Ruling the unruly: larval passiveness enabled size inequality between ant queens and workers

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Abstract

Differences between reproductive and worker castes are essential to eusocial societies. The morphological chasm between castes is highly heterogeneous across the ant phylogeny. The low size dimorphism of basal ant lineages suggests that evolutionary innovations occurred during the diversification of ants, potentially enabling their major evolutionary transition from simple eusocial societies to superorganisms. We measured queen and worker body volumes of 321 ant species belonging to 124 genera and 13 subfamilies to investigate the correlation between dimorphism and a panel of traits linked to complex eusociality. Using comparative phylogenetic methods, we show that higher dimorphism is associated with large colony sizes, distinct worker subcastes, and the loss of worker ability to mate. However, these traits correlate with both dimorphism and body volume variations of female castes. In contrast, larval morphology associated with passive larval behavior correlates only with dimorphism and not body volume variations of queens and workers. We argue that creating a large size differential between totipotent larvae with identical reproductive aspirations requires firm colony control over larval caste fate, which can only be exerted if larval nutrition is regulated by workers. We suggest that the evolutionary innovation of individualized worker-to-larva feeding, for example through trophallaxis, brought about passive larvae in some major ant lineages. Our results indicate that resolution of the conflict between larval reproductive aspirations and colony priorities was a necessary step in the evolution of complex eusocial societies.

Keywords: Major evolutionary transitions, caste dimorphism, larval caste fate, superorganism, larval morphology

INTRODUCTION

From the somatic and germline cells of multicellular organisms to the sterile workers and reproductive queens of social insect colonies, cooperation and conflict management between individual units is necessary for the functioning of the higher organizational unit. Division of labor is a central feature of such deeply cooperative systems (West et al. 2015). In eusocial Hymenoptera, adults are divided into reproductive and nonreproductive castes. Queens and males specialize in reproduction while workers specialize in food collection and brood care. This fundamental inequality in reproductive potential is key to the success of cooperative insect societies (Wilson 1971, Boomsma and Gawne 2018, Holland and Bloch 2020).

The striking ecological success of ants has been associated with extreme modifications of reproductive and worker caste morphologies, for example, a major evolutionary simplification of worker bodies once workers lost the need for flight (Peeters and Ito 2015, Peeters et al. 2020). Large size differences between queens and workers are associated with greater morphological divergence, leading to greater specialization of the castes in their respective tasks (Trible and Kronauer 2017). In addition, large size differences benefit dispersing young queens in the production of their first workers when they found new independent colonies (Peeters 2020).

The morphological chasm between queen and worker castes is highly heterogeneous across the ant phylogeny. At one extreme, early-branching lineages have small colonies of predatory specialists and minimal morphological differences between reproductives and workers. At the other extreme, ecosystem-dominating generalist species tend to have colossal colonies and high levels of queen:worker dimorphism (Peeters 1997, Keller and Peeters 2020). The establishment of such morphological divergence between female castes is unfortunately still unclear. Indeed, no broad comparative studies on size dimorphism have been conducted on ants to date. Consequently, the factors driving and enabling strong dimorphism and its consequences on ant evolution and ecology remain unclear.

Female larvae in social Hymenoptera are generally totipotent and can turn into either a queen or a worker. In a few species, this is specified as early as in the egg (Anderson et al. 2008, Weyna et al. 2021). In many ant species adult female caste is specified during larval development through ‘environmental’ (social) caste determination (Schwander et al. 2010). These distinct caste fates require different biochemical and gene expression pathways to be activated and repressed (Wheeler 1986, Rajakumar et al. 2018, Qiu et al. 2022), and most certainly different nutrition, at the very least in terms of quantity (e.g. *Apis mellifera*) and

royal jelly (Kucharski *et al.*, 2008)). In species where larvae are totipotent, each female larva is in conflict with the rest of the colony in that she could potentially have greater fitness by becoming a queen, while during the colony's growth phase, the rest of the colony benefits from the production of workers (Bourke and Ratnieks 1999, Wenseleers et al. 2003). Some authors have argued that the reproductive aspirations of larvae could influence dimorphism, although it has not yet been tested to the best of our knowledge (Wheeler and Wheeler 1976, Kaptein et al. 2005, Wolf and Seppä 2016).

Using comparative phylogenetic methods and a panel of ecological, behavioral, morphological, and life-history traits, we sought to unravel the origins and consequences of the queen-worker dimorphism in ants. We tested whether a greater dimorphism enabled more advanced eusociality and whether the reproductive aspirations of larvae can hinder the development of strong dimorphism. We finally analyze key traits that the ants acquired to build stronger asymmetries between queens and workers.

RESULTS

We wanted to understand what factors enabled the evolution of highly unequal phenotypic outcomes in ants, and what traits may have been consequences of this evolution. To understand the evolution of queen:worker caste dimorphism across the ant phylogeny we measured the body volume of one queen and one worker for 321 species in 124 genera and 13 subfamilies. We then analyzed the evolution of body volumes, consequent queen:worker (Q:W) dimorphism, and a panel of traits that may have enabled the evolution of highly unequal phenotypic outcomes in ants, or that may have been consequences of such asymmetry.

Queen and worker body volume evolution

Queen and worker body volumes shifted dramatically over evolution (Fig. 1), both increasing and decreasing in size. In our dataset, queen volume ranged from a maximum of 452 mm^3 in *Dorylus wilverthi* to 0.12 mm^3 in *Cardiocondyla obscurior*. Worker volumes ranged from 355 mm^3 in *Dinoponera gigantea* to 0.057 mm^3 in *Solenopsis picta*. Queen size evolved faster than did worker size (σ^2 *queen* = $8.4e10^{-3}$, σ^2 *worker* = $5.8e10^{-3}$, Likelihood Ratio Test: $p < 0.001$) indicating different levels of selection pressure on the two castes.

Queen:worker dimorphism is phylogenetically heterogeneous

Phylogenetic analysis of the evolution of queen:worker dimorphism revealed that dimorphism followed a lambda model of evolution with a phylogenetic signal λ of 0.87,

indicating that dimorphism is slightly less similar amongst species than expected from their phylogenetic relationships (Pagel 1999). Queen and worker body volumes followed the same model of evolution but with stronger phylogenetic signals λ (0.93 and 0.95 respectively, Table S1).

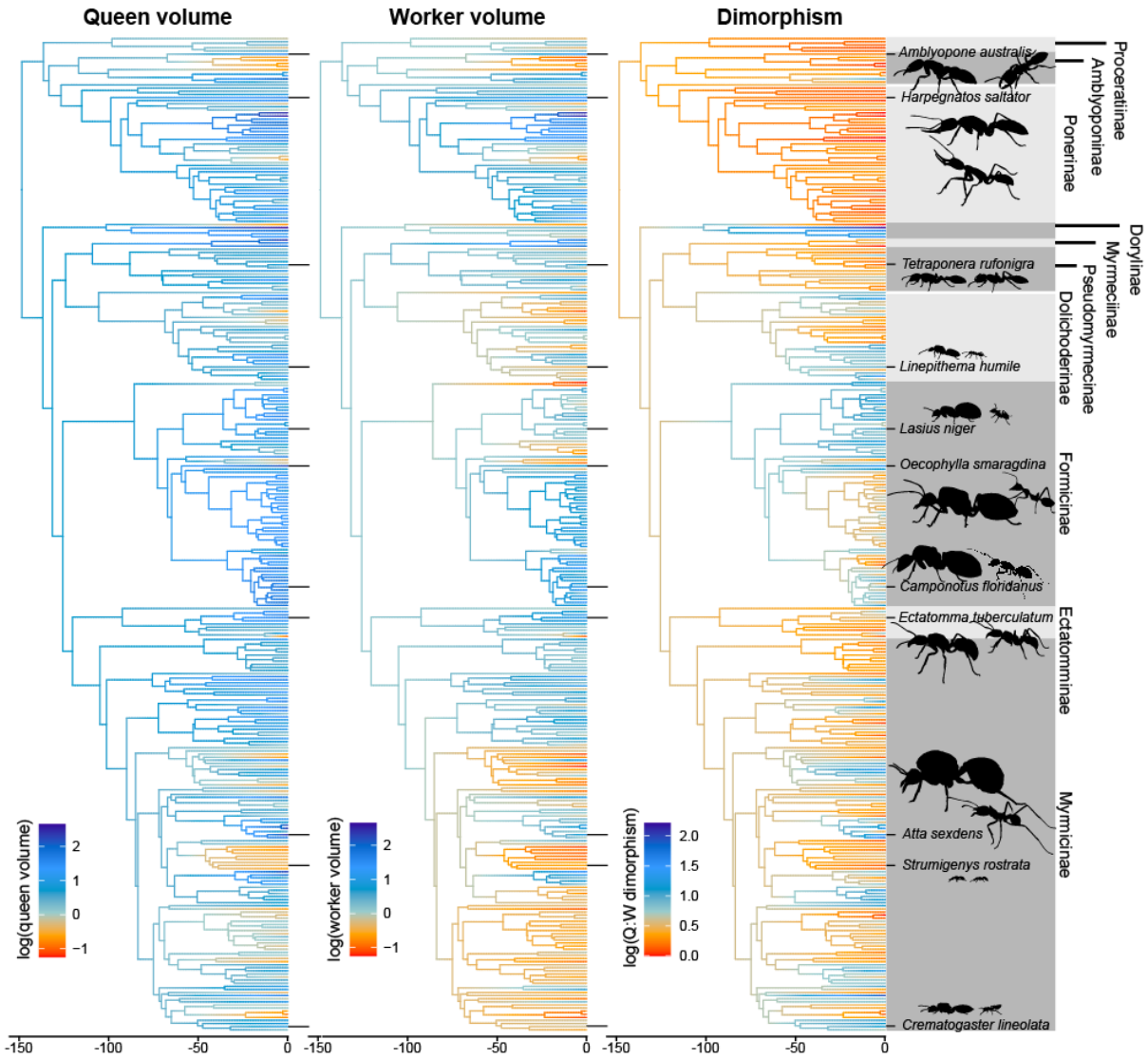

Figure 1. *Ancestral state reconstruction of queen volume, worker volume and dimorphism from $\log_{10}$ transformed values measured in 321 species (dataset 1 in Table S2). Colored branches indicate calculated values of traits. Silhouettes of queens (left) and workers (right) are shown in proportion to their measured size. Major subfamilies are labeled and time is expressed in Ma.*

Despite the fact that there is ample plasticity in the evolution of ant body volume (Fig. 1), the Poneroid clade stands in stark contrast to the Formicoid clade in that 90% of Poneroid species have low dimorphism (Q:W < 2) and no Poneroid species had strong dimorphism (Q:W > 3.5). Formicoids instead show a broad range of dimorphism with significantly higher average dimorphism values in Dorylinae, Myrmicinae, Formicinae, and Dolichoderinae

(one-way ANOVA: $F_{12,308}$ = 10.55, p < 0.001; Tukey's post hoc test: p < 0.05, Fig. S1). Formicoid lineages make up 80% of extant ant species and comprise the most abundant ants in the world (Borowiec et al. 2021). They have invaded many ecological niches, often display generalist feeding behaviors (e.g. eating both insect prey and aphid honeydew). In contrast, low dimorphism subfamilies (i.e. subfamilies of the Poneroid clade, Myrmeciinae, and Ectatomminae) are all primarily predators (Blanchard and Moreau 2017, Sosiak and Barden 2021) and have been proposed to be more similar to the last common ancestor to all ants (Peeters 1997, Barden and Grimaldi 2016).

Differences in the evolutionary patterns of dimorphism are noticeable between the Poneroid and Formicoid clades (Table S1) where Formicoid ants evolve two times faster than Poneroid ants (σ^2 = 2.8e10^{-3} *vs.* σ^2 = 1.1e10^{-3}, Likelihood Ratio Test: p < 0.001).

High size dimorphism is associated with traits of advanced eusociality

Given these associations between the high dimorphism of Formicoid ants and other aspects of advanced eusociality, we wanted to assess whether queen:worker dimorphism is associated with more complex social organization and reduced social conflict. We collected a series of traits that are often associated with more advanced eusociality: absence of workers with full reproductive potential (e.g. gamergates, Monnin and Peeters 1999), trophallaxis (Meurville et al. bioRxiv), diet including sugary liquids (Meurville et al. bioRxiv), colony size (Holbrook et al. 2011, Ferguson-Gow et al. 2014), and presence of polymorphic workers (Boomsma and Gawne 2018).

Our Phylogenetic Generalized Least Squares (PGLS) analysis revealed that larger colony size (p = 0.0041) and worker polymorphism (p = 0.0001) were positively correlated with greater Q:W body size dimorphism, while species with workers that can mate were negatively correlated with high dimorphism (p = 0.0011). However, each of these traits also correlated with both worker and queen volumes (see Table S3 for detailed results of PGLS analyses), indicating that the relationship with dimorphism might be a byproduct of other selection pressures acting on body volumes. Our other tested traits, trophallaxis (p = 0.57) and a diet including sugary liquids (p = 0.72) did not significantly correlate with dimorphism. Our results show that strong dimorphism is associated with some traits found in complex eusociality and negatively associated with worker totipotency (Fig. 2A).

Why strong dimorphism never evolved in several lineages of ants remains unclear. Given the large evolutionary fluctuations in size within female castes (Fig. 1), size plasticity is unlikely to be the limiting factor for dimorphism. Instead, rapid positive selection at the emergence of

Formicoids (Romiguier et al. 2022) is suggestive of one or several evolutionary innovations that allowed greater freedom in size between castes.

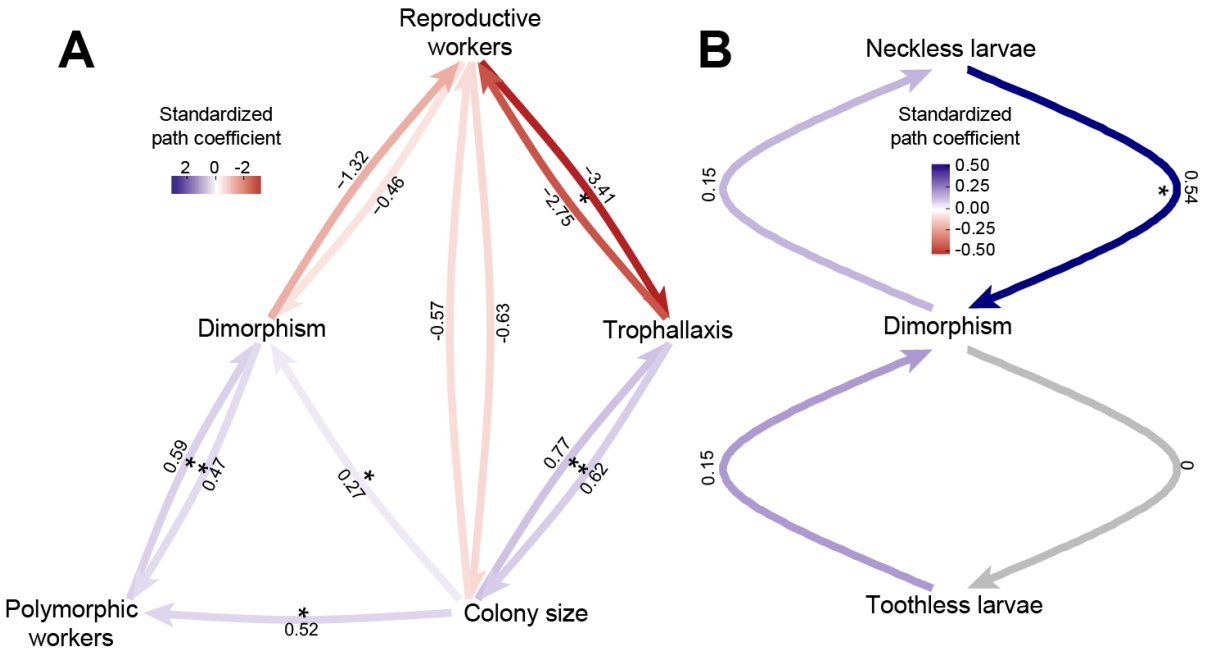

Figure 2. *Representation of correlation and causality of traits with dimorphism. Phylogenetic path analysis based on the PGLS model-averaging with the standardized regression coefficient of dimorphism relationships with* ***A****) social complexity traits (n = 220, dataset 3 in Table S2) and* ***B****) larval morphologies (n = 202, dataset 4 in Table S2). Blue edges indicate a positive correlation while red edges mark a negative correlation. Stars indicate significance based on 95% confidence intervals (Table S4).*

Lack of larval control over caste fate as a critical innovation in ant evolution

Ants being holometabolous insects, adult size is fundamentally dependent on nutrition received in the larval phase (Mirth and Riddiford 2007). Thus, creating a non-homogenous distribution of larval feeding should be the key to building different female castes. For most ants, larvae are totipotent and capable of developing into either a queen or a worker (Schwander et al. 2010). Thus, each female larva is in conflict with the rest of the colony: she has individual reproductive aspirations that do not match either the colony's growth target or colony-level reproductive aspirations (Bourke and Ratnieks 1999, Wenseleers et al. 2003).

For optimal cooperation at the scale of the colony (the superorganism), the feeding of larvae – and therefore larval caste fate – should be managed by workers rather than the larvae themselves (Wenseleers et al. 2003). The degree of alloparental care through feeding varies

extensively between lineages, ranging from some species that simply deposit of a freshly killed prey item in the vicinity of several larvae that feed on it in scramble competition (e.g. Locher et al. 2009) to other species where nursing workers regurgitate highly modified liquids (LeBoeuf et al. 2016, 2018) directly to larvae at regular intervals and in time-calibrated one-to-one interactions (e.g. Cassill and Tschinkel 1995). The former mode of larval feeding leaves room for the reproductive aspirations of larvae in that it allows scramble competition for resources and thus larvae can follow their own reproductive aspirations rather than those of the colony. We hypothesize that this should block the development of strong body size dimorphism.

Larval morphology is known to be a signature of larval diet (Petralia and Vinson 1979, Masuko 2008). We selected two traits proposed to be indicative of larval passiveness (Wheeler and Wheeler 1976). First, the lack of an elongated neck should indicate larvae with little capacity for self-directed motility for feeding, and instead, passive reception of food provided by adult workers. Second, we associated the lack of medial teeth on larval mandibles with feeding on soft food (e.g. liquid passed by trophallaxis or eggs), while larvae with medial teeth on their mandibles likely chew through tougher food. We take these traits as proxies of larval passiveness and use the number of passive traits (i.e. zero, one, or two) in larvae of a given species to test their relationship with dimorphism. PGLS linear regression revealed that larval passiveness was correlated with high dimorphism (p = 0.0199, Fig. 2B, Fig. 3) while remaining uncorrelated to either queen or worker body volume (*queen:* p = 0.95, *worker:* p = 0.16), indicating that larval passiveness is correlated to Q:W asymmetry rather than to worker or queen body size.

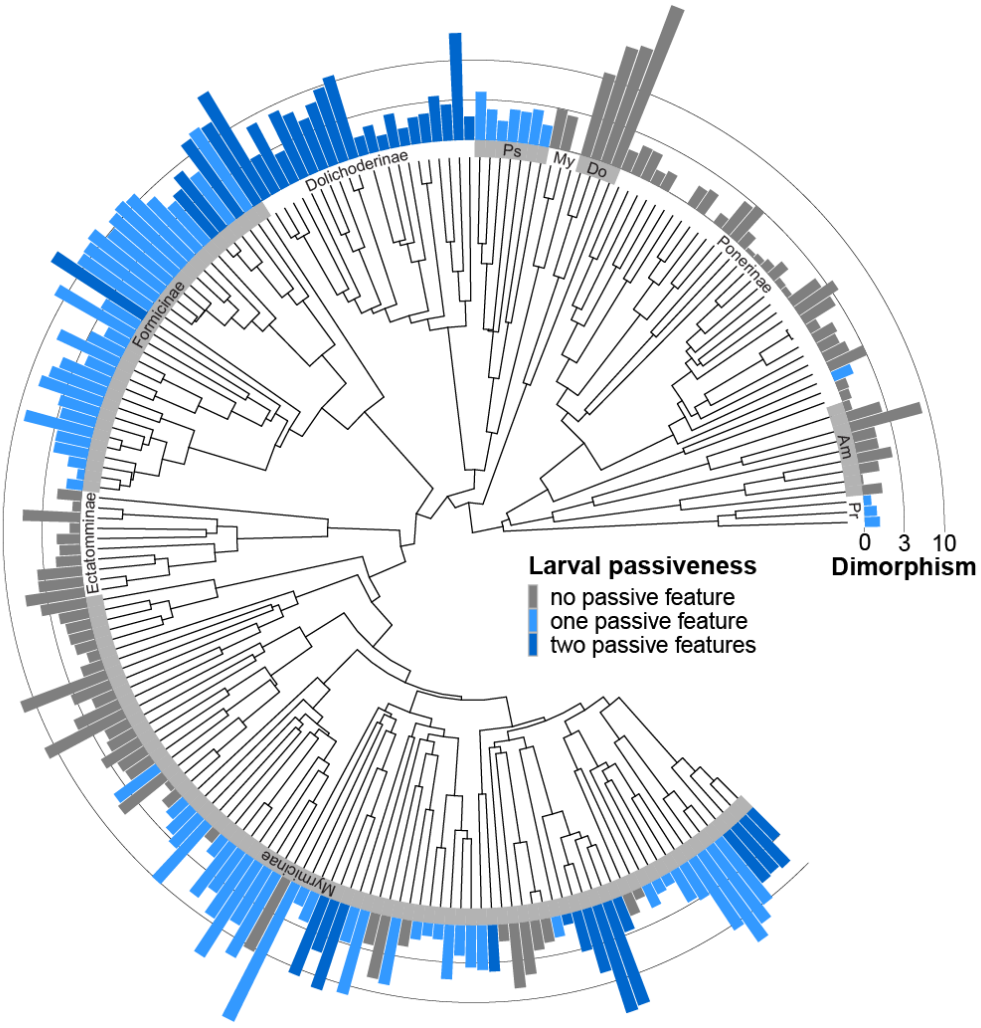

Figure 3. *Phylogenetic representation of dimorphism values according to the presence of passiveness features in larvae for 202 species (dataset 4, Table S2). Am: Amblyopone, Do: Dorylinae, My: Myrmeciinae, Pr: Proceratiinae, Ps: Pseudomyrmecinae.*

Ancestral state estimations of neckless or toothless larvae (Fig. 4) show that these larval passiveness features evolved primarily in the Formicoid clade, especially Formicinae, Myrmicinae, and Dolichoderinae, three speciose subfamilies with generally high dimorphism (mean: 6.59±0.57 Q:W, Fig. 1). We find multiple independent evolutions of neckless larvae and toothless larvae in Formicinae, Dolichoderinae, Pseudomyrmicinae and Myrmicinae, but also in some Ponerinae and Proceratini.

Regurgitation behavior between nestmates (i.e. trophallaxis) and the presence of sugary food in the diet are two traits that might promote passive larval morphology by replacing independent larval feeding on prey. We tested the evolutionary relationship between these traits using Pagel's (1994) method and found that the presence of passive features in larval morphology were positively correlated with the evolutionary shift to sugary liquid in the diet

(p = 0.031) and to trophallaxis (p = 0.010) (see also Table S5 and S6), although these feeding traits were not correlated to dimorphism (see Table S3).

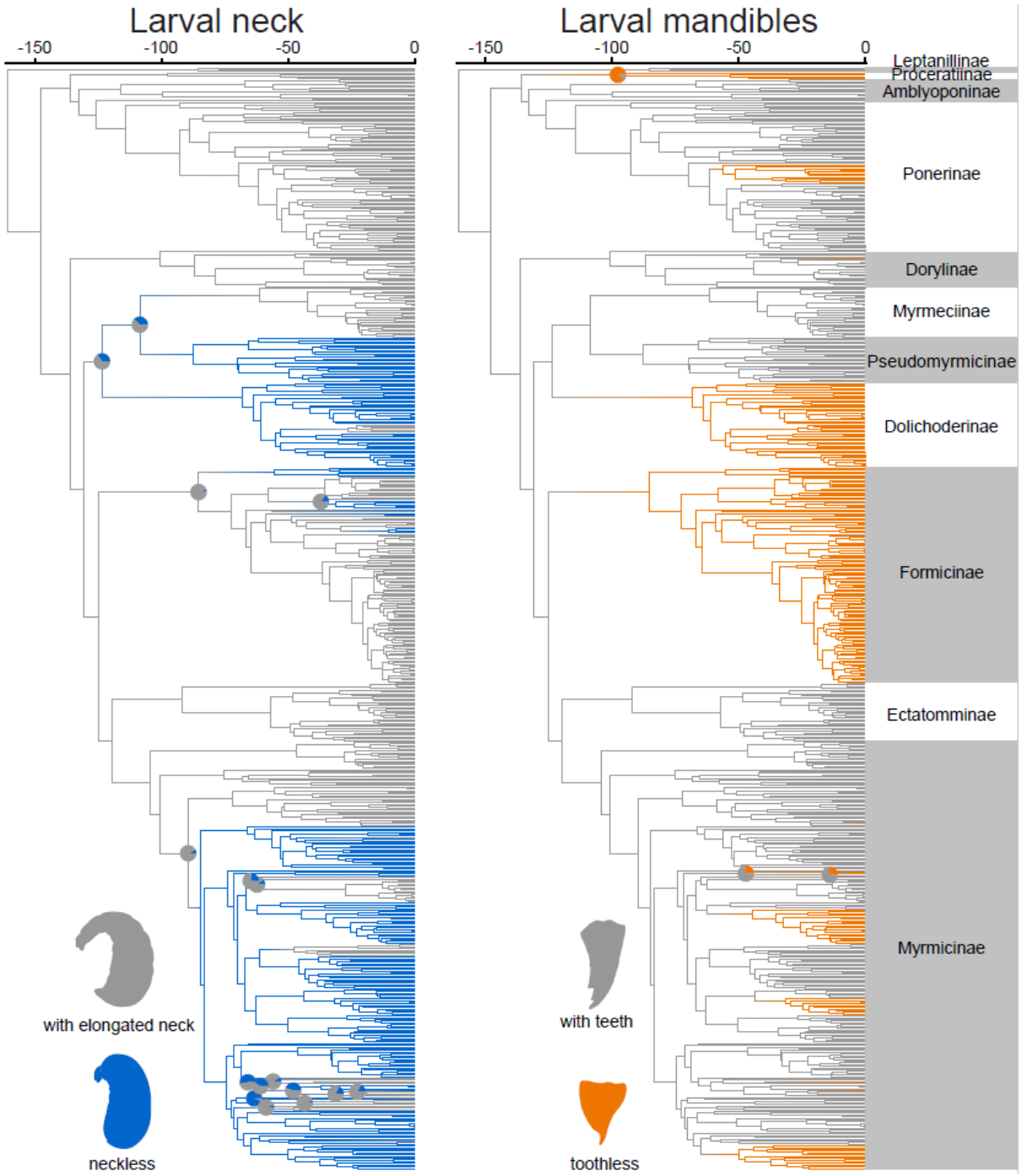

Figure 4. *Ancestral state reconstructions of larval neck and mandible shape evolution for 636 species (dataset 2 in Table S2) over the ant phylogeny. Major subfamilies are labeled and time is expressed in Ma. Pie charts are plotted on nodes where the confidence < 95%.*

DISCUSSION

Our results show that the ant phylogeny holds a wide range of dimorphism levels that have evolved in conjunction with fundamental social traits. Low queen:worker dimorphism was the ancestral state for ants; dimorphism increased dramatically in several Formicoid lineages, where it co-evolved with large colony size, worker polymorphism and loss of worker totipotency. We propose and provide evidence to support the idea that larval passiveness was a key evolutionary innovation that allowed the evolution of extreme dimorphism.

Putting control of larval fate into the hands of workers was a critical change point in the major evolutionary transition of ants from cooperative societies to full superorganisms, and could be considered a key trait of superorganismality. The possibility of building strong caste dimorphism enables ant societies to increase colony size through the production of cheap workers (Peeters and Ito 2015), to develop subcastes of workers for different tasks (Wilson 1980) and produce massive dispersing queens that can found new independent colonies with no external food supply, avoiding predation (Peeters 2020). These outcomes are made possible through the reduction in reproductive conflict between larvae. Looking at the major evolutionary transition in individuality from single-celled to multicellular organisms, it is likely a similar shift in individual to collective priorities had to be accomplished through cell non-autonomous fate determination during development (Du et al. 2015, Veit 2019). This may be a fruitful avenue for future research on the mechanisms that allowed the evolution of multicellularity.

The existence of passive larvae is somewhat surprising given ants' evolutionary origins and fitness incentives. The Hymenopteran phylogeny places ants between two groups of wasps whose larvae feed only on arthropods as ecto- or endoparasitoids (Peters et al. 2017, Labandeira and Li 2021). Ant larvae can be assumed to be ancestrally independent feeders on insect prey, corroborating our result for an ancestrally active larval morphology (Fig. 4). Given the scramble competition for food between prey-feeding larvae, the most passive larva within a colony will have less chance to become a reproductive queen able to transmit its passive larval genes. Rather from one perspective, the unruliest larvae should be favored by natural selection. Furthermore, the evolution of individualized larval feeding puts a larger care burden on workers, which could be detrimental to colonies. Thus, the existence of passive larvae testifies that the benefit to the queen's fitness – and indirectly, the benefit to each member of the colony – from evolving passive larvae and worker control was so great that it overcame notable evolutionary barriers.

Individualized feeding is the critical innovation that removes food competition between larvae and enables passive larval morphologies. In adopting this new feeding pattern, workers strengthen their control over larval nutrient intake and assert stronger regulation of their caste fate (Fig. 5). We found that passive larval traits correlated not only with dimorphism but also with the use of trophallaxis and sugary liquids in the diet. Contrary to prey, liquids can't be shared without individualized care. However, the use of liquid food for larval rearing is likely to require prior processing into a more consolidated form to meet the nutritional requirements of larval growth (Dussutour and Simpson 2009, Csata and Dussutour 2019). Such food improvement can be achieved by nitrogen-fixing bacterial symbionts in the guts of workers (Russell et al. 2009, Jackson et al. 2022) or through metabolic labor performed by workers, transforming exogenous food into endogenous ant proteins (Hakala et al. 2021). This transformation of sugary liquids requires the ingestion of the liquid by workers and consequent sharing of the products through trophic eggs or regurgitation (trophallaxis). Trophallaxis broadly evolved within the major highly dimorphic lineages (Meurville et al. bioRxiv), and larval growth regulators have been reported in the trophallactic fluid of several species (LeBoeuf et al. 2016, 2018). This provides additional clues for how workers modulate larval development.

Factors other than larval morphology clearly influence dimorphism variation across the ant phylogeny. There exist low dimorphism species with passive larvae and conversely high dimorphism species with unruly larvae (Fig. 3). Success or failure during solitary colony foundation was likely a major selection pressure that increased dimorphism. Greater dimorphism puts more resources in dispersing queens who can then produce cheaper, sterile workers to quickly achieve a minimally viable colony. Other dispersal solutions exist that do not require high dimorphism (Hakala et al. 2019). Strong dimorphism in species without passive larval morphology is likely to come about through other methods of larval caste fate regulation. Some species have caste fate partially or fully determined by genetic factors, biasing larval totipotency (Anderson et al. 2008, Weyna et al. 2021). In some ant taxa (mostly Amblyoponinae), adults mildly injure their larvae and feed their hemolymph. This non-destructive cannibalism pilfers nutrients from larvae, and could allow adults to create stronger dimorphism (Masuko 1986), potentially explaining the unusually large dimorphism in two species of Amblyoponinae (Ito 2010, Peeters and Molet 2010). In *Eciton,* where dimorphism is exceptionally large, sexual larvae are reared in cohorts separate from worker-destined larvae (Trible and Kronauer 2017), allowing either coordinated larval cannibalism or differential allocation of food to larvae. Despite these examples of alternative strategies, we found that species with more passive larvae tend to exhibit higher dimorphism.

The reproductive division of labor in eusocial insect societies requires building differences between genetically equivalent individuals. Our results highlight the importance of controlling larval growth to build a morphological chasm between castes. In honeybees, which have evolved worker and queen castes similar to ants, worker-destined larvae are reared in size-delimited cells preventing unchecked growth. If honeybee larvae are reared outside of the colony, a full range of intermediates between workers and queens are observed (Linksvayer et al. 2011, Leimar et al. 2012). For ants, where larvae are not enclosed, this control must be exerted through alloparental care. Although some amount of alloparental care toward larvae was likely already present in early ant eusociality (Boudinot et al. 2022, Snir et al. 2022), our results indicate that adopting new feeding modes for larval care enabled ants to take an important step in building strongly differentiated morphological castes (Fig. 5).

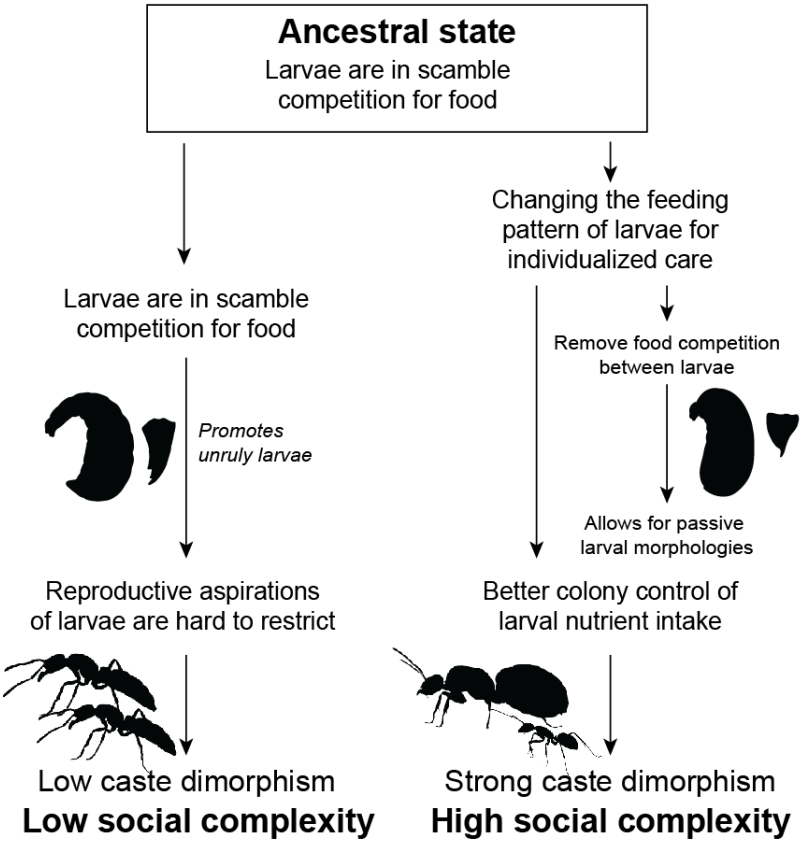

Figure 5. *An evolutionary scenario for the acquisition of passive larval morphology and its link with dimorphism and social complexity.*

MATERIAL AND METHODS

Data sampling

We examined the evolution of body volumes of queen and worker castes and their dimorphism, and their relationship with a panel of ecological, behavioral, morphological, and life-history traits. Because of little overlap in our datasets of species whose dimorphism, social complexity and larval morphology data are available, we had to parse our data to test the effects of a set of traits on others. Details of the number of species used for each test are detailed in Table S2. The total species per dataset and each species' sources are provided in Supplementary material 1.

Caste body volumes and dimorphism

We measured body size of ant queens and workers for 321 species in 124 genera over 13 subfamilies. Species were selected on the basis of presence of available image data (Antweb.org), available data for our traits of interest in the literature, phylogenetic data and optimally wide taxon sampling (Fig. S2).

Using the Antweb.org photo database, we measured one queen and one worker per species, favoring type specimens where possible (56 % of our dataset are from type specimens). As our goal was to evaluate the maximum size difference between queens and workers, intermediate forms of workers such as soldiers were not measured. Ergatoid queens (i.e. wingless at adult emergence) were not measured either, except when they represented the only known reproductive form for the species (viz. several Dorylinae and *Blepharidatta conops*) (Peeters 2012).

We measured the length, width and height of the mesosoma as well as the length and width of the gaster and the head using ImageJ (Collins 2007) (v.1.53a) (Fig. 6). Because measurements are sensitive to tagma orientation, head height was impossible to properly measure on numerous mesosoma-pinned specimens. Head height was therefore estimated as 0.67926 x head width according to the known strong allometric correlation (Sosiak and Barden 2021). Gaster length and width were taken from measured ellipses that matched as closely as possible the shape of the gaster, balancing the gaster areas inside and outside the ellipse when required (Fig. 6). Body volumes and dimorphism were estimated as:

$$\boldsymbol{V_{head}} = L_{head} \times W_{head} \times H_{head}$$

$$\boldsymbol{V_{mesosoma}} = L_{mesosoma} \times W_{mesosoma} \times H_{mesosoma}$$

$$V_{gaster} = \frac{Lgaster}{2} \times \frac{Wgaster}{2}^{2} \times \frac{3}{4}\pi$$

$$V_{total} = V_{head} + V_{mesosoma} + V_{gaster}$$

$$\textbf{Size dimorphism} = \frac{Vtotal\ queen}{Vtotal\ worker}$$

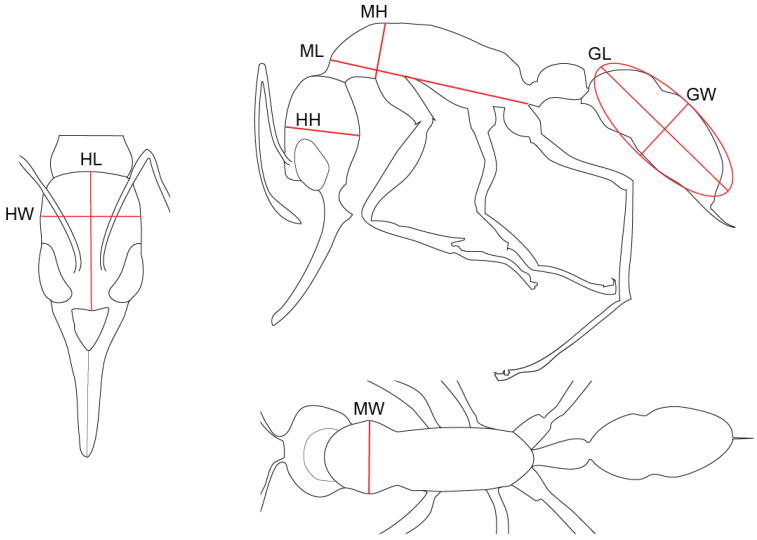

Figure 6. *Morphological measurements used in the present study: head high (HH), head length (HL), head width (HW), mesonotum high (MH), mesonotum length (ML), mesonotum width (MW), gaster length (GL), gaster width (GW).*

For species with no morphologically distinct reproductive caste (e.g. *Diacamma*), only one individual was measured and categorized both as queen and worker, resulting in a dimorphism value of 1. Statistical analyses were performed and graphs were generated with the software R (v.4.1.3) (Development Core Team R 2013). All our measurement data are provided in supplementary material 1 and the coordinates of each measurement on the images in supplementary material 2. Dimorphism ratio and queen and worker volume values were used as $\log_{10}$ transformed in the phylogenetic analyses following recommendations (Garland Jr et al. 1992, Mongiardino Koch et al. 2015).

Social complexity traits

We collected trait data for trophallaxis, sugary liquid in the diet, presence of reproductive workers (i.e. gamergates) and colony size from Meurville et al. bioRxiv, a dataset representing 417 species, of which 270 from 97 genera over 14 subfamilies are in our final dataset (Table S2). We did not collect these data for additional species. Following Meurville et al. bioRxiv, to face heterogeneity in data collection, we split colony size by quartile which were used as a continuous trait (1st quartile: under 60 individuals, 2nd quartile: 60-268, 3rd quartile: 269-2000, 4th quartile: 2001-3060x10^5). The presence of polymorphic workers was compiled from La Richelière et al. (2022).

Larval morphologies

To assess larval passiveness in relation to dimorphism, we assembled data on larval morphology for 636 species from 165 genera over 13 subfamilies, mainly from the pioneering work of Wheeler and Wheeler (1976, 1986) and complemented by some more recent larval descriptions (Supplementary material 1). We collected larval data with the aim to evaluate larval dependency on workers for food. We make two assumptions relating Wheeler and Wheeler's larval morphological features to larval dependence. We take the lack of an elongated neck in larvae to indicate a low capacity for self-directed motility for feeding, suggesting passive reception of food given by adult workers in agreement with the observations of Wheeler and Wheeler (1976). We associate the lack of medial (= subapical) teeth on larval mandibles with feeding on soft food (e.g. liquid passed by trophallaxis, eggs), in opposition to denticulate larval mandibles, which are likely associated with chewing through tougher food. We assume that these traits are indicative of larval passiveness. Other aspects of larvae could also be informative (sclerotization of mandibles, rate of movement, dynamic range of neck, feeding behavior) but there is little to no available data for these traits. More details of larval classification procedure are provided in Appendix 1.

Phylogenetic data

We used the maximum credibility tree produced by Economo et al. (2018) as a reference for phylogeny as it was the largest and most recent ant phylogeny at the time. Species names modified due to taxonomic revisions have been corrected according to Antwiki.org valid species names in 2022.

Modeling trait evolution

To investigate the temporal dynamics of morphological evolution over the diversification of ants, we followed a similar strategy to that of Cooney et al. (2017) (based on measurements of extant taxa and assumes constant niche space and no or constant extinction throughout the radiation). For each trait (i.e. body volume of queens and workers and their associated dimorphism), we assessed the phylogenetic signal in the data by calculating Pagel's lambda and Blomberg's *K* with the R package phytools (Revell 2012) (v.1.2.0). We then tested the fit of nine models of trait evolution for each trait. We applied a Brownian motion model, a single-optimum Ornstein–Uhlenbeck model, an early burst model, a white noise model, a rate trend model, a lambda model, a kappa model, and a delta model of trait evolution using the function fitContinuous of the R package Geiger (Harmon et al. 2008) (v.2.0.7). The

models were compared by their Akaike information criterion (AIC). All results obtained from trait evolution analyses are summarized in Table S1.

Differences in evolutionary rates (σ^2) of traits were assessed following the Adam's method (2013) implemented in the package mvMORPH (Clavel et al. 2015) (v.1.1.4). We compared the log-likelihood of two Brownian motion based models, one where the two traits to compare evolve at distinct evolutionary rates, and another where the traits are constrained to evolve at a common evolutionary rate. The log-likelihood values of the two models were compared using the Likelihood Ratio Test hosted in mvMORPH (Clavel et al. 2015) (v.1.1.4).

Ancestral state reconstructions

Ancestral state reconstructions of queen and worker body volumes were performed using the function anc.ML in the R package phytools (Revell 2012) (v.1.2.0), where we estimated evolutionary parameters and ancestral states using likelihood given a Brownian-motion model of evolution of continuous traits. To find the best evolutionary model fitting the evolution of our phenotypes, the ancestral state estimations were run on lambda-transformed trees according to the previously calculated phylogenetic signal (see above) of the reconstructed trait. These transformed trees were obtained from the original species tree using the function rescale of the R package Geiger (Harmon et al. 2008) (v.2.0.7). Anc.ML function was computed with a Brownian motion model of evolution with all other settings left at the default. We then projected reconstructed ancestral states onto the original species tree.

We performed ancestral state reconstructions for larval body shape and mandible shape at each node using the function make.simmap in the R package phytools (Revell 2012) (v.1.2.0). This utilizes a stochastic character mapping approach (Huelsenbeck et al. 2003, Bollback 2006). For both traits, we ran 500 simulations upon the model of trait evolution “All rates different” (ARD), referring to rates of trait state changes and all other settings were left at the default. The percentage of simulations for which each trait state was inferred at each node and we plotted the probability of node stats as a pie chart when under 95%.

Correlation between traits

We used phylogenetic regression analysis to test the correlation between traits taking into account relatedness among taxa. Phylogenetic regression assumes that branch length is proportional to the residual error in the model (Felsenstein 1985, Revell 2010). As such, we performed phylogenetic generalized least squares (PGLS) using the function gls of the R package nlme (Pinheiro et al. 2018) (v.3.1-155). PGLS analyses were performed on the

lambda transformed trees, using the function rescale of the R package Geiger (Harmon et al. 2008) (v.2.0.7), according to the phylogenetic signal of the tested answer variable (see **Modeling trait evolution**), a classical method to implement phylogenetic signal in PGLS analysis (Symonds and Blomberg 2014). We used the stepAIC function in the R package MASS (Venables and Ripley 1997, Ripley et al. 2013) (v.7.3-55) to compare models via stepwise addition and removal of predictors and their interactions. Log-transformed dimorphism values were additionally root-squared transformed in order that residuals fit a normal distribution.

To refine our understanding of how traits influenced dimorphism, we used phylogenetic path analysis (von Hardenberg and Gonzalez-Voyer 2013) using the R package Phylopath (van der Bijl 2018) (v.1.1.3). This method allows the comparison of models of possible causal relationships between traits while testing for direct or indirect effects using the d-separation method (Shipley 2000, 2009) and considering the non-independence of the traits due to phylogeny through phylogenetic generalized least squares analysis (PGLS). We build 14 models to analyze relationships between dimorphism and complex sociality traits, and ten models to analyze relationships between dimorphism and larval morphologies (Figs S3, S4). Because we did not identify a single best model (Figs S5, S6), we averaged the best models using the average() function in the phylopath package. This resulted in a model reflecting the results of the best models.

We tested the correlation between the presence of passiveness features in larvae with the use of trophallaxis and the presence of sugary liquid in the diet using the function fitPagel hosted in the R package phytools (Revell 2012) (v.1.2.0). A function designed to fit Pagel's (1994) method for testing for an evolutionary relationship between two binary characters. The evolutionary relationships between traits were investigated by building additional models where one trait depends on the other. The model fits were then compared by their Akaike information criterion (AIC) (Table S5).

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