of their respective segment, just below the longitudinal wing muscles. The absence of wing muscles in workers allows dorsal elongation of the mid and hind external trochanter muscles (IIscm6, IIIscm6), which attach either on the roof (Cataglyphis) or lateral walls (Euponera) of the rigid thoracic box. Queen-worker differences are minor for the forelegs: in both castes external trochanter muscles Iscm6 originate at the lower surface of the T1 furcal platform (Figure 3). These muscles, therefore, act antagonistically to the neck muscles originating at the upper surface of this platform, helping to pull the head and transmitting force effectively from the head-neck articulation to the front legs. Workers in both species have a relatively larger IIscm6 than queens, and in Cataglyphis Iscm6 and IIIscm6 are also larger.

Enlargement of the petiole muscles
As with the head, all muscles responsible for articulating the free part of the abdomen are housed inside the thorax. Four muscle pairs (two dorsal, two ventral) insert on the anterior narrow end of the petiole, which protrudes in the back of the thorax. By acting antagonistically to each other, these muscles control both vertical and horizontal movements of the abdomen.

Our segmentations show that the two dorsal pairs differ strongly between queens and workers due to the presence/absence of the flight apparatus (Figure 3). Muscles IA1 and IA2 originate on the roof of the propodeum (middle and lateral, respectively), but because of the posterior end of the horizontal wing muscles in queens, these petiole muscles are squeezed in the narrow space between the posterior phragma and the exoskeleton (Additional file 1). In workers, the lack of wing muscles means that muscles IA1 and IA2 fill the propodeal cavity entirely. Although relative muscle volumes are similar between queens and workers (Figure 4), differences stand out in the fibre geometry in *E. sikorae*. First, IA1 has longer fibres in the worker than in the queen despite the smaller size of workers. In addition, IA1 fibres attach at significantly lower angles in the worker, resulting in greater efficiency (Figure 5). Second, IA2 fibres are longer in the queen and attachment angles are not significantly different between worker and queen. However, the worker has 30% more muscle fibres with a broad distribution of angles, revealing how much this muscle can fan out in the absence of wing muscles.

Muscles IA1 and IA2 differ markedly between the workers of the two different subfamilies (Figure 4). In *Euponera* workers, these petiole muscles originate on the entire roof of the elongated propodeum. In contrast, in the dome-shaped propodeum of *Cataglyphis*, the equivalent muscles do not extend forward and are relatively smaller in the worker than in the queen; instead the anterior roof is the origin for the external trochanter muscles (IIIscm6) of the hind legs (Additional file 7).

Comparison with other flightless insects

Flight is the ancestral state across the insect tree of life, and its evolutionary loss occurred multiple times independently [24]. While a broad survey of flightlessness across insects is beyond the scope of this paper, we considered whether the modifications described here are specific to social as opposed to solitary lifestyles. In general, the lack of massive wing muscles does not lead to the same modifications of the thorax in solitary taxa. Our micro-CT of a female mutillid wasp shows strikingly enlarged abdomen muscles (there is no petiole in Mutillidae), attached to most of the dorsal roof and reaching the anterior mesonotum (Table 1). This is congruent with the origin of the external trochanter of midlegs on the mesofurca, instead of the propodeum roof. Given that mutillids are parasitoids that search for hosts underground and oviposit on the spot [25], this difference in thorax rearrangement likely reflects the absence of any selective pressures for prey transport. Indeed, added strength is not necessarily adaptive in solitary insects that are not central place foragers. In flightless moths (Lepidoptera) [26], ovaries reach into the posterior thorax, and the posterior phragma is retained to shield the ovaries; head muscles are not affected compared to flying males. Beetles (Coleoptera) fly with their hind wings only, hence flight musculature is confined to their enlarged T3 and does not conflict with the neck articulation. A functional
approach makes it possible to interpret the internal adaptations associated with wing loss across insects showing divergent groundplans.

Discussion

Modifications in the thorax of wingless ant workers

Our micro-CT comparison of queens and workers in two distant subfamilies reveals that the evolutionary loss of the flying engine typical of insects allowed for a remodelling of the thoracic skeleton and associated muscles to power earthbound activities. We showed worker-specific modifications in the three endoskeletal furcae that are key for muscle attachment as well as transmission of load between mandibles, legs and abdomen. Our measures of muscle volumes and pennation angles confirmed the conspicuous pattern of repositioning and enlargement seen in 3D segmentations. We highlight the importance of indirect muscles in the prothorax that move the head sideways by shifting two ventral plates (propleura). These adaptations for strength likely result from the selective pressures of central place foraging, that necessitates carrying or dragging food to the nest. Strength is not a general feature of flightless insects but rather an adaptation for maximizing the acquisition of resources for ant colonies. In contrast, solitary mutillid wasps use vacant space in the thorax for much enlarged abdomen muscles, allowing for better performance as a stingig parasitoid.

Our comparative survey across phylogenetically disparate species revealed a consistent pattern of expanded muscles with altered geometry (Table 1). While all ant workers have a similarly modified thorax relative to queens, there are differences reflecting trade-offs in abilities across lineages. Many ponerines, especially "tank-like" ones such as *Euponera*, need more strength to manoeuvre the sting and hold the heavy abdomen level. By contrast, formicines like *Cataglyphis* are highly gracile, fast-running ants with a relatively small gaster, thin cuticle, and they lack a sting. *Euponera* workers use the expanded propodeal roof for petiole muscles only, while in *Cataglyphis* the more dome-shaped propodeum is used as attachment surface for both the petiole and hindleg muscles (Figure 3, Additional file 7).

Functional morphology of the thorax in ants

We describe here the morphological redesign of the thorax, and present hypotheses for their functional implications, but it remains a challenge to directly link morphology with performance. A comprehensive modeling effort focusing on the ant thorax is needed to definitively link structure and function, but this presents a formidable challenge. Although we measured muscle volumes and fiber angles, mathematical analysis of how these changes relate to greater strength or faster contraction speed remains difficult in insects. Moreover, cuticle parameters such as thickness and elasticity need to be integrated in the analysis, but our results indicate that cuticle thickness changes within one individual. Hence simplifying thickness to single values for each individual is a source of errors that complicates mathematical models. Future studies need to refine the biomechanical connection between skeletomuscular specialization of each subsystem (neck, legs and petiole) and overall strength.
The ability to carry heavy prey or liquid food, and to manipulate objects, allows a broader range of potential food items (arthropods live or dead, seeds, honeydew or other sweet secretions) and nest constructions. While activities inside the nests may be less reliant on athletic abilities, foraging encompasses carrying and dragging food, running to escape predators, and the ability to defend and hunt (e.g. mandible dexterity, stinging or formic acid spraying). Given that ant queens retain the ability to disperse by flight in most species, workers were free to combine morphological adaptations for ground labour and social skills. So many benefits derive from the loss of flight in ant workers that, until now, musculoskeletal modifications have been overlooked. In addition to obvious energy savings related to absent wing muscles, both manufacture and maintenance [27], the loss of flight in workers was a decisive innovation that revolutionized colonial economy - cheaper workers allow for more populous colonies when this is adaptive [28].

Conclusions

The attributes of individual phenotypes are likely just as crucial to the evolution of social life as genetic caste differentiation mechanisms, chemical communication, kin-recognition systems, and collective behaviour algorithms. Both morphology and social behaviour need to be integrated to contrast the colonial benefits of having wingless workers in ants as opposed to winged workers in social bees and wasps. All worker castes in Hymenoptera evolved specializations for tasks complementary to those of the queen caste, but the chasm is much less dramatic in social wasps and bees because the workers need to fly. Our insights in the adaptations of a thorax rearranged for strength on six legs reveal that ants maximized the merits of having queen and worker castes, allowing for a much greater divergence in body size compared to social bees and wasps. We propose this redesign of the worker phenotype for ground labour combined with the benefits of sociality is key to understanding the ecological success of ants.

Declarations

Acknowledgements. We thank Serge Aron for providing specimens of Cataglyphis, Francisco Hita-Garcia for the scanned Mutillidae, Denis Brothers for identification of the Mutillidae, Nathan Lecocq De Pletincx for morphometric data on C. savignyi. We thank the OIST Imaging Section for access to the CT-Scanner.

Funding. Subsidy funding to OIST (A.K., E.P.E., R.A.K., and G.F.); collaboration agreement between FCiencias-ID (Portugal) and OIST (R.A.K.); JSPS KAKENHI (JP17K15180) grant (E.P.E.). AB was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No. 754290, “Mech-Evo-Insect”).

Availability of data and materials

All datasets are deposited on Zenodo https://dx.doi.org/xxxx

Authors’ contributions. C.P., R.A.K. and E.P.E. conceived of the study. C.P. and R.A.K. designed the experiments; A.K., J.K. and G.F. conducted the experiments; C.P., R.A.K., A.K., G.F., J.K. and E.P.E. analysed
the data; and C.P., R.A.K., A.B., G.F., A.K. and E.P.E. wrote the manuscript. All authors read and approved the final version of the manuscript. C.P. and R.A.K. contributed equally.

All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript

**Competing interests.** We have no competing interests.

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**Tables**

**Table 1.**
Thorax modifications resulting from the lack of wing muscles in additional taxa from three ant subfamilies (Dorylinae, Formicinae, Myrmicinae) and a female mutillid wasp (subfamily Mutillinae, tribe Smicromyrmini or Trogaspidiini) examined by micro-CT and SEM.

| Subfamily | Species             | Fusion of external plates (SEM)? | Angle of profurca platform? | Origin of direct head muscle Idlm1? | Indirect muscle Idvm5 enlarged? | Origin of mid- & hindlegs trochanters muscles? | Enlarged petiole muscles? |
|-----------|---------------------|----------------------------------|-----------------------------|-------------------------------------|-------------------------------|-----------------------------------------------|--------------------------|
| Formicin ae | Colobopsis truncata | articulate pronotum and mesonotum | more vertical               | mesonotum roof                      | pronotum larger               | mesonotum roof                                      | entire roof               |
| Dorylinae | Dorylus wilverthi   | promesonotal fusion              | more vertical               | mesonotum roof                      | pronotum larger               | mesonotum roof                                      | entire roof               |
| Myrmicinae | Carebara perpusilla | promesonotal fusion              | more vertical               | absent *                            | pronotum larger               | mesonotum roof                                      | entire roof               |
| Myrmicinae | Melissotarsus carii | promesonotal fusion              | more vertical               | absent *                            | extremely large               | mesonotum roof                                      | highly reduced *          |
| Myrmicinae | Messor barbarus     | promesonotal fusion              | more vertical               | mesonotum roof                      | pronotum larger               | mesonotum roof                                      | entire roof               |
| Myrmicinae | Pheidole oculata     | promesonotal fusion              | more vertical               | mesonotum roof                      | pronotum larger               | mesonotum roof                                      | entire roof               |
| Mutillinae | Unidentified        | promesonotal fusion              | tilted back                 | absent #                            | no, smaller than petiole      | mesofurca for midlegs                                | past midlegs only         |

* associated with miniaturisation

# linked to hypognathy

**Figures**
Figure 1

Figure 2
Figure 3
Figure 5

IA1

W<Q (p<0.05)

Fiber Count

IA2

W≠Q (p=0.33)

Attachment Angle

Caste

Queen

Worker

Figure 5
Distributions of fibre angles for the dorsal petiole muscles (top IA1; bottom IA2; see Additional file 7) differ between the Euponera sikorae queen (grey) and worker (red). Fibres with an angle of 0° contract in the same direction as the force exerted by the muscle, hence lower angles mean more efficient fibres. Dashed line indicates mean angle. According to Mann-Whitney rank sum tests for non-normally distributed data, attachment angles in IA1 are significantly lower in worker than in the queen ($W = 27911$, $p < 0.05$) while not significantly different in IA2 ($W = 32485$, $p = 0.33$).
p<0.05). There is no significant difference in distribution of angles between worker and queen in IA2 (W = 18231, p=0.33).

Figure 7

Differences in the proportion of prothorax muscles (left) and muscles of the legs and abdomen (right) between worker and queen in Euponera sikorae and Cataglyphis savignyi. In Cataglyphis, head and leg muscles are relatively larger in the worker, but petiole muscles are bigger in the queen. Measurements of muscle volumes were obtained from mCT scans (Supplementary Table 1).
Impact of loss of wing muscles on geometry of leg muscles and petiole levators (Euponera sikorae). (A) Wing muscles in queens fill the thoracic cavity, limiting the size of adjacent structures. (B) In the absence of wing muscles the external trochanter muscles (orange) of the midlegs (IIscm6) and hindlegs (IIIscm6) are extended to originate high on the roof of the mesothorax (T2) and the lateral walls of propodeum (IA) respectively, while the levators of the petiole (green) increased in volume to fill up almost the entire propodeal cavity.

Figure 9

Tension muscles (red) connect head, furcae, and petiole along the thorax in ant workers (Euponera sikorae). Furcae (blue) are skeletal structures that relay force/stress inside the thorax. A direct muscle (Idlm1, mauve) originates dorsally at anterior edge of the mesonotum and pulls the head up. External trochanter muscles (orange, right legs only). Posteriorly, muscles arising on metafurca insert on the anterior end of the petiole, pulling the abdomen to maintain horizontality. T1, prothorax. T2, mesothorax. T3, metathorax. F1, profurca. F2, mesofurca. F3, metafurca. Cx1, Cx2 and Cx3 are coxae (= first segments) of the fore-, mid- and hindlegs respectively.
Figure 10

Caste differences in fusion of thoracic plates and presence/absence of wing muscles (Cataglyphis savignyi). (A) A large mesonotum (purple) in queens freely articulates dorsally, and (C) carries huge wing muscles (purple) that occupy most of the thoracic cavity. (B) The mesonotum in workers is much reduced and fully fused with the posterior section of the thorax to form a rigid box-like structure (blue). (D) Neck muscles in workers (red, orange, yellow) expand in the absence of wing muscles within the enlarged anterior thoracic cavity (B, red). T1, prothorax. T2, mesothorax. T3, metathorax. IA, propodeum.