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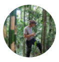
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Innovation in ant larval feeding facilitated queen–worker divergence and social complexity

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Building differences between genetically equivalent units is a fundamental challenge for all multicellular organisms and superorganisms. In ants, reproductive or worker fate is typically determined during the larval stage, through feeding regimes managed by adult caretakers. However, the feeding care provided to larvae varies significantly across ants, as does phenotypic divergence between queen and worker castes. Here, we employed comparative phylogenetic methods and causal inference to investigate the relationships between larval feeding care, caste size dimorphism, and social complexity across ant diversity. We digitized the life's work of George and Jeanette Wheeler, cataloging the larval morphology of over 700 species, and we compiled data on species diets and larval feeding behaviors from the literature and our own observations. We measured queen–worker size dimorphism in 392 species and gathered data for colony size, worker polymorphism, and worker reproduction. Our analyses revealed that ancestral active-feeding larvae evolved passive morphologies when adults began feeding them individually, typically with processed material and often following a shift to nonpredatory diets. Greater queen–worker size dimorphism coevolved with larval passiveness, alongside traits indicative of increased social complexity, including larger colony sizes, worker subcastes, and a reduction in workers' reproductive potential. Likelihood comparisons of causal phylogenetic models support that extended alloparental care facilitated stronger caste dimorphism, which, in turn and along with increased colony sizes, promoted higher social complexity. Our results suggest that enhanced adult control over larval development enabled greater phenotypic specialization within colonies, with profound implications for social evolution.

caste fate conflict | maternal care | major evolutionary transitions | larval morphology | social evolution

Significance

Ants are among the rare organisms to have extended reproductive division labor beyond the cells of a multicellular organism. However, the degree of specialization between reproductive and worker castes varies considerably between ant lineages. In this study, we demonstrate that strong caste dimorphism in ants coevolved with social complexity, and this strong caste dimorphism was achieved by asserting adult control over larval development. We conclude that this enhanced control over larval caste fate was a critical junction in the evolution of ants.

From the specialization of germline and somatic tissue in multicellular organisms to the sterile workers and reproductive queens of social insect colonies, reproductive division of labor is a central feature of deeply cooperative systems (1–3). In social Hymenoptera, adults are divided into reproductive and nonreproductive castes (2). Queens specialize in reproduction while workers specialize in food collection and brood care.

In ants, the size difference between queens and workers reflects the degree of caste specialization into their respective tasks (4). Higher caste dimorphism is associated with bigger colony sizes (5) and benefits dispersing queens during colony foundation where smaller workers are comparatively cheaper to produce (6). Despite the purported advantages of higher caste dimorphism for social life, the morphological gap between queen and worker castes is highly heterogeneous across the ant phylogeny, covering a wide spectrum. At one extreme, early-branching lineages often have small colonies of predatory specialists with minimal morphological differences between reproductives and workers. At the other extreme, speciose ecosystem-dominating generalist species tend to have colossal colonies and high levels of queen–worker dimorphism (7, 8). Ants are among the most dominant organisms in nearly all terrestrial ecosystems, contributing significantly to biomass and abundance. As ecological engineers, they play critical roles in soil decomposition and act as keystone species, shaping communities around them (9). Thus, understanding their evolutionary pathways and the development of caste dimorphism is crucial, not only for evolutionary biology but also for understanding the origin of their broad ecological impacts. To our knowledge, however, no broad comparative studies have yet been conducted on caste determination in ants, leaving unclear the evolution of dimorphism and its consequences on ant evolution. While previous studies have explored associations between caste polymorphism and colony size across ants (e.g., refs. 10, 11), comprehensive analyses remain limited regarding the influence of ecological factors—such as feeding behavior and diet—on the evolution of caste dimorphism and its broader consequences on ant social complexity.

The larval stage is critical in building caste dimorphism. Female embryos of social Hymenoptera are generally totipotent and can turn into either queens or workers. While

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The authors declare no competing interest.

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caste fate is specified as early as in the egg in a few Formicinae and Myrmicine species (12–16), the prevailing belief is that "environmental" (social) caste determination plays a major role during larval development for most ant species (13, 17). In holometabolous insects, building size differences rely on a nonhomogenous distribution of larval feeding, regardless of developmental input (18). Beyond size, these differences in nutrition can activate or repress different biochemical and gene expression pathways (15, 19, 20) that are also influenced by hormonal titers, for example, of juvenile hormone (21, 22).

For optimal cooperation at the colony scale, larval caste fate – and therefore the feeding of larvae – should be managed by workers rather than the larvae themselves. Larval totipotency is expected to bring about an intense conflict between each larva and the rest of the colony. Specifically, larvae would benefit by becoming queens rather than workers, thereby gaining greater direct reproduction, whereas the colony more often benefits by producing workers (23–25). In cases where caste is likely to be self-determined, as in Melipona bees, many females selfishly choose to become queens at the expense of colony productivity (26–29). In some stingless bees, larvae can even break into neighboring cells to obtain additional food and consequently develop into queens (30, 31). Nutritional caste control can be highly effective in preventing excess queen production (26). Honeybees employ size-delimited cells to rear worker-destined larvae, preventing unchecked growth. However, if honeybee larvae are reared outside of the colony, they exhibit a wide range of intermediates between workers and queens (32, 33). In ants, where larvae are not enclosed, such control can only be exerted through alloparental care.

Although alloparental care toward larvae was likely already a key feature of early ant societies (34–36), the extent of feeding care varies widely among ant lineages. Larvae of some species are exceptionally autonomous, feeding directly on entire prey retrieved by adults (e.g., ref. 37). Whereas other species exhibit completely passive larvae fed by nurse workers that regurgitate liquids rich in endogenously produced components (38, 39) in regular and time-calibrated one-to-one interactions (e.g., ref. 40). Semiautonomous larvae exhibit active feeding behavior and several authors have hypothesized that this less-supervised feeding method leaves more room for the reproductive aspirations of larvae, limiting the possibility to build dimorphism in these species (e.g., refs. 41–43). In those species, adults sometimes even aggress their larvae to prevent them from becoming queens (43, 44), though developmental mechanisms remain unclear.

In this macroevolution study, we combined data on morphology, ecology, life history traits, and behavior across hundreds of species to unravel the evolution of caste size dimorphism in ants. Our analyses revealed that ancestral active-feeding larvae evolved passive morphologies as adults began feeding them individually, typically with processed material and following a shift to nonpredatory diets. Greater queen–worker size dimorphism coevolved with larval passiveness, alongside traits indicative of increased social complexity, including larger colony sizes, worker subcastes, and a reduction in workers' reproductive potential. Likelihood comparisons of causal phylogenetic models indicate that larval passiveness, through stronger caste dimorphism, promoted higher social complexity. Overall, our study analyzes the causes and consequences of pronounced size asymmetries between queens and workers.

Results

To investigate the role of alloparental care in building caste dimorphism, we first sought to establish larval morphology as a proxy for alloparental care by analyzing the evolution of larval morphology in combination with larval feeding habits and adult diet. Next, we analyzed the evolution of caste dimorphism and tested its correlation with larval morphology and traits characteristic of extreme social complexity.

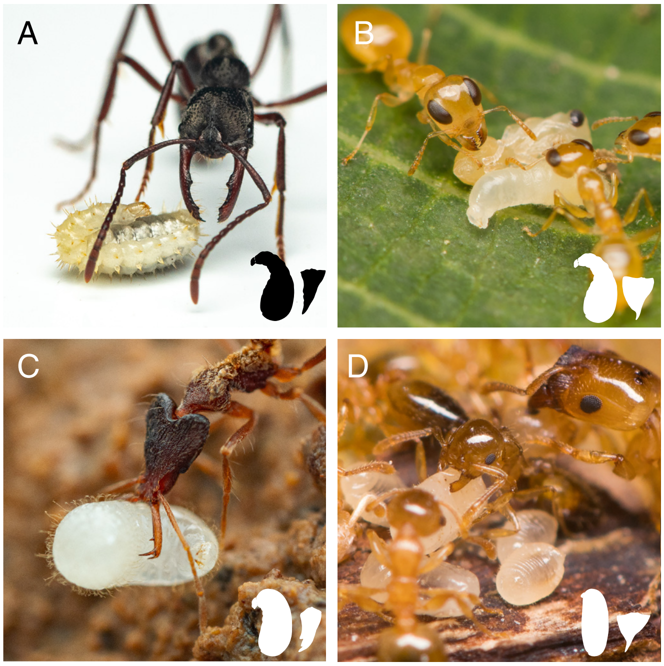

Fig. 1. A sample of the diversity of ant larval morphology. (*A*) *Myopias* sp.: larva with elongated neck and developed mouthparts. (*B*) *Gesomyrmex howardi*: larva with elongated neck and reduced mouthparts. (*C*) *Strumigenys* sp.: larva with reduced neck and developed mouthparts. (*D*) *Crematogaster cylindriceps*: larvae with reduced neck and reduced mouthparts. Photos by François Brassard and used with permission. Larval silhouettes represent Wheeler and Wheeler's classification for the designated species (*SI Appendix*, Fig. S1 and Table S1 and S2).

Alloparental Care, Larval Morphology, and Diet Evolution. Because details about alloparental care and larval feeding are scarce in the literature (45), we used larval morphological features as proxies for larval feeding habits. Ant larval morphology is extremely diverse (46) (Fig. 1), and is known to provide a signature of larval feeding methods (e.g., refs. 47–49). George C. Wheeler and Jeanette Wheeler underscored, throughout their extensive 66-y study on ant larvae, that passive larval morphological features correlate with greater adult assistance in larval feeding. Specifically, the lack of an elongated neck indicates larvae with reduced capacity for self-directed feeding, and instead, passive reception of food provided by adult workers. Second, the poor development of larval mandibles indicates feeding on soft food (e.g., on liquid passed by trophallaxis or on eggs), while larvae with medial teeth on their mandibles likely chew through tougher food (46, 50, 51).

To test this hypothesis proposed by Wheeler and Wheeler, we assembled data for the presence of an elongated neck and medial teeth (according to their classification, see *SI Appendix*, Fig. S1 and Tables S1 and S2) in the larvae of 733 species to analyze the evolution of these two key morphological features. We tested whether these larval features indeed correlated with larval feeding habits using Pagel's (52) method on a set of 99 species spanning 71 genera where both behavior and morphology were available (*SI Appendix*, Fig. S2). Specifically, we categorized whether larvae of these species are usually fed semiautonomously with limited alloparental care (viz., actively feeding upon unprepared prey left at their disposal or receiving large chunks of food items) or, conversely, if their feeding primarily involves strong adult care (viz., regurgitation, preparation of premasticated pellets, distribution

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of trophic eggs, etc.) (see Dataset S1 for quotes and classifications). We found a significant positive correlation between the evolution of neckless and toothless larvae and the evolution of more supervised alloparental feeding (Pagel's, both $P < 0.001$, *SI Appendix*, Table S3). Additionally, we found supervised larval feeding habits significantly correlated with nonpredatory adult diets (Pagel's, $P < 0.001$, *SI Appendix*, Table S3).

To clarify the evolutionary relationships between adult dietary shifts, larval feeding habits, and larval morphologies, we employed phylogenetic path analysis. With this method, plausible evolutionary models of causal relationships between traits are tested by assessing which of the plausible models is best supported by the distribution and sequence of these traits' evolution over the phylogeny in question. Only plausible models are tested to avoid attributing causality to spurious correlations (e.g., umbrellas cause rain). Here, we built five plausible models linking adult dietary shifts, larval feeding habits, and larval morphologies (Fig. 2*A*). In order to test the support for each model while accounting for phylogenetic uncertainty, we developed an approach derived from the original phylopath package (53) to assess models across many trees instead of relying on a single tree ("multitree phylogenetic path analysis," *Materials and Methods*). One of our five models was significantly better than the others (Fig. 2*B*). This model suggests that ancestrally active-feeding larvae evolved passive morphologies when adults began feeding them individually, regardless of the type of food, yet that the emergence of individualized feeding was promoted by shifts in adult diet (Fig. 2 *C* and *D*).

Evolution Patterns of Larval Morphology. We performed ancestral state reconstructions of larval morphology (neckless-toothless, necked-toothless, neckless-toothed, necked-toothed), according to three models with different constraints on discrete character state changes and selected the best model using the Akaike information criterion weight (AICw). The symmetric model was the best fit (AICw = 0.75, symmetrical transitions for each trait) compared with all-rates-differ (AICw = 0.22) and equal-rates (AICw = 0.02, all transition rates equal). The ancestral state reconstruction of larval morphology revealed nine independent evolutions of neckless and toothless larvae from a necked-toothed ancestor (Fig. 3). The evolutionary pattern of acquisition of neckless and toothless morphology aligns with the Wheelers' respective associations of these morphologies with an individualized distribution of food and specialization in soft foods (see above), and highlights the different pathways taken by ant lineages to feed their young.

Neckless larvae have predominantly evolved in Dolichoderinae, Pseudomyrmecinae, and Myrmicinae. Current evidence indicates that Dolichoderinae primarily nourish their larvae with trophic eggs and trophallaxis (Dataset S1), while Pseudomyrmecinae larvae mostly feed on pellets prepared by adults (55). In lineages of Myrmicinae that acquired neckless larvae early in the subfamily's evolution, this morphology is found in extant species exhibiting a generalist diet and feeding their larvae through regurgitation and pellets (e.g., *Solenopsis*, *Monomorium*, *Temnothorax*, *Pheidole*). However, larvae of some species regained an elongated neck, in particular in species exhibiting primarily predatory diets (e.g., *Orectognathus, Epopostruma, Eurhopalothrix, Myrmecina, Terataner*). Conversely, necklessness was followed by the subsequent loss of medial teeth in larvae of lineages that largely ceased feeding their larvae with prey (e.g., fungus-growing ants, *Cephalotes*, *Crematogaster*). Neckless larvae have evolved in species exhibiting individualized distribution of food, regardless of the nature of the food.

Toothless larvae evolved in six subfamilies: at or near the root of the subfamilies Formicinae, Dolichoderinae, Proceratinae, and more sparsely in Myrmicinae, Ponerinae, and Dorylinae (Fig. 3). Aside from Dorylinae and Ponerinae, these ant species with toothless larvae are all documented to predominantly feed their larvae through trophallaxis, eggs, or fungal staphyla (Dataset S1), aligning with larval specialization on soft food. Within Formicinae, our dataset revealed that the only observed reversions to necked and toothed larvae occurred in *Myrmoteras* and possibly in *Proformica*. *Myrmoteras* has undergone dietary and behavioral reversion toward specialization on prey capture and displays phylogenetically uncharacteristic low dimorphism between queens and workers (56). Within Dorylinae, toothlessness evolved only in the army ants.

We hypothesized that shifts in adult control over larval nutrition might have facilitated the development of different adult female morphologies by allowing the colony's workers to direct larval fate according to the colony's needs and maturation. To test this hypothesis, we focused on the most fundamental female morphological difference in ants, between queens and workers. While queens and workers show a broad array of morphological differences, we simplified their differences by focusing only on volumetric body-size dimorphism.

Evolution of Queen–Worker Volume Dimorphism. Ant species exhibit a large range of queen–worker dimorphism. Our taxon sampling includes a wide range of dimorphisms, ranging from species with similar-sized castes (e.g., in *Myrmecia, Neoponera, Prionopelta*), to species having queens over one hundred times bigger than their smallest workers (e.g., in *Atta, Carebara, Dorylus, Tranopelta*). Phylogenetic analysis of the evolution of queen–worker dimorphism revealed a lambda model of evolution with a phylogenetic signal λ of 0.85 (*SI Appendix*, Table S4), indicating that dimorphism is slightly less similar among species than expected given their phylogenetic relationships (57).

Despite considerable variability in ant queen–worker dimorphism, the Formicoids stand in stark contrast to the Poneroid and Leptanillomorphs clades (Fig. 3). This same branch in the ant phylogeny was previously found to have sustained rapid positive selection (58). Notably, 90% of sampled poneroid species and 100% of the sampled Leptanillomorphs exhibit low dimorphism ($Q{:}W_{volume} < 3$). In contrast, formicoid ants display extreme variation in dimorphism, with significantly higher average dimorphism values in Dorylinae, Myrmicinae, Formicinae, and Dolichoderinae than in Poneroids (one-way ANOVA: $F_{11,376} = 15.812$, $P < 0.001$; Tukey's post hoc test: $P < 0.05$, *SI Appendix*, Fig. S3).

These stark differences cannot be attributed to potential limits in the body volume evolution. Queen and worker body volumes shifted dramatically, both increasing and decreasing in all ant lineages (Fig. 3) with no clade-specific difference in evolutionary rates (Likelihood Ratio Test: both $P = 1$). Queen and worker body volume evolution were best fit with an Ornstein–Uhlenbeck and a delta model of evolution with phylogenetic signals λ of 0.87 and 0.89 respectively (*SI Appendix*, Table S4). Queen volume evolved faster than did worker volume (σ^2 *queen* $= 9.3e10^{-3}$, σ^2 *worker* $= 5.8e10^{-3}$, Likelihood Ratio Test: $P < 0.001$) indicating putative different levels of selection pressure on the two castes.

High Queen–Worker Size Dimorphism Is Associated with Larval Passiveness and Traits of Advanced Sociality. While body size of queens and workers can shift rapidly in all lineages, the difference between queen and worker size remained consistently low in some groups while variable in others. We next investigated the evolution of the size gap between these castes: queen–worker dimorphism. We found that strong dimorphism predominantly evolved within the subfamilies exhibiting passive larvae (Figs. 3–5).

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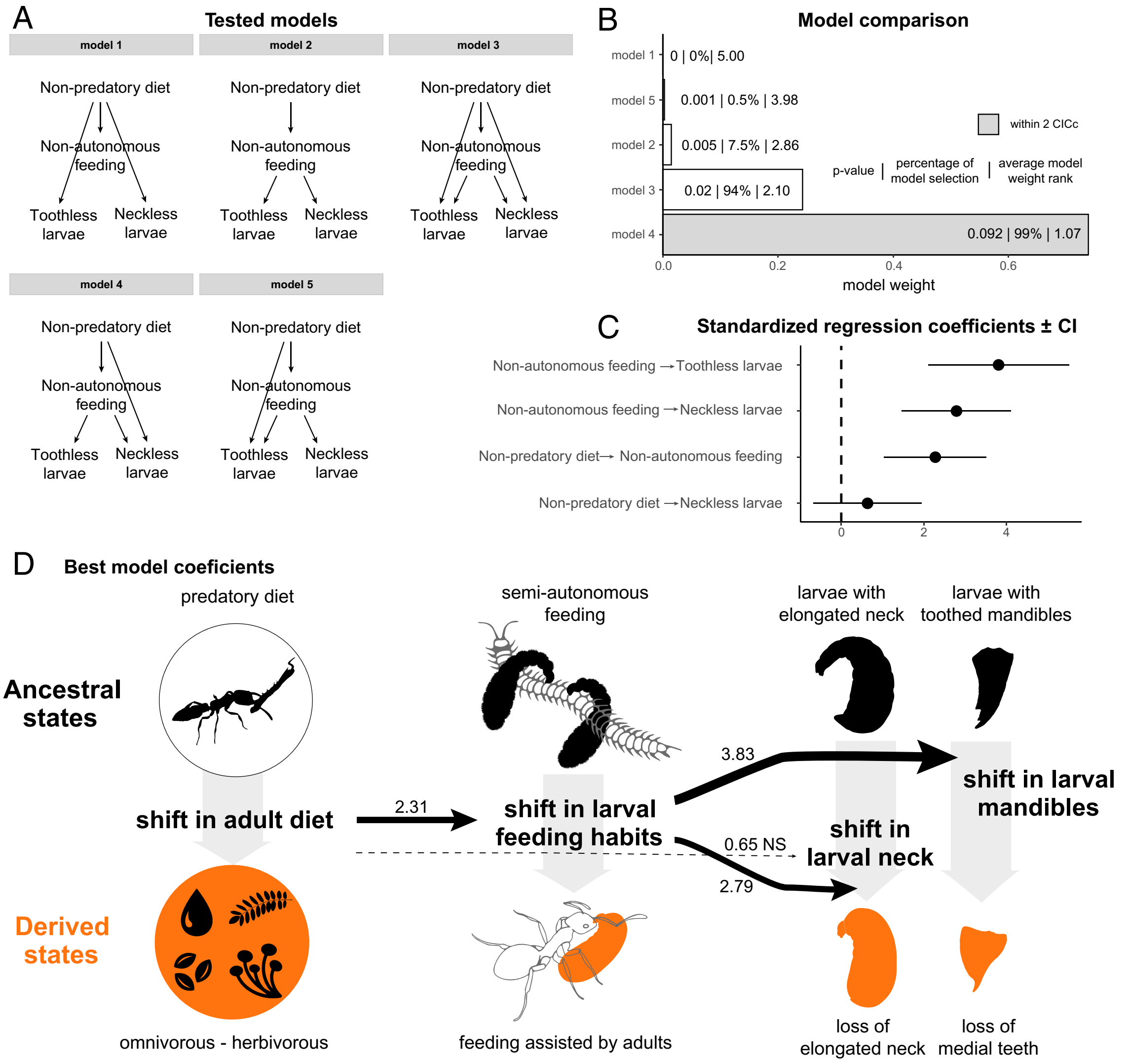

Fig. 2. Species diet, larval feeding habits, and larval morphologies are interconnected. Multitree phylogenetic path analysis performed on the dataset of 99 species (71 genera) and run over 200 phylogenetic trees. (*A*) Directed acyclic diagrams of the compared models. (*B*) Model comparison and selection (in gray) with details of *P*–values (significance indicates model rejection), percentage of model selection (best models within a range of 2 CICc), and average model weight rank over the multiple phylogenies used. (*C*) Standardized regression coefficients ± CI of the selected model (model 4) estimated over the multiple phylogenetic trees—these coefficients indicate the strength of the relationship between traits. (*D*) Causal relationships between variables from the best supported model. Arrow values indicate the phylogenetic path coefficients, and the dashed line indicates nonsignificant interactions (i.e., 95% CI including 0). Predatory ancestral diet is supported by Smith et al. (54), and ancestral larval morphology is supported by Fig. 3.

Formicoid lineages make up 80% of extant ant species and ants of these lineages are the most abundant ants worldwide (59). They have successfully colonized diverse ecological niches, often exhibiting generalist feeding behaviors, including both insect prey and nectar or honeydew consumption. In contrast, low dimorphism subfamilies (ants from the poneroid clade, but also Myrmeciinae and Ectatomminae) are primarily predators (60, 61).

Given these associations between the high dimorphism of most formicoid ants and other aspects of advanced sociality, we wanted to assess whether queen–worker dimorphism was associated with larval passiveness traits (necklessness and toothlessness), more complex social organization, and dietary shifts. We compiled a series of traits typically associated with advanced sociality, including the absence of workers with full reproductive potential [e.g., gamergates (62), colony size (63, 64), and presence of polymorphic workers (2). We treated the number of passive traits in larval morphology as ordinal data (i.e., zero, one, or two). Additionally, we gathered dietary information, contrasting genera that are primarily predators with those that are omnivores or herbivores.

Our Phylogenetic Generalized Least Squares (PGLS) analysis revealed that larval passiveness ($P = 1.2e10^{-3}$), larger colony size ($P = 8.7e10^{-5}$), and worker polymorphism ($P = 7.1e10^{-8}$) were positively correlated with greater queen–worker size dimorphism (Fig. 4). Workers with full reproductive potential were negatively correlated with queen–worker size dimorphism ($P = 0.018$). Predatory diet did not significantly correlate with queen–worker size dimorphism ($P = 0.57$). The detailed results of PGLS analyses including the relationship of these variables with queens and workers body volume are provided in *SI Appendix*, Table S5.

What Led to High Social Complexity? To disentangle the causal relationships between these traits, we constructed plausible evolutionary models to address two key questions about the evolution of social complexity (Fig. 5*A*). First, we wanted to understand the role of enhanced adult control of larval feeding in the evolution of social complexity—was enhanced care a cause or a consequence of increased social complexity? Second, we wanted to know which led to greater worker polymorphism: increased colony size or queen–worker dimorphism.

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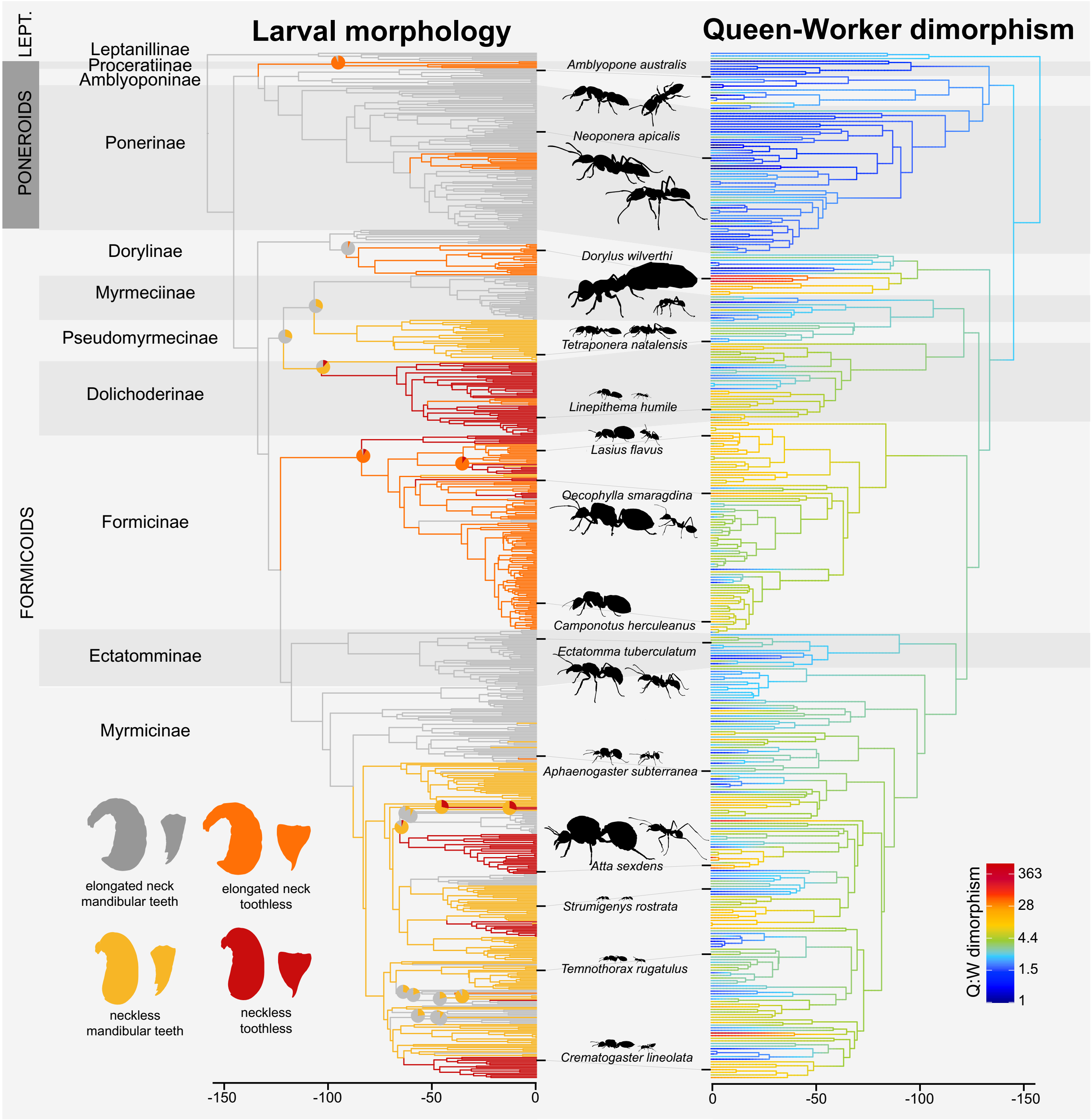

Fig. 3. Ancestral state reconstructions of (*Left*) larval morphology for 733 species and (*Right*) queen–worker volume dimorphism measured on 392 species. Colored branches indicate predicted values of traits. Pie charts are plotted on nodes where the confidence < 95%. Silhouettes of queens (*Left*) and workers (*Right*) are shown in proportion to their measured size. Major subfamilies are labeled in alternating tones with details of their clade: e.g., "LEPT." = Leptanillomorphs. Time is expressed in Ma, and the color bar on the *Right* panel indicates the ratio between queen and worker body volumes.

We built four fundamental models (Fig. 5*A*) to test these scenarios, using the proxies of colony size and worker polymorphism for social complexity and passive larval morphologies as a proxy for enhanced alloparental care. These models were evaluated and compared using multiphylopath. We included three core trait relationships that consistently improved model estimations (65): the influence of diet on passive morphologies, and the influence of both dimorphism and colony size on worker polymorphism (Fig. 5*A*). These core relationships were derived from significant correlations identified in our PGLS analysis (Fig. 4), our causal models linking diet and larval morphology (Fig. 2) and supported by previous literature (60, 66).

Sensitivity analyses. From the four backbone models (Fig. 5), we also tested three alternatives in the relationship of colony size with species diet and larval passiveness (*SI Appendix*, Fig. S5). In addition, we conducted our analyses with both species-level data (240 species, 95 genera) and colony-size data averaged at the genus level, allowing a larger dataset but lower resolution (363 species, 128 genera). The models and results presented in Fig. 5 reflect the four main model types of the alternatives with the best support

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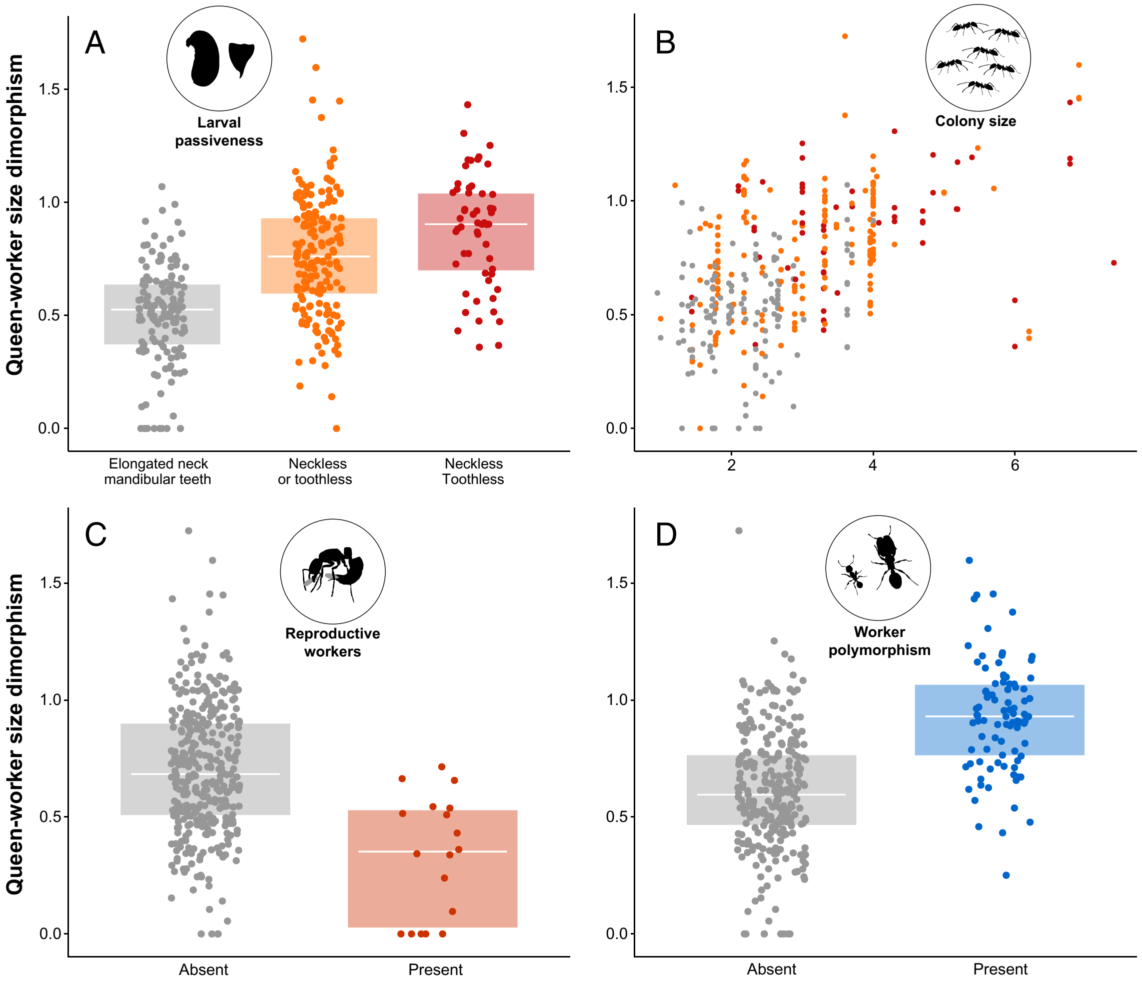

Fig. 4. Queen–worker size dimorphism correlates with several social complexity traits. Significant pairwise correlations between queen–worker size dimorphism and (*A*) passive larval morphologies, $P = 1.2e10^{-3}$, (*B*) colony size, $P = 8.7e10^{-5}$, (*C*) worker reproductive autonomy, e.g., gamergates, $P = 0.018$, and (*D*) worker polymorphism, $P = 7.1e10^{-8}$. Queen–worker size dimorphism values are displayed as root squared and log-transformed. Colony size data were log-transformed, and dots were colored according to larval morphology.

based on species-level data (see *SI Appendix*, Fig. S5 and S6 for details on other models tested).

Enhanced care is more likely a cause than a consequence of social complexity. Across all scenarios and whether the species- or genus-level dataset was used, the models that consistently received the strongest support were models where enhanced alloparental care was a cause rather than a consequence of the evolution of social complexity (Fig. 5*A*, see also *SI Appendix*, Fig. S6). Interestingly, our analysis could not determine whether increased queen–worker dimorphism or colony size was the primary driver of social complexity. Queen–worker dimorphism was favored in the larger genus-level dataset, while colony size was favored in the reduced species-level dataset. Moreover, in both cases, the difference in support was small, preventing the rejection of either. This suggests that caste size dimorphism and colony size are deeply interwoven, likely representing two key factors in the evolution of ant sociality.

Discussion

While early ants were already capable of building distinct queen and worker morphologies (34, 35), our study suggests that species became able to significantly increase caste dimorphism, and reap considerable fitness benefits, by obtaining greater control over larval feeding. We found that low queen–worker size dimorphism and actively feeding larvae were the ancestral states for ants. Dimorphism increased dramatically in several formicoid lineages, where it coevolved with larval passiveness, large colony sizes, worker polymorphism, and loss of worker reproductive potential. The shift of larvae from active feeders to passive food receptacles was promoted by preinnovations shifting adult diets toward new food sources that required individualized distribution. We propose that shifting the ancestral feeding pattern of larvae toward extended and individualized nutritional alloparental care was a key evolutionary innovation that allowed the evolution of extreme dimorphism with notable impacts on social complexity.

How Could Passive Larvae Have Evolved? The evolution of passive larvae is somewhat surprising given ants' individual fitness incentives. The parasitoid evolutionary origins of ants (67), consistent with our ancestral state reconstructions, would have required active larval morphology for independent feeding on adult-acquired prey. In a hypothetical early ant colony where larvae fed actively on prey in the nest without individualized care [e.g., *Stigmatomma* (49, 68)], each diploid larva should aim to be the largest such that she could develop into a reproductive queen and ensure her own fitness gains. Thus, the most passive larva within a colony has little opportunity to become a reproductive queen transmitting passive larval genes. Instead, the most unruly larva should be favored (e.g., ref. 69). Furthermore, the additional care required by passive larvae puts a larger care burden on workers.

Dietary shifts likely provided the preinnovations that enabled individualized care. We found that passive larval morphologies

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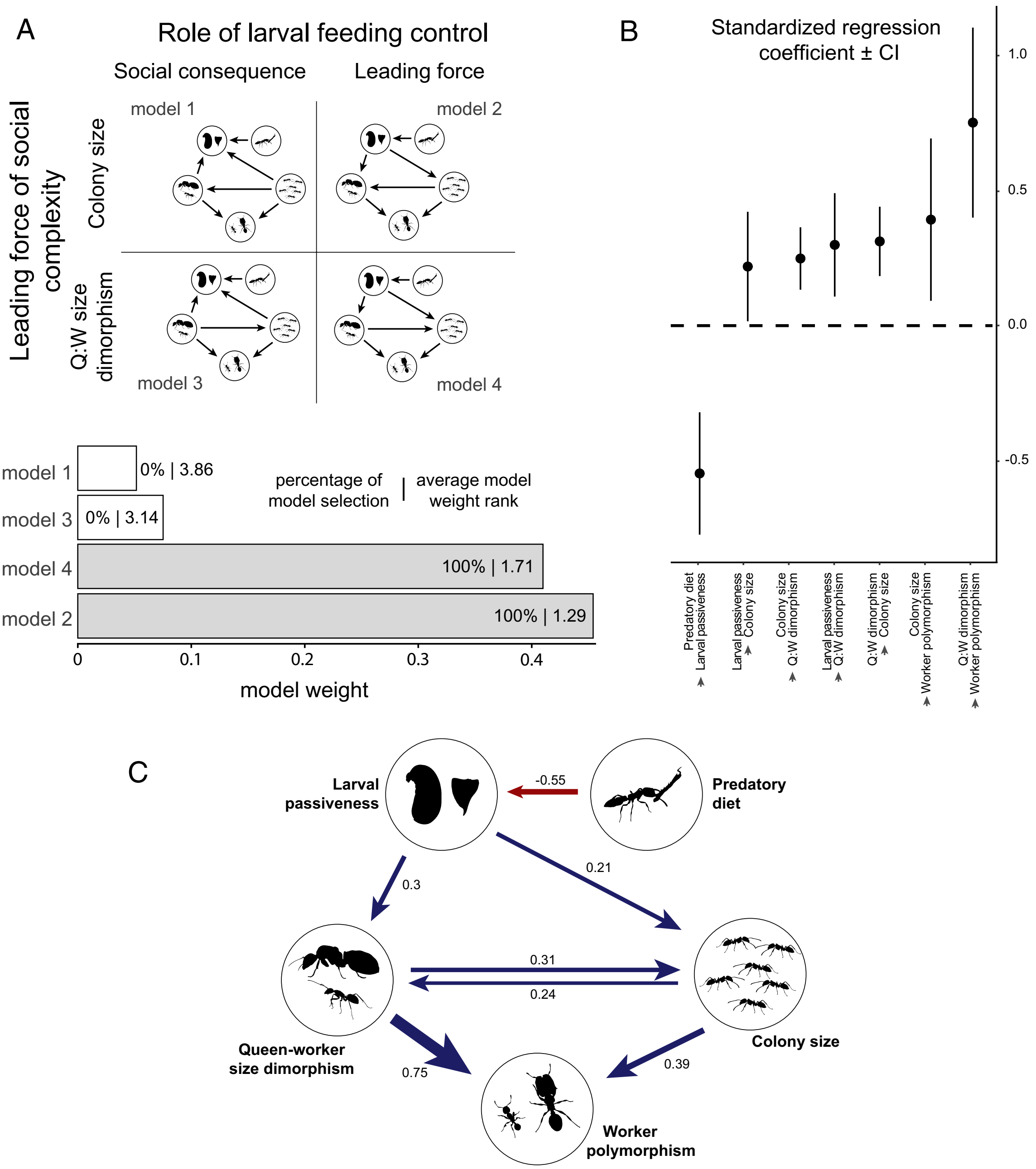

Fig. 5. Causal inference of relationships across enhanced alloparental care, dimorphism, and social complexity. Multitree phylogenetic path analysis performed on a dataset of 240 species (95 genera) and run over 200 phylogenetic trees. (*A*) Model comparison. Model weight estimation and comparison over multiple phylogenetic trees with details of the percentage of trees for which a model was selected (best models within a range of 2 CICc) and average model weight rank. *P*–values (whose significance indicates model rejection) were all above 0.05. (*B*) Standardized regression coefficients ± CI estimated over multiple phylogenetic trees from averaging the best models (models 2 and 4). (*C*) Causal relationships between variables from averaging the best models (models 2 and 4). Arrow values indicate the phylogenetic path coefficients. Blue edges indicate positive correlations while red edges mark negative correlations. Results from alternative scenarios and with a larger dataset with colony size averaged at the genus level are presented in *SI Appendix*, Fig. S5 and S6.

were associated with more supervised feeding habits, which in turn were linked to dietary shifts away from a predatory diet. Throughout ant diversification, numerous species have transitioned from predatory to omnivorous or even fully herbivorous diets (60, 70). While prey items can be shared among larvae in joint feasts or distributed into large pieces, most derived diets are unshareable. Sugary liquids, trophic eggs, Beltian and Mullerian bodies, fungal staphyla, and some seeds, due to their material nature and small size, require adults to feed larvae individually and in small quantities through one-to-one interactions. Thus, whereas ancestral ant adults could drop large pieces of prey on or near active larvae and remove them at their discretion, shifts in adult diet necessitated incremental and individualized feeding of larvae. However, transitioning to alternative food sources while maintaining nutritional support for larval growth required physiological or ecological innovations, often achieved through symbiont acquisition or metabolic division of labor (*SI Appendix*, *Supplementary Discussion*).

Change toward more supervised and individualized larval feeding habits had three crucial outcomes. First, it reduced the opportunity for larvae to overfeed so as to influence their caste fate (but see ref. 71). Second, it dramatically reduced potential direct food

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competition among larvae. These are two mechanisms promoting active larval morphologies. Third, shifts toward a nonpredatory diet likely changed the nutrient flows within the colony. While predatory species often rely on larvae to process their high-protein meals and redistribute nutrients to adults, the converse occurs in carbohydrate- or lipid-based diets (72–74). Hence, shifts toward obligate individual feeding granted adults greater control over larval nutrition and facilitated the evolution of morphologically passive larvae, less capable of manipulating their own nutrient intake (Fig. 6). Both paved the way for great queen–worker dimorphism and its myriad benefits for ecological dominance.

Passiveness allows but does not necessitate extreme dimorphism. While the amount of food received by autonomous larvae on a carcass can still be adjusted by workers, for example, by removing larvae from prey, building dimorphism in this manner would require strong and consistent social organization. In contrast, the enhanced parental care associated with derived larval diets gave adults significantly greater control over the nutrients consumed by each larva (22, 38–40, 75). This level of control likely provided a valuable mechanism for managing the selfish reproductive aspirations of larvae and created more asymmetric nutritional states, channeling larvae into distinct adult morphologies (queen, worker, or various worker subcastes).

Alongside behavioral adaptations, physiological adaptations underlie adults' control over larval development. In numerous species, passive larvae are extensively fed through trophic eggs or regurgitated liquids whose components are produced by the adults themselves (Dataset S1, (76)). Trophic eggs have been reported to influence caste determination (77) and regurgitated fluids have been found to contain larval growth regulators that adjust adult body size (22, 38, 39), providing mechanisms of how workers can modulate larval development through personalized nutrition.

Factors other than larval feeding influence queen–worker dimorphism. Notably, there are low-dimorphism species featuring passive larvae and high-dimorphism species with active larvae. Colony foundation and queen dispersal provide significant constraints on the evolution of dimorphism. During solitary colony foundation larger dispersing queens can produce comparatively cheaper workers to quickly achieve a minimally viable colony (6). However, other dispersal solutions exist that do not require high dimorphism (78), or that remove limitations to queen volume (e.g., no need for flight in Army-ant Dorylinae).

Strong dimorphism in species without passive larval morphology can also arise through less common mechanisms of caste fate regulation. In some ant taxa, adults mildly injure their larvae and feed on their hemolymph. This nondestructive cannibalism pilfers nutrients from larvae and may enable the creation of nutritional asymmetries that can result in size dimorphism (79). Larval hemolymph feeding has been found to be particularly frequent in *Leptanilla japonica* and *Myopopone castanea*, which both exhibit phylogenetically unexpected high dimorphisms (37, 80). This possible mechanism of creating dimorphism requires deeper investigation. In some species, caste fate is influenced partially or entirely by genetic factors or maternal effects, which bias the totipotency of diploid larvae or completely predetermine it to one caste fate or another (13, 14, 81). Although much remains to be discovered, strictly predetermined caste fate has so far only been documented in five Myrmicine genera and one Formicine genus, in lineages that had already established significant caste dimorphism (81, 82). However, the developmental literature is heavily biased toward species with strong worker dimorphism, which means that the absence of evidence for maternal effect determination outside these species does not necessarily imply its absence. Given this uncertainty, alternative scenarios can be considered. One possibility is that larval nutrition determination is ancestral, with maternal effects being a rare derived state in species with strong worker dimorphism and high social complexity. Alternatively, maternal effects could be a more common derived state in many contexts, or could even be an ancestral trait, with various species independently evolving larval nutrition determination. Future work should explore these possibilities, as better understanding of the role of maternal effects in caste development

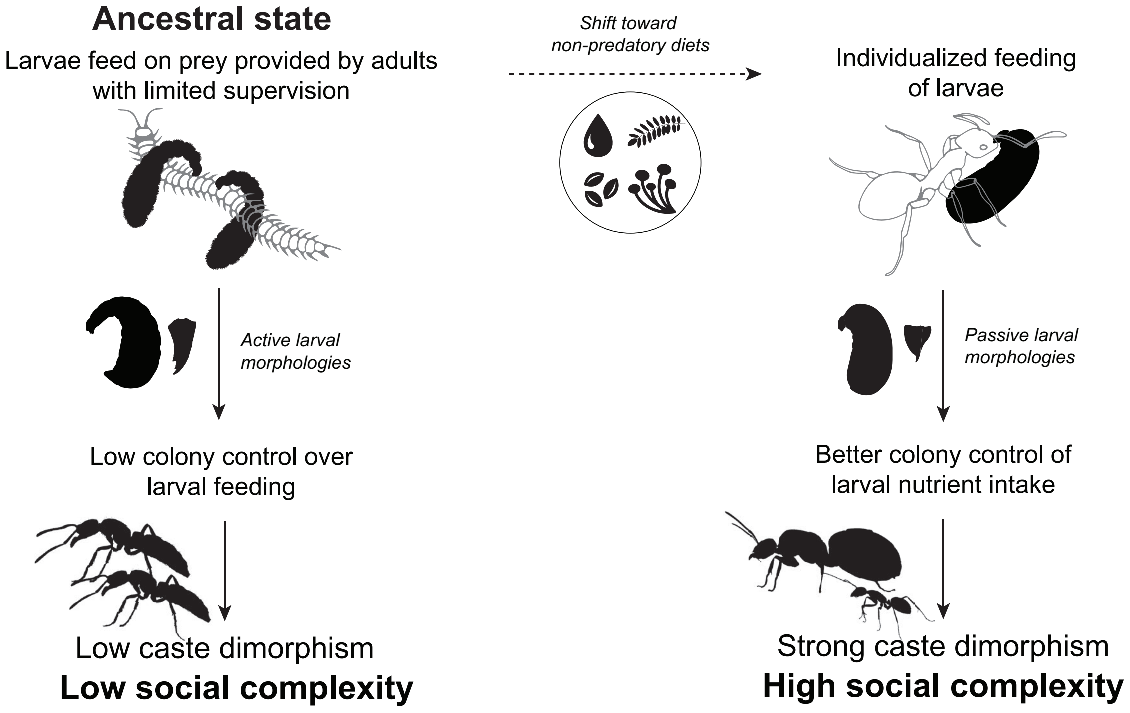

Fig. 6. Summary of the evolutionary relationships between larval feeding habits, caste dimorphism, social complexity, diet, and larval morphology.

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could significantly enhance our understanding of caste evolution across ant lineages.

Benefits of larval passiveness. Putting larval fate under adult control was a critical change point in the social transitions of ants. If alloparental care toward broods enabled early ant societies to build the first female castes (34, 36), individualizing adult control over larval nutrition allowed ants to build stronger caste dimorphism and take a major step in their social evolution. The ability to precisely channel larvae into different castes enabled greater reproductive and worker caste specialization, along with subcastes of workers specialized for different tasks (83), increased colony size (4, 84), and production of massive dispersing queens able to found new independent colonies with no external food supply, reducing predation risk (6). These outcomes were made possible through stronger colony-level control over larval development. Considering the major evolutionary transition from single-celled to multicellular organisms, a comparable shift in individual to collective priorities likely necessitated nonautonomous fate determination during development (1, 85). This may be a fruitful avenue for future research on the mechanisms underlying major evolutionary transitions in individuality.

Materials and Methods

Caste Body Volumes and Dimorphism. To examine the evolution of dimorphism and its relationship with a panel of ecological and life-history traits, we measured body size of ant queens and workers in 392 species spanning 147 genera over 14 subfamilies (Dataset S2). Species were selected based on the presence of both queen and worker image (Antweb.org), available data for our traits of interest in the literature, phylogenetic data, and optimally wide taxon sampling (*SI Appendix*, Fig. S4).

Using the Antweb.org photo database, we measured one queen and one worker per species, favoring type specimens when 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*, (86). Due to the lack of pictures for both queen and worker of Leptanillinae species with phylogenetic data on Antweb.org, we measured queens and workers body volume for one *Leptanilla* and two *Protanilla* species from six specimens illustrated in refs. 87 and 88. While ideally, we would have measured queen–worker pairs from the same colony or locality, the availability of imaged specimens would have significantly restricted our sample size. Therefore, we utilized the available images to sample the widest possible range of species. Consequently, our measures of dimorphism might be influenced by geographical effects on queens and workers from different localities (e.g., refs. 89 and 90). However, this effect should be randomly distributed across our dataset and thus have a limited impact compared to the actual species effect on dimorphism variation.

We measured the length, width, and height of the mesosoma as well as the length and width of the head and the gaster using ImageJ (91) (v.1.53a) (Fig. 7). Because measurements are sensitive to tagma orientation, head height was impossible to properly measure on numerous mesosoma-pinned specimens. We therefore used the dataset of Sosiak and Barden (61) to estimate the best proxy for this metric. Head length explained 95% of the head height variation (n = 299), allowing to approximate head height as 0.608 × head length. 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:

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

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

$$V_{gaster} = \frac{L_{gaster}}{2} \times \frac{W_{gaster^2}}{2} \times \frac{3}{4}\pi,$$

$$V_{body} = V_{head} \times V_{mesosoma} \times V_{gaster},$$

$$Size\ \ dimorphism = \frac{V_{body\ queen}}{V_{body\ worker}}.$$

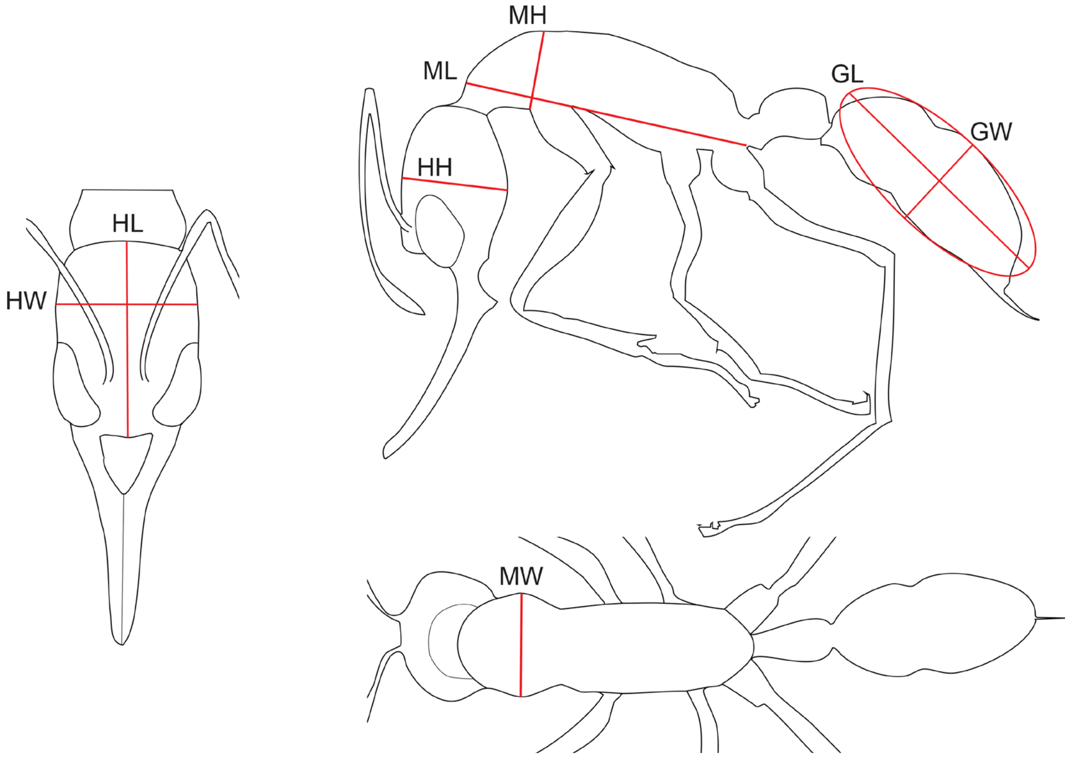

Fig. 7. Morphological measurements used in the present study. Head high (HH), head length (HL), head width (HW), mesonotum high (MH), mesonotum length (ML), mesonotum width (MW), gaster length (GL), and gaster width (GW).

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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. Species where the measured worker happened to be bigger than the measured queen [e.g., *Mystrium oberthueri* (92) and other species with similar-sized queens and workers] were also categorized with a size dimorphism value of 1.

Statistical analyses were performed and graphs were generated with the software R (v.4.1.3) (93). All our measurement data are provided in Dataset S2. Queen and worker volume values were used as $\log_{10}$ transformed and dimorphism ratio was used as $\log_{10}$ and root-square transformed in our analyses to fit a normal distribution (94, 95).

Larval Alloparental Care and Morphologies. Since there is limited information available in the literature about alloparental care and larval feeding across the ant phylogeny, we wanted to examine the relationship between larval feeding habits and their morphologies. Our dataset included information on the larval feeding habits of 99 species spanning 71 genera and 12 subfamilies (Dataset S1). Specifically, we categorized whether larvae of these species usually fed semiautonomously with limited alloparental care (viz., actively feeding upon unprepared prey left at their disposal or receiving large chunks of food items) or, conversely, if their feeding primarily involves strong adult care (viz., regurgitation, preparation of premasticated pellets, distribution of trophic eggs, etc.) (see Dataset S1 for quotes and classification).

Over a span of 66 y, Wheeler and Wheeler performed a thorough analysis of larval morphology in more than 700 ant species. Among the global ant larva diversity, they identified 13 general body shapes and 20 general mandible shapes in which they classified ants at the genus level, describing, among other features, the presence or absence of an elongated neck and medial teeth on the mandible (46, 96) (see *SI Appendix*, Fig. S1 and Tables S1 for details about these categories).

According to Wheeler and Wheeler's statements about the role of neck and mandibles in larval feeding (*Results*), we retrieved the presence of an elongated neck and medial (subapical) teeth on mandibles in larvae to associate these features with their feeding habits. We assembled data on larval morphology for 733 species from 202 genera, mainly from the pioneering work of Wheeler and Wheeler and complemented by some more recent larval descriptions. Other aspects of larvae might also be informative (e.g., mandible size, mandible sclerotization, rate of movement, dynamic range of neck), but there are little to no available data for these traits.

To associate larval morphologies with species from Wheeler and Wheeler's work, we retrieved morphological categories associated with genera by Wheeler and Wheeler and the list of species they studied (46, 96). We associated species with the morphologies of their genus and updated the names of species according to the latest taxonomic revisions (AntCat.org).

Wheeler and Wheeler described two larval mandible morphology categories as "with or without medial teeth" (*SI Appendix*, Table S2). For the species falling into these categories, we thoroughly examined their detailed larval descriptions to determine the presence or absence of medial teeth in these species. The resulting database and classification details are provided in Dataset S3.

These morphological traits display little evolutionary lability (Fig. 2). Within our dataset, among the 113 genera represented by multiple species (with an average of 5.7 species per genus), only five genera displayed intragenera variability in neck or mandible morphology (viz., *Cephalotes*, *Myopias*, *Neivamyrmex*, *Nylanderia*, *Tetramorium*). As such, we matched the species in our dimorphism dataset with larval morphology extrapolated from the genus level, except species belonging to genus with variable larval morphologies that we kept at the species level.

Social Complexity and Life History Traits. We collected several traits related to the social and ecological evolution of ants. We gathered data on the presence of polymorphic workers at the species level from ref. 97. Polymorphism was considered absent in 11 species from our dataset not included in ref. 97, as polymorphism has been recorded from 0 to 2% in species within their genera. Information on genera with species exhibiting workers with full reproductive potential (gamergates) was retrieved from Antwiki.org. Species in our dataset belonging to these genera were classified as gamergate, and otherwise classified as non-gamergate. We used the classification of Blanchard and Moreau (60) to retrieve if a genus is mainly predaceous or not. We gathered colony size data (Dataset S4) from various databases and primary literature for a total of 762 records encompassing 621 species across 135 genera for which we had dimorphism and larval morphology data. We calculated the median per species and then the median per genus that we extrapolated to the species of our dataset for the statistical analysis.

Phylogenetic Data. We used the phylogenetic data produced by Economo et al. (98) as a reference for phylogeny as it was the largest and most recent ant phylogeny data at the time. Specifically, these data include 1) a Maximum Clade Credibility (MCC) backbone tree reconstructed from the sequence alignment of 679 specimens, 2) a set of 200 topologies in which almost all (~15k) ant species were randomly grafted around the leaves of the same genus from backbone trees, and 3) one MCC ~15k species grafted tree. Species names were updated according to the latest taxonomic revisions (AntCat.org, release March 2023). Species of our dataset missing in the grafted trees were included at the position of another unused species of the same genus.

Modeling Trait Evolution. We investigated the temporal dynamics of morphological evolution over the diversification of ants using the MCC backbone tree, whose leaves are fully supported by molecular data. For each trait (i.e., body volume of queens and workers and queen–worker dimorphism), we assessed their phylogenetic signal in the data by calculating Pagel's lambda and Blomberg's *K* with the R package phytools (99) (v.1.9.16). We then tested the fit of nine models of continuous 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 (100) (v.2.0.10). The models were compared by their log-likelihood and Akaike information criterion (AIC). Results obtained from trait evolution analyses are summarized in *SI Appendix*, Table S4.

Differences in evolutionary rates (σ^2) of traits were assessed following Adam's method (101) implemented in the package mvMORPH (102) (v.1.1.7). We compared the log-likelihood of two Brownian motion-based models, one where the two traits or clades to compare evolve at distinct evolutionary rates, and another where the traits or clades 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 (102) (v.1.1.7).

Ancestral State Reconstructions. Ancestral state reconstructions were performed on the MCC ~15k species grafted tree. We performed ancestral state reconstructions for larval body shape and mandible shape at each node using the function *fitMk* in the R package phytools (99) (v.1.9.16). This utilizes a stochastic character mapping approach (103, 104). We compared three evolutionary models of state transitions (equal-rates, symmetric, and all-rates-different) using their AICw. Ancestral state reconstructions of the Q:W dimorphism were performed using the function *anc.ML* in the R package phytools (99) (v.1.9.16), where we estimated evolutionary parameters and ancestral states using likelihood given a Brownian-motion model of evolution of continuous traits. To incorporate the best-fitted lambda evolutionary model and account for the phylogenetic signal of dimorphism (as detailed above), ancestral state estimations were performed on a lambda-transformed tree. The tree was transformed according to the calculated phylogenetic signal of dimorphism using the function *rescale* of the R package Geiger (100) (v.2.0.10). We then projected reconstructed ancestral states onto the original species tree.

Correlation between Traits. We tested the evolutionary correlation between larval feeding pattern and larval morphology features (i.e., presence of an elongated neck and medial teeth) on the MCC ~15k species grafted tree using the function *fitPagel* hosted in the R package phytools (99) (v.1.9.16). A function designed to fit Pagel's (52) method 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 AIC (*SI Appendix*, Table S3).

We used PGLS analysis to test the correlation between traits taking into account relatedness among taxa. PGLS uses a covariance matrix to weigh least squares and incorporate phylogenetic relatedness into the analysis, assuming that branch length is proportional to the residual error in the model (105, 106). As such, we performed a PGLS analysis using the function *gls* of the R package nlme (107) (v.3.1.157) on

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the mean covariance matrix between species from the 200 phylogenetic trees. Before calculating the covariance matrix, trees were lambda transformed, using the function *rescale* of the R package Geiger (100) (v.2.0.10), according to the phylogenetic signal of the tested answer variable (*Modeling Trait Evolution*), a classical method to implement phylogenetic signal in PGLS analysis (108). 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 (109) implemented in the R package Phylopath (53) (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 (110, 111) and considering the nonindependence of the traits due to phylogeny through PGLS analysis. We built several plausible models and estimated their parameters on the MCC tree. Following van der Bijl (53), we selected models with the minimum corrected Akaike Information Criterion (CICc) within a range of 2 CICc and with no significant *P*-values indicating conditional independencies. To account for uncertainties in our phylogenetic data, we also retrieved the model selection over the 200 grafted trees to corroborate results from the MCC tree. Additionally, we edited the function *average* from the phylopath package to handle a set of trees instead of a single one and to estimate coefficients of variables' relationships arising from model selection over the 200 grafted trees and average them using the function *average_DAGs*. We provide all our datasets and R code to conduct these analyses in the Zenodo repository (https://doi.org/10.5281/zenodo.14746149) (112).

Data, Materials, and Software Availability. R codes and datasets have been deposited in Zenodo (https://doi.org/10.5281/zenodo.14746149) (112). All other data are included in the manuscript and/or supporting information.

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Supporting Information for

Innovation in ant larval feeding facilitated queen-worker divergence and social complexity

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This PDF includes:

- Supplementary discussion
- Figures S1 to S6
- Table S1 to S5
- Legends for Datasets S1 to S4
- SI references

Other supporting materials for this manuscript include the following:

- Datasets S1 to S4

SUPPLEMENTARY DISCUSSION

Changing larval diet requires other changes

Transitioning to alternative food sources is a complex process. Since ant larvae arose from a prey-feeding background, any new food still must fulfill nutritional requirements for larval growth; i.e., rich in protein and nitrogen (1, 2). Different ant lineages overcame this challenge through diverse innovations. For instance, sugary liquids can be enriched by nitrogen-fixing bacterial symbionts inside the digestive tracts of workers (3–5), or through metabolic labor performed by workers (6, 7). Both innovations entail worker ingestion, followed by sharing through trophic eggs or regurgitation, two features common in species with passive larvae. Other ants, including fungus-growing ants and some Pseudomyrmecinae, forged relationships with external symbionts such as fungi or plants that provided nutritional support for larval growth (8–10).

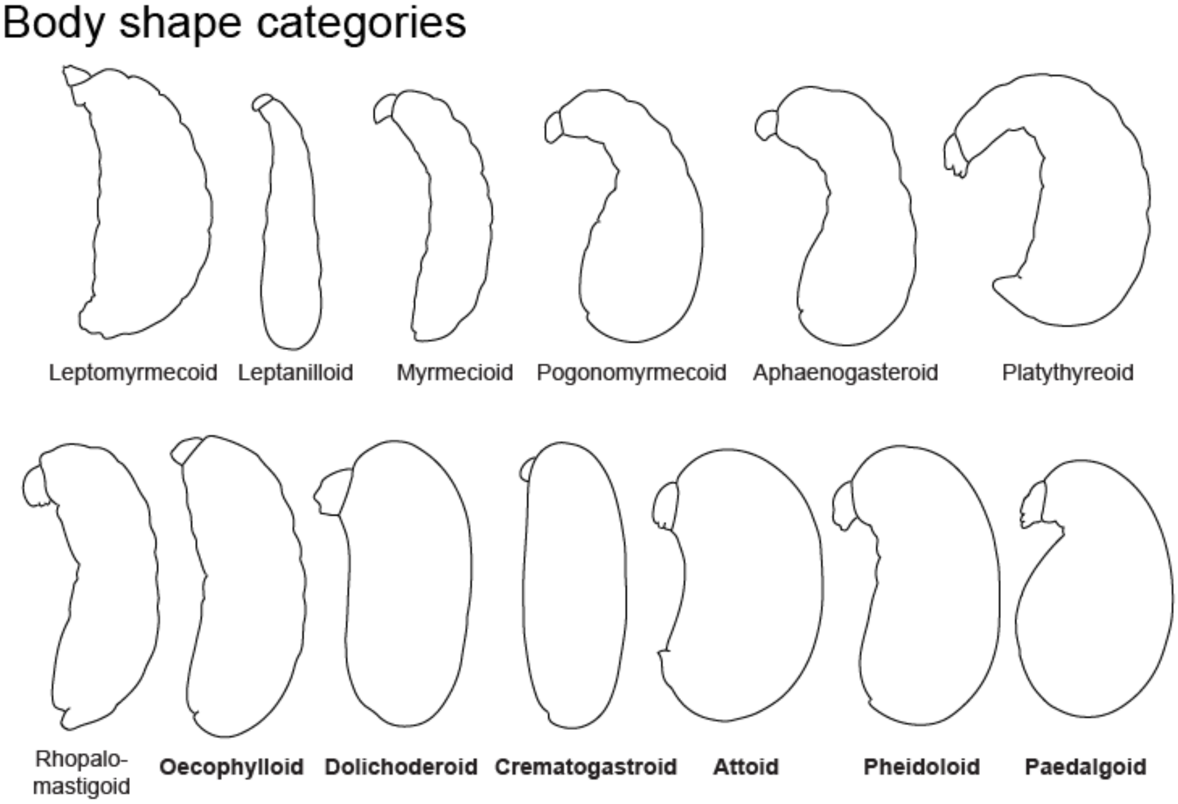
Behavioral and temporal innovations strengthening adult control over larvae

Additional behavioral adaptations likely contributed to the evolution of passive larvae. Age-related changes in larval diet have been reported in several species, with young larvae receiving regurgitate while late-stage larvae are provided with prey (e.g., 11, 12). Besides the potential limited ability of young larvae to handle prey, the pressure to monitor larval feeding may also decrease as their developmental trajectory settles (13). Turning prey into pellets instead of leaving larvae consuming it directly ensures individualized and monitored feeding, albeit placing a greater burden on workers. These innovations were likely largely facilitated by the use of alternative food sources for larvae, reducing the amount of prey to process and associated with larger colony sizes (14). Larger colonies often have greater task organization (15), allowing the evolution of other facilitators such as sorting larvae by stage (16) and nurse larval-stage specialization (17), which likely strengthen the regulation of larval growth. In addition, forming cohorts of larvae that become either all queens or all workers may be another way for adults to efficiently manipulate larvae' caste fate. By separating larvae spatially within the nest or temporally (e.g., seasonality), the colony can overcome the challenge of having to create inequality among larvae with similar caste fates. However, the sporadic recording of these behaviors precludes macroevolution analysis at this time.

Several remaining mysteries on the evolution pattern of passive larvae

Regarding these associations between species diets, larval feeding habits, larval morphology and size dimorphism, several questions remain unanswered on the evolution pattern of passive larvae. Regarding the consistent evolution of toothless larvae in dorylinae Army-ants, Wheeler and Wheeler pondered in 1984 "*Why do larvae of the world's most carnivorous ants have mandibles incapable of chewing solid food?*". Although Wheeler and Wheeler (18) speculated that these larvae might digest prey externally before sucking the resulting liquid, the mystery remains unsolved, and the cause and consequence of such mouthpart reduction are even more obscure. This particular adaptation may arise from their diet largely composed of other ants' brood (19). On the other hand, several species of *Leptogenys* and *Myopias* have also evolved toothless morphologies in otherwise actively feeding larvae. The case of these species, however, might stem from specialization on prey with extremely thick cuticles, such as millipedes, which restricts larvae to feed only on the internal soft tissues, as described by Brown (20) and Ito et al. (21). Finally, it is interesting to note that many species retain elongated necks in lineages that have first evolved toothless larvae (e.g., Formicinae) While the necessity for mandibular teeth may primarily relate to food texture handled by larvae, the pressures influencing larval necks can be considerably more diverse. Larval opportunities for enhanced mobility, food-begging behaviors, brood cannibalism, and handling larger prey may favor the presence of necks in larvae (22, 23). Additionally, the already-increased worker control over larval feeding in those lineages may alleviate pressure for entirely passive larvae.

SUPPLEMENTARY TABLES AND FIGURES



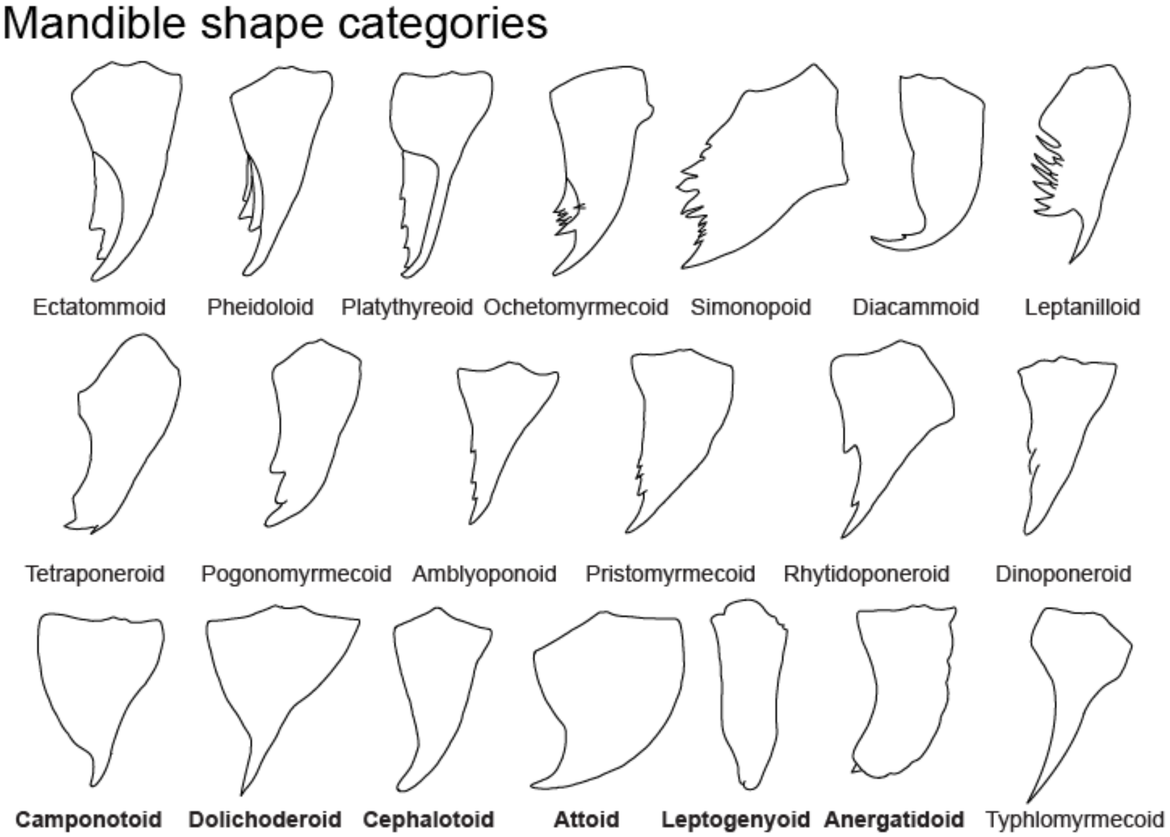


Figure S1. Ant larval body and mandible shape categories proposed by Wheeler and Wheeler (24, 25). Names in bold are categories considered ‘passive’ according to the absence of elongated neck and absence of mandibular teeth following their description (see Tables S1 and S2). Although the Typhlomyrmecoid mandible category appears toothless in the Wheeler and Wheeler representation shown, the description of the category does not mention the absence of medial teeth and the larvae of the concerned genera (*Apomyrma* and *Typhlomyrmex*) are described and represented with medial teeth (26, 27).

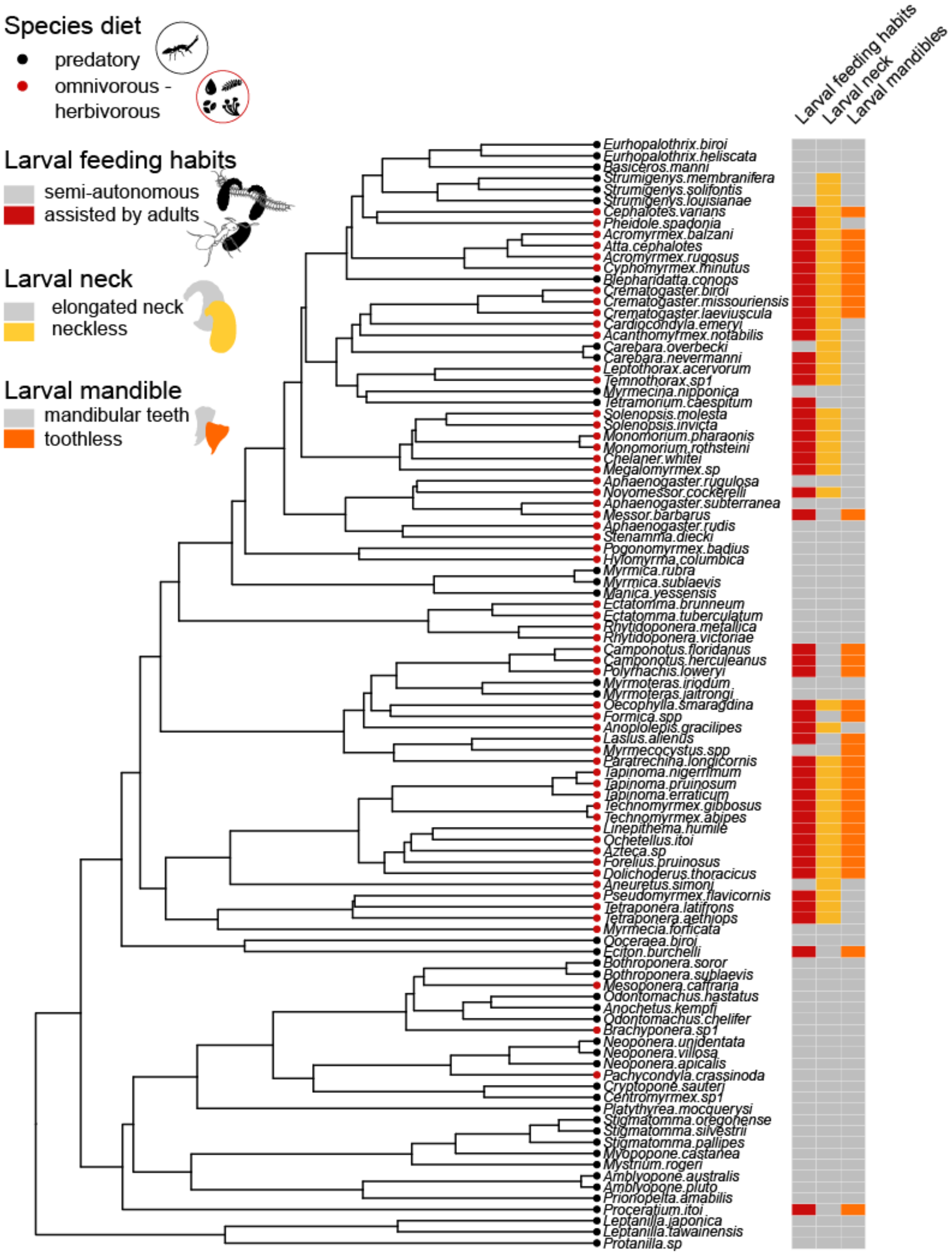


Figure S2. Phylogenetic representation of the 99 species covering 71 genera with both data for larval feeding habits, larval morphology, and species diet.

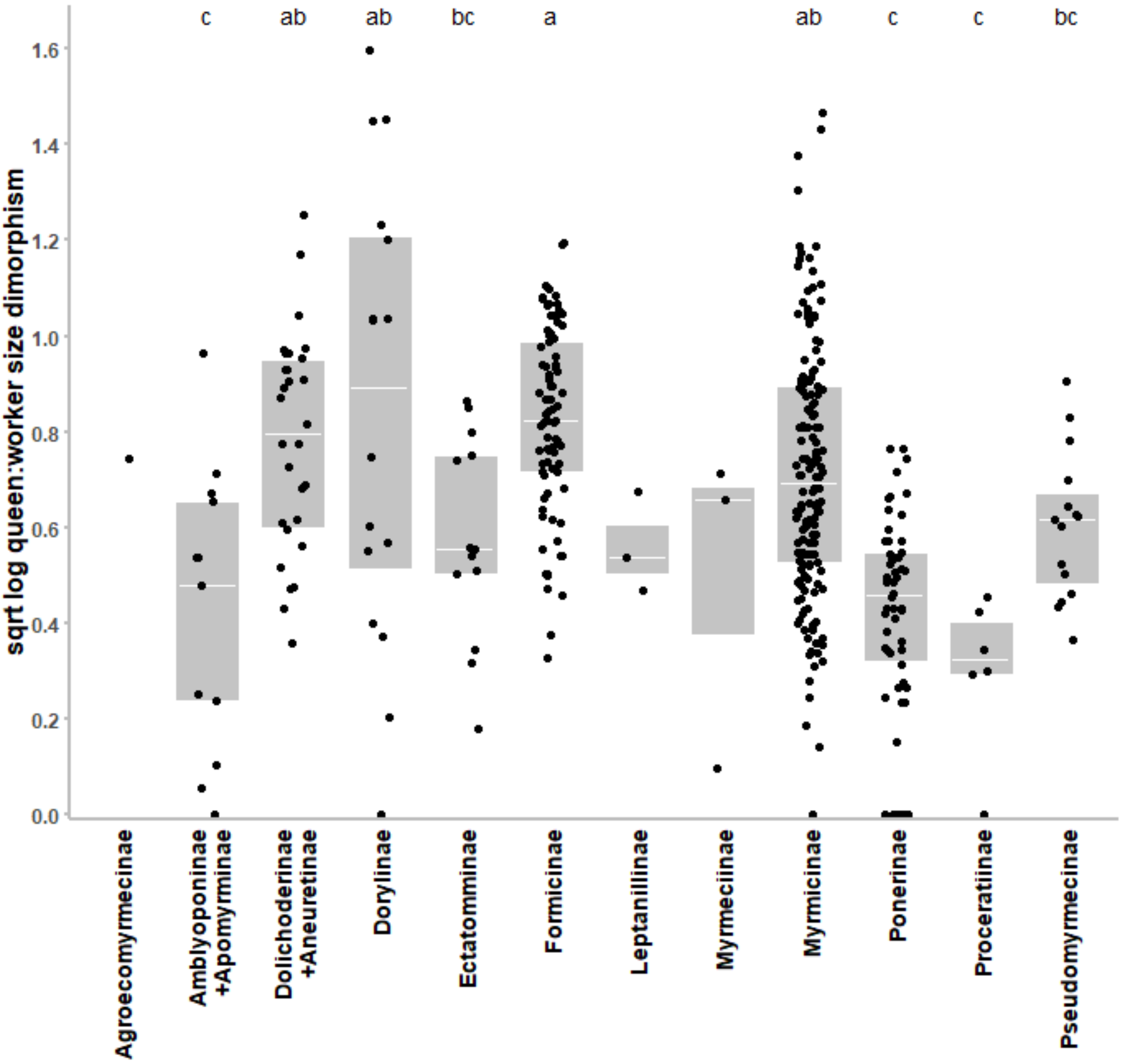


Figure S3. Dimorphism values measured in 392 species distributed in the different ant subfamilies. Ant subfamilies differ in their dimorphism values (one-way ANOVA: $F_{11,376}$ = 15.812, $p < 0.001$). Different letters indicate significant differences between subfamilies (Tukey's post-hoc test: $p < 0.05$). Groups containing less than 6 species were not considered in the statistical analysis. Dimorphism values were $\log_{10}$ and root-squared transformed in order that residuals fit normal distribution according to the Shapiro test.

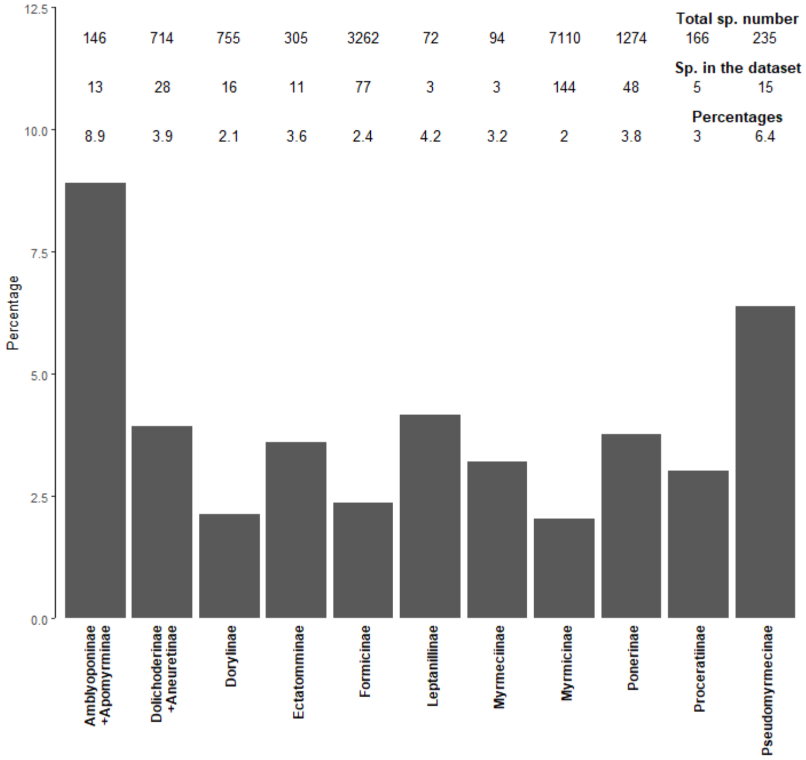


Figure S4. Representation per subfamily of the 363 species (128 genera) present in our dataset for PGLS and phylogenetic path analysis. Total number of species data is sourced from Antcat.org (September 2023).

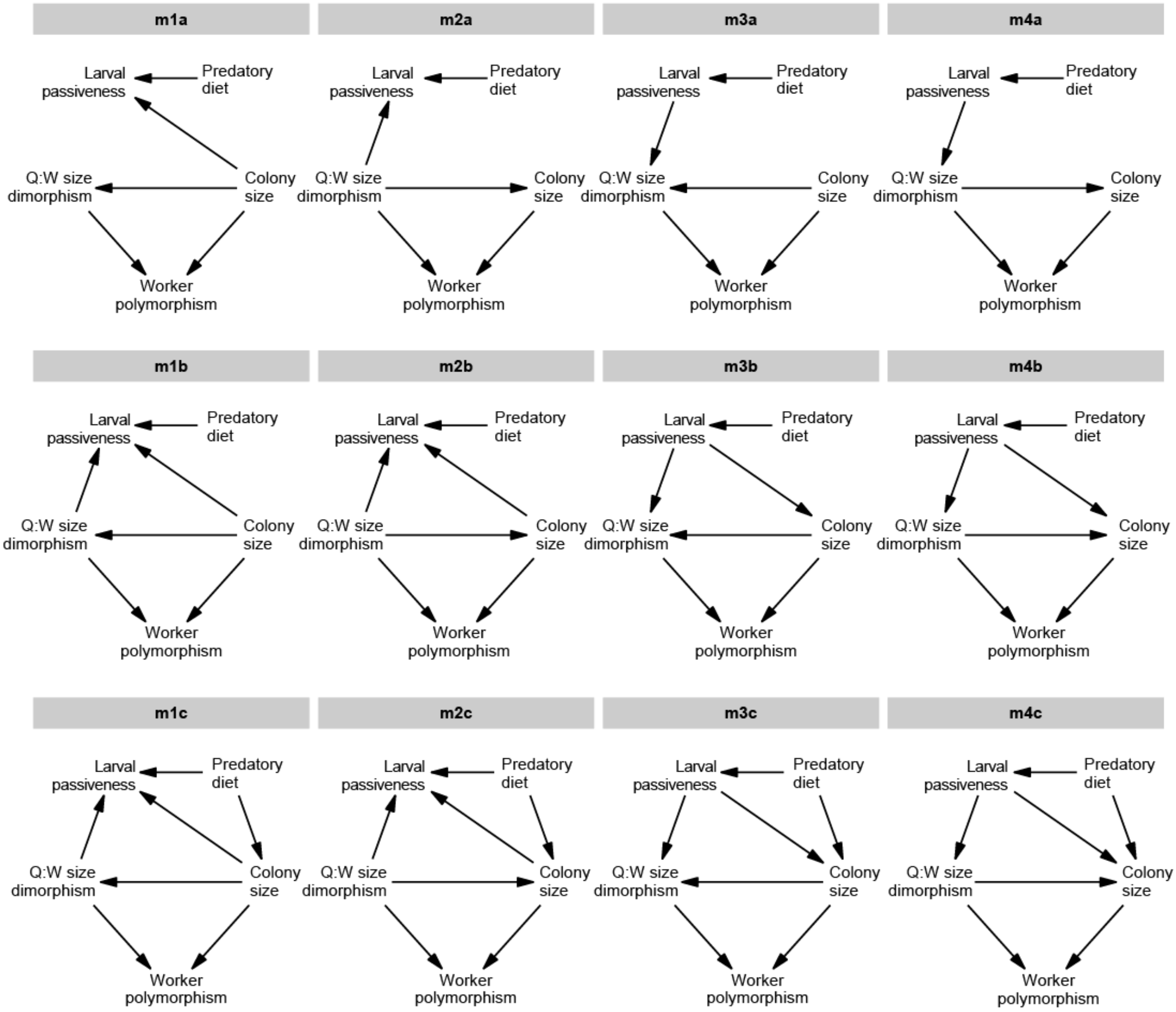


Figure S5. Plausible evolutionary models evaluated and compared using a phylogenetic path analysis. These models were designed to address 1) whether enhanced parental care for larvae, reflected by passive larval morphologies, was a driving force behind increased social complexity (m3 and m4) or whether it arose as a consequence of increased social complexity (m1 and m2); and 2) whether greater queen-worker dimorphism was a primary driver of higher sociality (m2 and m4), or whether colony size played the leading role (m1 and m3). We tested several alternatives with variations in the impact of larval passiveness on colony size (a and b vs. c) and the influence of a non-predatory diet on colony size (a vs b and c).

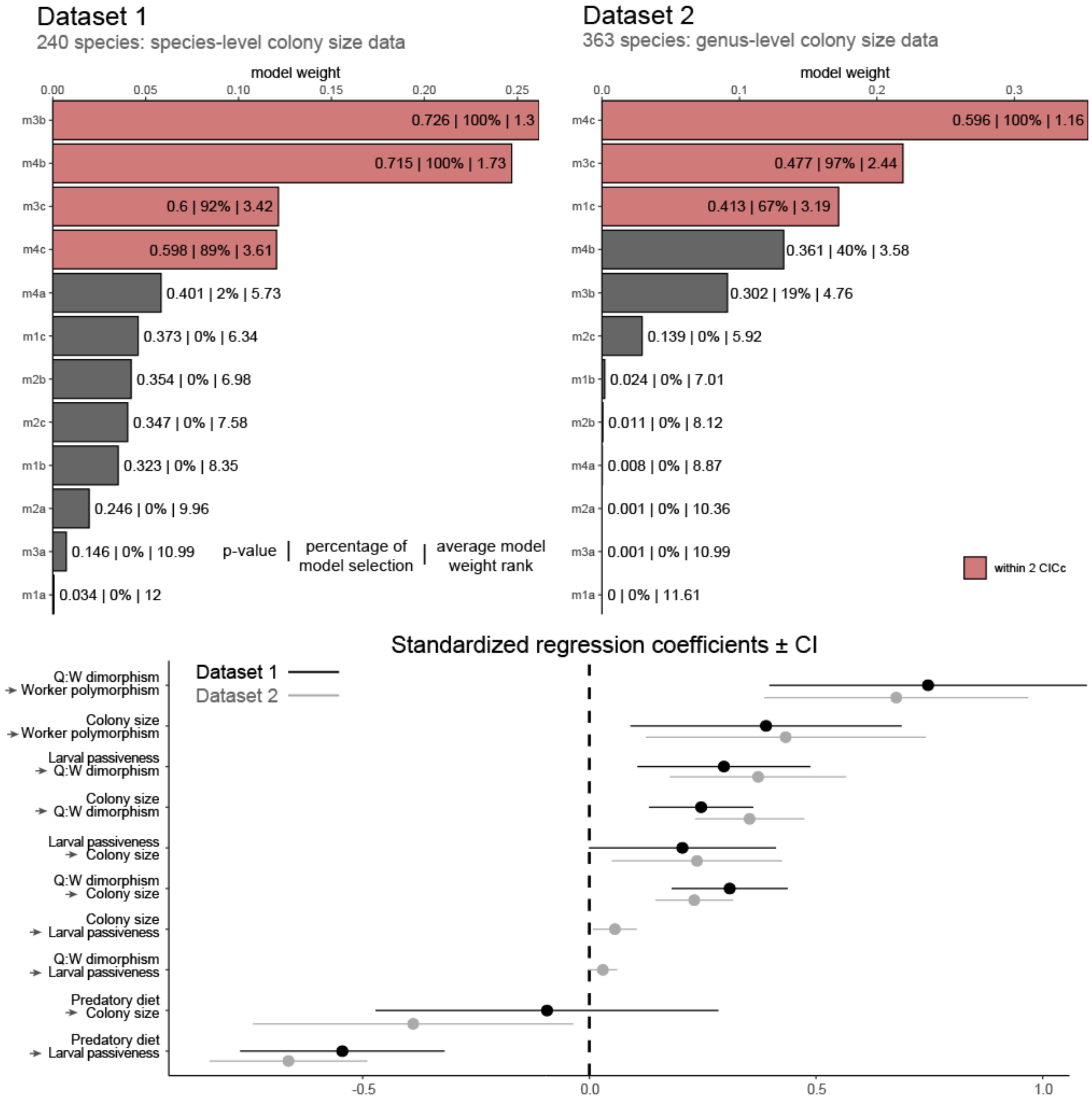


Figure S6. Phylogenetic path results for the tested models across two datasets. Tested models are displayed in Fig. S5. Model weight estimation and comparison over 200 phylogenies with details of P−values (whose significance indicates model rejection), percentage of model selection (best models within a range of 2 CICc), and average model weight rank. Standardized regression coefficients ± CI estimated over 200 phylogenetic trees from averaging the best models (in red).

Table S1. Descriptions of the ant larvae' body shape categories provided by Wheeler and Wheeler (24, 25)**.** Are in bold the elements indicating larval passivity.

Pogonomyrmecoid	Diameter greatest near middle of abdomen, decreasing gradually toward head and more rapidly toward posterior end, which is rounded; thorax more slender than abdomen and forming a neck, which is curved ventrally.
Pheidoloid	Abdomen short, stout and straight; head ventral near anterior end, mounted on **short stout neck**, which is the prothorax; ends rounded, one end more so than the other.
Dolichoderoid	Short, stout, plump, straight or slightly curved, with both ends broadly rounded; anterior end formed by enlarged dorsum of prothorax; head ventral, near anterior end; **no neck**; somites indistinct. Diameter approximately half the distance from labium to anus.
Attoid	Short, very stout, plump, slightly curved, with both ends broadly rounded; anterior end formed by the enlarged dorsum of prothorax; head ventral, near anterior end; **no neck**; somites indistinct; diameter approximately equal to distance from labium to anus.
Myrmecioid	Elongate and rather slender; curved ventrally; without a differentiated neck; diameter diminishing only slightly from fifth abdominal somite to anterior end.
Crematogastroid	Elongate-subelliptical; head applied to ventral surface near anterior end; **no neck**; somites indistinct.
Aphaenogastroid	Slightly constricted at first abdominal somite, diameter increasing gradually toward middle of thorax and of abdomen; thorax arched ventrally but not forming a distinct neck; posterior end broadly rounded.
Platythreoid	Both ends directed ventrally from a straight body; terminal somite taillike.
Leptanilloid	Elongate, slender and club-shaped.
Leptomyrmecoid	Elongate, stout and slightly curved; diameter greatest at third and fourth abdominal somites, decreasing rapidly toward either end; 3 posterior somites small and directed ventrally; prothorax sharply differentiated into 2 parts, the anterior wedge-shaped (longer below) and abruptly depressed below posterior part; head on anterior end with mouth parts directed anteriorly; somites distinct.
Oecophylloid	Plump, sausage-shaped, slightly curved; diameter nearly uniform**; no neck**; head on anterior end.
Rhopalomastigoid	Diameter nearly uniform; slightly constricted between first and second abdominal somites; body bent ventrally from this constriction; terminating posteriorly in a conspicuous knob; head ventral, near anterior end.
Paedalgoid	Abdomen subspherical; thorax forming a stout **very short neck**, which is directed ventrally; anus ventral, quite far forward and with a posterior lip.

Table S2. Descriptions of the ant larvae' mandible shape categories provided by Wheeler and Wheeler (24, 25)**.** Are in bold the elements indicating larval passivity. For species falling into categories described as "with or without medial teeth" we examined their detailed larval descriptions to determine the presence or absence of medial teeth in these species.

Ectatommoid	Subtriangular; with a medial blade arising from the anterior surface and bearing one or two medial teeth; apex curved medially to form a tooth.
Camponotoid	Subtriangular; base broad (width at least 2/3 the length) ; apex forming a round pointed slightly curved tooth.; **no medial teeth** (or rarely one small one).
Dolichoderoid	Basal part inflated and narrowed more or less abruptly to the distal part, which is slender, sharp-pointed and straight; **no medial teeth** or blade.
Pogonomyrmecoid	Subtriangular; with three conspicuous medial teeth, which are approximately in the same plane.
Amblyoponoid	Narrowly subtriangular; without a blade; straight or with the apex slightly curved medially; **with or without minute teeth** on medial surface,
Pristomyrmecoid	Subtriangular; no medial blade; apical tooth curved medially and usually acute; subapical medial teeth small
Pheidoloid	Subtriangular; with two or three subapical teeth not all in the same plane; apex curved medially to form a tooth
Platythyreoid	Narrowly subtriangular; with a medial blade arising from the anterior surface; **with or without medial teeth**; apex curved medially to form a tooth.
Cephalotoid	Apex rounded and curved medially; **no medial teeth.**
Attoid	Broad, short and stout; apical portion abruptly attenuated and curved medially to form a sharp-pointed apical tooth; **no medial teeth.**
Dinoponeroid	Narrowly subtriangular; distal portion strongly curved posteriorly; with one or two medial teeth.
Diacammoid	Falcate; with the base dilated; with or without minute medial teeth, apex forming a long sharp-pointed tooth.
Tetraponeroid	Short, stout and blunt; apical tooth short and directed medially; with one lateral subapical tooth and one blunt subapical medial tooth.
Rhytidoponeroid	Basal half greatly inflated and terminating medially in a large tooth, which is directed ventrally; distal half very narrow and forming a long slender apical tooth; one or two small medial teeth.
Leptanilloid	Outer border furnished with several long slender sharp-pointed teeth; apical tooth long, slender, sharp-pointed and directed laterally.

Typhlomyrmecoid	Basal half greatly dilated; distal half extremely narrow and straight, terminating in a long slender curved apical tooth.
Anergatidoid	Elongate-lobose; slightly curved medially; with an apical denticle but **no teeth.**
Leptogenyoid	Subconical; apex rounded; a small subapical denticle projecting posteriorly; **no teeth.**
Ochetomyrmecoid	With a large (about 1/3 total length) sharp-pointed apical tooth; with anterior and posterior subapical teeth, between which are numerous long needle-like teeth.
Simoponoid	Subtrapeziform; without a blade; masticatory border convex and bearing one apical and several medial teeth, which are sharp-pointed and of approximately the same size, and numerous small subapical teeth.

Table S3. Coevolution model comparisons for larval feeding habits, larval morphology, and species diet. Results from Pagel's (28) method for testing for evolutionary relationships between binary characters on a dataset of 99 species (71 genera) shown in Figure S2.

y \ x	**Supervised larval feeding habits**				
	model	**d.f.**	**log(L)**	**AIC**	**weight**
Neckless larvae	independent	4	-81.79004	171.5801	7.9438E-06
	interdependent x & y	8	-66.10942	148.2188	0.9394206
	x dependent of y	6	-73.88248	159.765	0.00292179
	y dependent of x	6	-70.90029	153.8006	0.05764969
Toothless larvae	independent	4	-81.59694	171.1939	9.3427E-06
	interdependent x & y	8	-67.25114	150.5023	0.2907999
	x dependent of y	6	-70.91164	153.8233	0.05526485
	y dependent of x	6	-68.44078	148.8816	0.6539259
Non-predatory diet	independent	4	-84.89965	177.7993	0.00111907
	interdependent x & y	8	-75.35664	166.7133	0.2858611
	x dependent of y	6	-76.4453	164.8906	0.7111222
	y dependent of x	6	-82.3715	176.743	0.00189771

Table S4. Comparison of model fits for different models of trait evolution and phylogenetic signal for body volumes and dimorphism. Models were estimated with a subset of 103 species on a tree whose phylogenetic relationships are fully supported by molecular data.

	Model comparison log-likelihood difference (AIC difference)									**Phylogenetic signal**		
Trait	Brownian motion	Ornstein-Uhlenbeck	early busrt	white noise	rate trend	mean trend	lambda	kappa	delta	λ	K	σ^2
Queen volume	-5(8)	0(1)	-5(10)	-7(11)	-1(3)	-5(10)	-3(6)	-5(9)	**0(0)**	0.867	0.56	0.0093
Worker volume	-2(2)	**0(0)**	-2(4)	-19(36)	0(0)	-2(4)	-1(3)	-2(4)	0(0)	0.894	0.77	0.0058
Q:W dimorphism	-4(6)	-1(2)	-4(8)	-8(15)	-1(3)	-4(8)	**0(0)**	-1(2)	-1(2)	0.847	0.61	0.0034

Table S5. Details of values from PGLS analysis conducted on dimorphism, queen volume and worker volume on 363 species over 128 genera (df = 357). Dimorphism values were log$_{10}$ and root-squared transformed in order that residuals fit normal distribution according to the Shapiro test.

	square root log Q:W dimorphism				**log queen volume**				**log worker volume**			
	λ = 0.847				*λ = 0.867*				*λ = 0.894*			
Trait	**Value**	**Std.Error**	**t-value**	**p-value**	**Value**	**Std.Error**	**t-value**	**p-value**	**Value**	**Std.Error**	**t-value**	**p-value**
larval passiveness	0.115	0.035	3.247	**0.00128**	-0.052	0.087	-0.606	0.54457	-0.241	0.082	-2.945	**0.00344**
predatory diet	0.026	0.046	0.572	0.56747	-0.108	0.112	-0.967	0.33419	-0.204	0.106	-1.929	0.05451
log colony size	0.057	0.014	3.970	**8.7E-05**	0.238	0.035	6.870	**2.9E-11**	0.126	0.032	3.891	**0.00012**
worker polymorphism	0.181	0.033	5.504	**7.1E-08**	0.528	0.080	6.607	**1.4E-10**	0.273	0.075	3.650	**0.0003**
reproductive workers	-0.163	0.069	-2.363	**0.01864**	0.514	0.168	3.054	**0.00243**	0.579	0.159	3.641	**0.00031**

DATASET CAPTIONS

Datasets and code for analysis can be found in the Zenodo repository:
https://doi.org/10.5281/zenodo.14746149

Dataset S1 (separate file). Data for ant larval feeding habits retrieved from the literature, with quotes and classifications for the present study.

Dataset S2 (separate file). Data for ant queen and worker body volume and queen:worker dimorphism.

Dataset S3 (separate file). Data for ant larval morphology.

Dataset S4 (separate file). Data for ant colony sizes.

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