SUPERORGANISMAL ANISOGAMY: A COMPARATIVE TEST OF AN EXTENDED THEORY

Philip A. Downing[1], Jussi Lehtonen[2], Louis Bell-Roberts[3], Heikki Helanterä[1]

[1]University of Oulu, Finland, [2]University of Jyväskylä, Finland, [3]University of Oxford, UK

Correspondence: philip.downing@oulu.fi

TEASER

Behold the mighty ant queen and her tiny male consort. The eggs and sperm of their colony. But are they really? We investigate how well this metaphor works by developing theory to explain the evolution of anisogamy and comparatively testing it with data on queen-male size dimorphism in ants. Read on to discover some parallels and idiosyncrasies of evolution at life's different hierarchical levels.

ABSTRACT

Multicellular organisms and superorganisms (e.g., ant colonies) are both products of major evolutionary transitions in individuality, and they share many analogous traits. Theory developed to explain the evolution of one such trait, anisogamy, has recently been adapted to explain its superorganismal analogue: large egg-like queens and small sperm-like males. To test this theory with comparative data, we first extended it to incorporate variation in how colonies with multiple queens arise. We then used data from 732 ant species to investigate the effects of colony size, worker caste number, and queen number on queen size (thorax volume) and queen-male dimorphism. Queen size and queen-male size dimorphism both increased with colony size and number of worker castes, consistent with predictions. Contrary to predictions, queen size and queen-male size dimorphism were not associated with queen number. To further understand the commonalities and idiosyncrasies of evolution at different hierarchical levels, future work should consider the adaptive and non-adaptive causes for correlated evolution between queen and male, and egg and sperm sizes.

Keywords: mixed model, sexual size dimorphism, polygyny, group size, eusociality, gamete competition, castes

INTRODUCTION

A recurrent theme in the history of life on Earth has been for entities to transition from independent to group-level replication (Huxley 1912; Maynard Smith and Szathmáry 1995). The transitions from single celled to multicellular life and from solitary insects to superorganisms are archetypal examples. These major transitions required similar evolutionary problems to be solved and gave rise to many analogous traits (Bourke 2011; West et al. 2015; Boomsma 2022). Consequently, it has been argued that theory developed to explain the evolution of multicellular traits could be applied to their superorganismal equivalents, and vice versa (Wiernasz and Cole 2009; Helanterä 2016; Kennedy et al. 2017). This has been done verbally (Helanterä 2016) and mathematically modelled (Lehtonen and Helanterä 2020) for the superorganismal equivalent of anisogamy: large queens and small males. To validate the approach of applying theory from one major transition to another, we now need an empirical test of the model’s predictions.

The superorganismal anisogamy model (Lehtonen and Helanterä 2020) predicts that queen size is a function of three parameters: $x^* = \alpha + (\beta / K^2)$. The α parameter controls the relationship between individual size and survival, β controls the relationship between queen size and colony survival, and K is the number of queens. Male size is a function of just one parameter: $y^* = \alpha$. From this, the following predictions can be made. First, queen size should increase with colony size and worker caste number. This is because larger, more complex colonies need more starting resources to survive, resulting in a larger β term. Second, queen size should decrease rapidly with increasing queen number. When there are multiple queens (polygyny), colony survival depends on the sum of their resources. Third, queen size and male size should evolve independently of each other. This is because males do not contribute to colony survival once the queen is fertilised. Queens should, therefore, be larger than males in species with large colonies with multiple worker castes, but the size difference will depend on the number of queens per colony.

The superorganismal anisogamy model is analogous but not identical to gamete competition models for the evolution of anisogamy. While there are several such models (e.g., Kalmus 1932; Scudo 1967; Parker et al. 1972; Knowlton 1974), the most closely analogous to ours is the game theoretical model of Bulmer and Parker (2002). The α and β parameters of these two models are analogous: in Bulmer and Parker (2002) the α parameter controls the relationship between gamete size and gamete survival, and β controls the relationship between initial zygote size and offspring survival. There is no analogue for the number of queens (K) described above. Broadly speaking, in anisogamy theory β is hypothesised to be correlated with multicellular complexity, while α is not, because a gamete remains a single cell by definition, regardless of how complicated the adult organism is. Similarly, β here is hypothesised to be correlated with colony complexity, while α is not, assuming that the requirements of the body plan of an individual are not directly related to how complicated the colony is.

In the original superorganismal anisogamy theory (Lehtonen and Helanterä 2020), multi-queen colonies arise when two or more queens co-found a colony. This is known as primary polygyny. However, multi-queen colonies can also arise via secondary polygyny (Hölldobler and Wilson 1977; Bourke and Franks 1995). This occurs when new queens remain in their natal colony, join an existing colony, or when colonies fuse. As secondary polygyny appears to be more common in ants than primary polygyny (Boomsma et al. 2014; Bell-Roberts et al. 2024), we modified the original superorganismal anisogamy theory to determine what effect this has on the evolution of queen size and queen-male dimorphism. We describe this modification below and report how it changes the model's predictions. We then test these predictions using a phylogenetic comparative analysis across 732 ant species.

MODEL

The superorganismal anisogamy theory is built on the idea that dispersing males and future queens are analogous to the gametes of multicellular organisms, i.e. sperm/eggs or pollen/seeds. Once mated, queens form an incipient colony which is equivalent to a zygote. This zygote-like stage makes the superorganismal life cycle similar to that of multicellular organisms. Queen size is under selection because colony survival depends on colony growth rate – the number of workers produced (the equivalent of somatic growth). Larger queens produce more workers, increasing colony survival through the initial growth period.

The model was built by adapting gamete competition theory to fit the reproductive biology of superorganisms. Gamete competition theory examines the requirements for the evolution of anisogamy in relation to size-specific survivorship of gametes and zygotes. In the superorganismal case, we are interested in size-specific survivorship of queens, males, and incipient colonies, and how this leads to queen–male dimorphism.

Table 1. Model notation.

Variable / parameter	Notation	Notes
probability a queen founds a colony alone	p	
total resource allocation to queens and males	M	*allocation to queens and males assumed equal*
resources allocated to a single queen: resident value (mutant value)	$x\ (\hat{x})$	*determines queen size*
resources allocated to a single male: resident value (mutant value)	$y\ (\hat{y})$	*determines male size*
number of queen offspring: resident value (mutant value)	$n_x\ (\hat{n}_x)$	$n_x = M/x$
number of male offspring: resident value (mutant value)	$n_y\ (\hat{n}_y)$	$n_y = M/y$
parameter controlling relationship between individual size and individual survival (analagous to gamete survival)	α	*assumed to be the same for queens and males*
parameter controlling relationship between queen size and colony survival (analagous to zygote survival)	β	
individual survival to mating and colony founding	$g(z) = e^{-\alpha/z}$	*z stands for* $x, y, \hat{x}, \hat{y}$
colony survival	$s(z) = e^{-\beta/z}$	*z stands for* x *or* $\hat{x}$ *except when there are multiple queens*

We begin by writing the fitness functions for queens and males for the original model (these correspond to equations 2.1 and 2.2 in Lehtonen and Helanterä (2020), see Table 1 for notation):

$$g(\hat{x})s(\hat{x}) \tag{1.1}$$

$$g(\hat{y})\frac{n_x g(x)s(x)}{n_y g(y)} \tag{1.2}$$

The fitness of a mutant daughter is the product of her own survival $g(\hat{x})$ to colony founding and the survival of the colony $s(\hat{x})$. For a mutant son, average fitness is the quotient of the number of successfully founded colonies $n_x g(x)s(x)$ and the number of males surviving to mate $n_y g(y)$ (which yields the mean number of colonies founded by a mating male), multiplied by the probability $g(\hat{y})$ that the mutant son itself survives to mate. We incorporate secondary polygyny by introducing the parameter p which is the probability a queen founds a colony alone. Fitness of a mutant daughter is then found by multiplying mutant offspring fitness by the probability p a queen founds a colony alone. Now, the fitness of a mutant daughter is the product of the probability she starts a new colony alone p multiplied by the fitness defined in equation (1.1), plus the probability she takes advantage of an existing colony $(1-p)$, in which case she only has to survive to this point (probability $g(\hat{x})$) because the colony has already been successfully founded. Fitness of mutant sons follows the logic of equation (1.2), taking into account the fitness output from both newly founded colonies and 'exploited' colonies: fitness of a mutant son is simply its probability of surviving to mate, multiplied by fitness per male surviving to mate.

$$pg(\hat{x})s(\hat{x}) + (1-p)g(\hat{x}) \tag{2.1}$$

$$g(\hat{y})\frac{n_x\big(pg(x)s(x)+(1-p)g(x)\big)}{n_y g(y)} \tag{2.2}$$

The total fitness a focal mother gains via mutant offspring is:

$$\widehat{w}_x = \hat{n}_x\big(pg(\hat{x})s(\hat{x}) + (1-p)g(\hat{x})\big) \tag{3.1}$$

$$\widehat{w}_y = \hat{n}_y g(\hat{y})\frac{n_x\big(pg(x)s(x)+(1-p)g(x)\big)}{n_y g(y)} \tag{2.2}$$

We solved these equations numerically by substituting the functions from Table 1 and finding the values of the evolving traits that maximise fitness (see supplementary material for matlab code). We hold α constant, because this is assumed to be equivalent for queens and males but allow β to vary from one to nine and p between 0 and 1. The numerically calculated equilibria are shown in Figure 1.

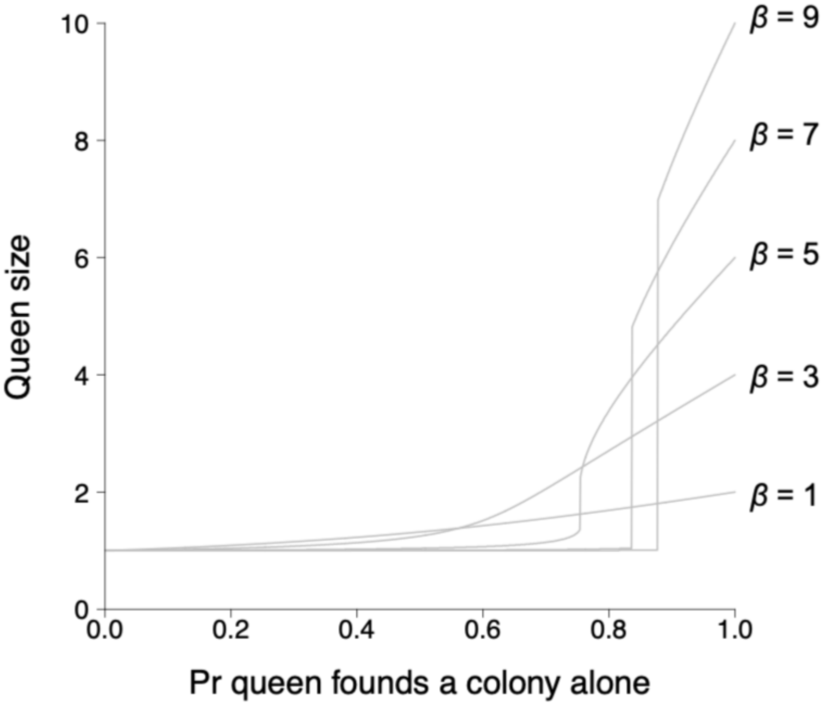

Figure 1. Queen size as a function of the probability a queen starts a new colony alone (p). The value of β increases from one to nine while α is fixed at one. The effect of increasing α is to shift the intercept of each slope upwards.

When queens always start a new colony alone ($p = 1$), the results coincide with the original model (queen size $x^* = \alpha + \beta$), which shows that queens are larger when β is higher. When queens sometimes forgo independent colony founding and instead join / remain in an existing colony ($p < 1$), queen size increases with p, but the rate of increase is moderated by β (Figure 1). When β is low, queen size increases gradually as the probability of founding a new colony alone increases. When β is high, queens remain small until a threshold probability of founding a new colony alone is reached ($p > 0.8$). At this point queens increase rapidly in size. When queens never start a new colony alone ($p = 0$), queen size is equal to α. This seems biologically unrealistic, however. If there are no primary foundresses in the population, secondary polygyny could not exist as there are no colonies to remain in.

Regarding predictions, if queens sometimes join or remain in an existing colony ($p < 1$), queens should be smaller, whatever the value of β. This will reduce the extent of queen-male size dimorphism in multi-queen colonies compared with single queen colonies. When colonies need more starting resources to survive, i.e. β is high – which is assumed to be the case for large, complex colonies – large queens are only expected if the probability of founding a colony alone is high. Overall, the original predictions that queen size should increase with colony size and worker caste number, but decrease with queen number, remain unchanged. These effects may be more difficult to detect under secondary polygyny, however, because of the threshold effect.

COMPARATIVE TEST

Methods

Overview

Our phylogenetic comparative analysis to test the predictions of the superorganismal anisogamy model involved three steps: i) collecting data on queen and male ant sizes from digital museum images; ii) collecting data on colony size, worker caste number, and queen number for as many of these species as possible from published databases; iii) building statistical models to determine the effects of colony size, worker caste number, queen number, and their interactions, on the evolution of queen size and queen-male dimorphism.

Data Collection

Queen and male sizes were estimated from images on Antweb. One queen and one male per species were measured, using type specimens when possible. Two measurements were taken for each individual: maximum mesosoma (alitrunk) width and length. Scale bars on the full-sized images allowed the measurements to be taken at a resolution of < 0.05 mm. Queen and male volumes were then approximated as mesosoma (thorax) volume $(width \times length)^{3/2}$ and natural log transformed. In total, we obtained data on queen and male sizes for 732 species (Table S1). Queen-male dimorphism was calculated as: log queen thorax volume – log male thorax volume. Positive values indicate that queens are larger than males, negative values that males are larger than queens.

Data on colony size, worker caste number, and queen number were extracted from the comparative database compiled by Bell-Roberts et al. 2024. This database consists of 794 ant species and was assembled using previous comparative analyses on ants (Hölldobbler and Wilson 1990; Hughes et al. 2008; Burchill and Moreau 2016; Blanchard and Moreau 2017), the Global Ant Genomics Alliance database (Vizueta et al. 2025), and via keyword searches in Google Scholar. Colony size is the number of workers in mature colonies and queen number is the average number of queens observed per colony. Worker caste number was treated as a binary trait with species having one or more than one non-reproductive caste. Species-level averages were taken when multiple estimates of these parameters were reported per species. In total, colony size estimates were available for 154 of the species in our data set, worker caste number for 187 species, and queen number for 52 species. For 46 species we had data on both colony size and queen number, and for 46 species we had data on both worker caste number and queen number. Consequently, sample sizes vary between analyses (Table S1). Our dataset excludes species that are supercolonial, social parasites or parthenogens, species with gamergates and species that use inter-lineage hybridization for caste determination. These species violate the assumptions of the superorganismal anisogamy model.

We sampled 50 trees at random from the posterior distribution of ant phylogenies from Economo et al. (2018). Each tree was pruned to match the 732 species in our data set using the *Ape* R package (Paradis and Schliep 2019). Phylogenetic uncertainty was account for by running all analyses described below across these 50 trees.

Statistical Analyses

We plotted the phylogenetic distribution of queen-male dimorphism onto one of the phylogenies chosen at random. Ancestral states were estimated on this phylogeny using maximum likelihood, corresponding to a Brownian motion model of trait evolution, in the *Phytools* R package (Revell 2024). This was done for data visualisation only. Bayesian Phylogenetic Mixed Models (BPMMs) with parameter expanded priors for random effects were used for our analyses (*MCMCglmm* R package, Hadfield 2010). We investigated the sensitivity of our results to the choice of prior by re-running all the models described below with an inverse Gamma prior. The results from these analyses are quantitatively similar and are reported in Table S2.

To estimate the phylogenetic correlation between queen size and male size, we used a multi-response BPMM. Our response variables were queen thorax volume and male thorax volume (both natural log transformed, Gaussian). The global intercept was removed to estimate trait-specific intercepts. We estimated the phylogenetic and residual (co)variances in these traits by fitting two 2 × 2 unstructured variance–covariance matrices (R code: *phylo_r_mods*). The phylogenetic correlation between queen size and male size was then calculated as $s_{x,y}/\sqrt{s_x^2 s_y^2}$. Where $s_{x,y}$ is the covariance between queen size and male size and s_x^2 and s_y^2 the variances. We estimated the major axis regression slope of the phylogenetic relationship (Warton et al. 2006) between queen size and male size as $\left(1/2s_{x,y}\right)\left(s_y^2 - s_x^2 + \sqrt{\left(s_y^2 - s_x^2\right)^2 + 4s_{x,y}}\right)$.

The effects of colony size, worker caste number, and queen number on queen size were determined using five models. In each model, queen thorax volume (natural log transformed) was the response variable (Gaussian) and phylogeny was included as a random effect. Colony size (natural log transformed) was the fixed effect in the first model (R code: *qn_col_mods*), worker caste number (binary) was the fixed effect in the second model (R code: *qn_caste_mods*), and queen number (natural log transformed) was the fixed effect in the third model (R code: *qn_num_mods*). In the fourth model, we fit the interaction between colony size and queen number (both natural log transformed; R code: *qn_int_mods*) and in the fifth model we fit the interaction between worker caste number and queen number (natural log transformed; R code: *qn_cqn_mods*). These interactions allow the relationship between queen size and our proxies of β (colony size and worker caste number) to be moderated by queen number, in line with our theoretical finding that large queens are only expected when β is high and queens have a high probability of founding a new colony alone.

To determine the effects of colony size, worker caste number, and queen number on queen-male dimorphism, we repeated these five models but with queen-male dimorphism in thorax volume (Gaussian) as the response variable (R code: *di_col_mods*, *di_caste_mods*, *di_num_mods*, *di_int_mods*, *di_cqn_mods*). We modelled the effects of colony size, caste number, and queen number separately, as well as their interactions, because main effects in models with interactions are conditional, representing the effect of each variable on the response when the other fixed effect is zero.

Each BPMM described above was run on each of the 50 ant phylogenies we sampled. The number of iterations per tree was 1 100 000 with a burn-in of 100 000 iterations and a thinning interval of 1000 iterations, giving a posterior distribution of 1000 iterations (and an effective sample size of 1000 for each parameter in our preliminary analyses). Twenty iterations were then sampled from each tree (every 50th) and combined to give 1000 iterations in total (i.e. 20 * 50) in the posterior distribution of each parameter. Model convergence was examined by calculating the degree of autocorrelation between posterior samples and by inspecting traces of posterior distributions. Posterior modes and 95% credible intervals (CI) from the posterior distributions of each parameter are reported in the results. Significance is based on whether the CI includes zero. We report the phylogenetic heritability (H^2 = phylogenetic variance / total variance) of each model in the results. Full details are provided in the supplementary R code.

Results

Queen thorax volume was larger than male thorax volume in 72% of the 732 measured species (Figure 2). There was a strong phylogenetic correlation between log transformed queen and male thorax volumes (phylogenetic r = 0.92, CI = 0.87 to 0.94, N = 732 species). Both queen and male thorax volumes (log transformed) showed high phylogenetic heritability (queen phylo H^2 = 0.82, CI = 0.74 to 0.89; male phylo H^2 = 0.83, CI = 0.74 to 0.89). This indicates that queens of closely related species tend to have similar thorax volumes, and likewise for males.

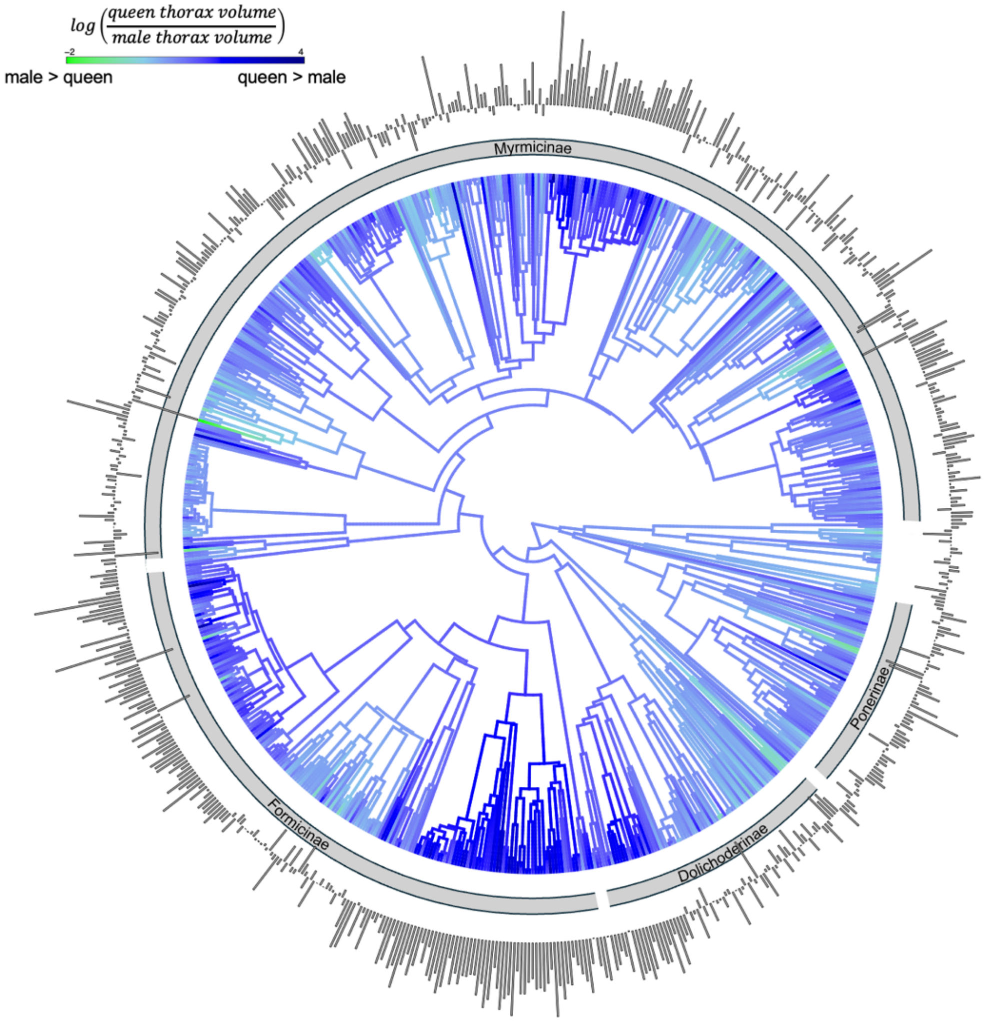

Figure 2. The phylogenetic distribution of queen-male dimorphism in thorax volume across 732 ant species. Outward-facing bars indicate that queens are larger than males, inward-facing bars that males are larger. Branches are coloured by the degree of ancestral dimorphism (blue: queen > male, green: male > queen).

The evolutionary allometry between log transformed queen and male thorax volumes was negative (major axis slope = 0.75, CI = 0.62 to 0.93, N = 732 species; Fig 3), indicating that increases in male thorax volumes have been proportionally larger than increases in queen thorax volume through evolutionary time.

Across species, queen thorax volume (log) increased significantly as colony (log) size increased (slope = 0.29, CI = 0.21 to 0.38, N = 154 species; Figure 4a; Table 2). Queens from species with two or more worker castes were significantly larger than those with one worker caste (difference = 1.27, CI = 0.77 to 1.96, N = 187 species, Figure 4b; Table 2). Queen thorax volume (log) was not associated with queen number (slope = -0.03, CI = -0.27 to 0.33, N = 51 species; Figure 3c; Table 2). The relationship between queen thorax volume (log) and colony size (log) was not moderated by queen number (interaction = 0.00, CI = -0.09 to 0.11, N = 46 species; Table 2) and neither was the relationship between queen thorax volume (log) and worker caste number (interaction = 0.31, CI = -0.36 to 0.84, N = 46 species; Table 2).

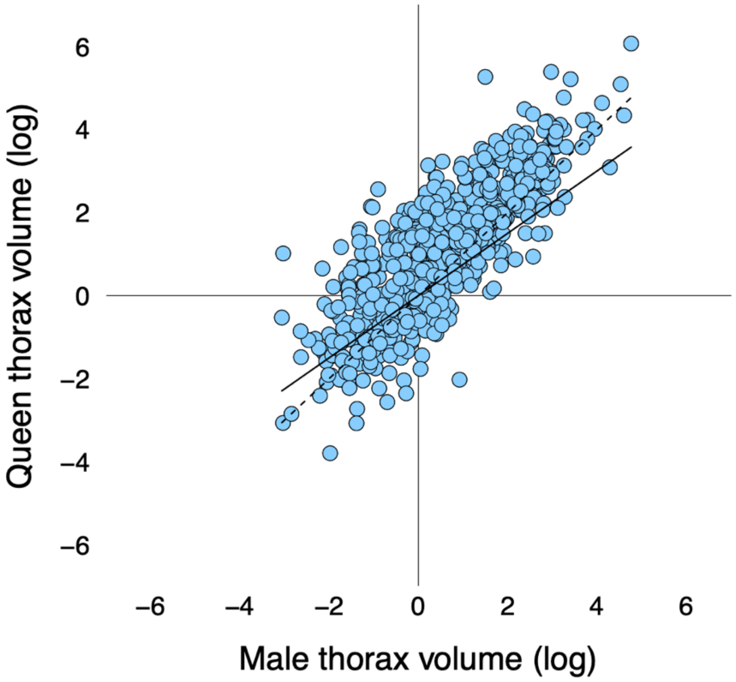

Figure 3. The evolutionary allometry between queen thorax volume (log) and male thorax volume (log) was negative. The solid line is the estimated major axis slope (0.75), the dashed line is the isometric slope (1). Each point is a different species (N = 732).

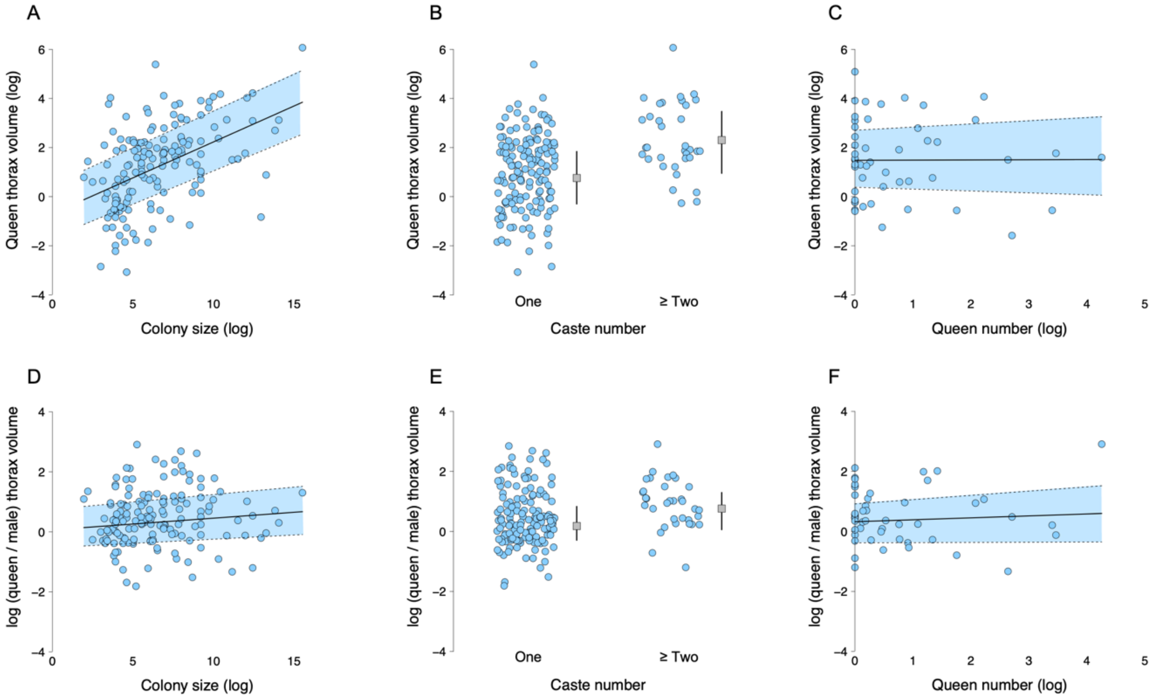

Figure 4. Queen thorax volume (log) was significantly larger in species that *a*) formed larger colonies (N = 154 species) and *b*) had more than one worker caste (N = 187 species), but *c*) was not associated with log queen number (N = 51 species). Queen-male dimorphism in thorax volume increased *d*) non-significantly with increasing log colony size (N = 154 species), *e*) significantly with worker caste number (N = 187 species), and *f*) was not associated with log queen number (N = 51 species). Each point is a different species. Regression lines and 95% CIs are plotted in A, C, D and F. The mean and 95% CI are shown in B and E.

Table 2. Parameter estimates from the five BPMMs examining queen thorax volume (log) in relation to colony size (log), worker caste number, queen number (log), and their interactions.

Fixed terms	Parameter	*Estimate*	*Lwr CI*	*Upr CI*	*Phylogenetic* H^2
log colony size (N = 154 species)	intercept	-0.76	-2.00	0.34	0.87
	slope	0.29	0.21	0.38	
caste number (N = 187 species)	intercept	0.76	-0.30	1.84	0.85
	slope	1.27	0.77	1.96	
log queen number (N = 51 species)	intercept	1.58	0.38	2.70	0.90
	slope	-0.03	-0.27	0.33	
log colony size x log queen number (N = 46 species)	intercept	-0.09	-1.77	1.35	0.87
	colony size slope	0.25	0.06	0.46	
	queen number slope	0.16	-0.77	0.80	
	interaction	0.00	-0.09	0.11	
caste number x log queen number (N = 46 species)	intercept	1.36	0.40	2.28	0.85
	caste number slope	0.68	-0.06	2.15	
	queen number slope	-0.19	-0.51	0.27	
	interaction	0.31	-0.36	0.84	

Queens were increasingly larger than males as colony size increased (slope = 0.04, CI = -0.02 to 0.09, N = 154 species; Figure 4d; Table 3), although this relationship was not statistically significant. Dimorphism was significantly higher in species with two or more worker castes than those with one worker caste (difference = 0.33, CI = 0.04 to 0.75, N = 187 species; Figure 4e; Table 3). Queen-male dimorphism was not associated with queen number (slope = 0.07, CI = -0.14 to 0.26, N = 51 species; Figure 4f; Table 3) and the relationship between queen-male dimorphism and log colony size was not moderated by queen number (interaction = -0.03, CI = -0.11 to 0.04, N = 46 species; Table 3). The relationship between queen-male dimorphism and worker caste number was moderated by queen number: dimorphism tended to decrease with queen number in species with one worker caste, whereas dimorphism increased with queen number in species with more than one worker caste (interaction = 0.54, CI = 0.20 to 1.09, N = 46 species; Table 3).

Table 3. Parameter estimates from the five BPMMs examining queen-male dimorphism in thorax volume in relation to colony size (log), worker caste number, queen number (log), and their interactions.

Fixed terms	Parameter	*Estimate*	*Lwr CI*	*Upr CI*	*Phylogenetic* h^2
log colony size (N = 154 species)	intercept	0.13	-0.68	0.73	0.76
	slope	0.04	-0.02	0.09	
caste number (N = 187 species)	intercept	0.18	-0.29	0.83	0.75
	slope	0.33	0.04	0.75	
log queen number (N = 51 species)	intercept	0.29	-0.38	0.92	0.74
	slope	0.07	-0.14	0.26	
log colony size x log queen number (N = 46 species)	intercept	0.33	-0.67	1.28	0.70
	colony size slope	-0.03	-0.15	0.13	
	queen number slope	0.35	-0.14	0.94	
	interaction	-0.03	-0.11	0.04	
caste number x log queen number (N = 46 species)	intercept	0.38	-0.05	1.09	0.67
	caste number slope	-0.18	-0.98	0.52	
	queen number slope	-0.20	-0.48	0.11	
	interaction	0.54	0.20	1.09	

DISCUSSION

The superorganismal anisogamy model predicts that ant queens should be largest in species with large colony sizes, multiple worker castes, and one queen per colony. In contrast, male size is predicted to be unaffected by colony size and queen number, leading to sexual size dimorphism which should covary with these parameters. Consistently, we found that queens are typically larger than males (in 72% of species), and large queens tend to occur in large colonies (Figure 4a) and in colonies with multiple worker castes (Figure 4b) and that queens were increasingly larger than males in species with more than one worker caste (Figure 4e). However, neither queen size (Figure 4c) or queen-male dimorphism (Figure 4f) was associated with queen number. Queen size and male size were strongly phylogenetically correlated (Figure 3).

According to our theoretical model, large queens should occur most often in species with a high probability of independent colony founding. We did not find a relationship between queen number and queen size, however. There are several explanations for this theory-data mismatch. First, queen number might not capture the probability of independent colony founding accurately enough. We assumed that queen number is negatively associated with the probability of independent colony founding, which is likely to be correct, but we lack an empirical understanding of how steeply this relationship changes. Even in species where a large proportion of queens disperse, queen number may be high. We also note our dataset lacks species with extreme queen numbers. In *Formica aquilonia* and *F. polyctena,* for example, several hundred queens per colony is typical (Rosengren et al. 1993). Second, some monogynous and polygynous species might found their colonies through temporary social parasitism (Buschinger 2009; Borowiec et al. 2021). If queens invade heterospecific nests and parasitize the resident workers, they are less dependent on their own resources for colony founding, and under weaker selection for increased size. Third, within-species variation in queen number could mask mean differences in queen size. Many ant species are socially polymorphic (Boomsma et al. 2014; Boulay et al. 2014; Bell-Roberts et al. 2024) and there is evidence that queens from multi-queen colonies are smaller than those from single-queen colonies e.g. in *F. truncorum* (Sundström 1995) and *F, selysi* (Meunier and Chapuisat 2009) but not in *Myrmica ruginodis* (Wolf et al. 2018). Fourth, queen number is a labile trait in evolutionary time (Boomsma et al. 2014; Boulay et al. 2014) whereas morphological evolution is more constrained. Changes in size may lag behind changes in queen number. Finally, our theory might need revising.

Our proxy for queen size might also make it difficult to detect an association between queen size and number. Thorax volume is thought to be a good approximation of the energetic resources available to an ant queen during colony founding, due to the histolysis of flight muscles (Peeters 2012). However, independent colony founding also uses energy from fat reserves in the abdomen (40-60 % of a queen's mass), and it is possible that queen-male differences in total body size including abdomen would be more pronounced, even if large flight muscles evolved to carry this abdominal weight (Helms & Kaspari 2015). Either way, measures of abdomen volume during colony founding are not available for many species (Helms & Kaspari 2015) and museum images are not appropriate for approximating this since collected queens may or may not be from established colonies. That said, our finding that queen size is associated with both colony size and worker caste number suggests that our proxy is capturing the necessary variation.

An unexpected result was the significant interaction between worker caste number and queen number (Table 3). Queen-male dimorphism tended to decrease with queen number in species with one worker caste but increase with queen number in species with more than one worker caste. This result is in the opposite direction to that predicted. In more complex colonies, those with more than one worker caste, large queens were only expected when the probability of independent colony founding is high. An outlier in our dataset appears to be driving this relationship: *Crematogaster pygmaea*. This ant species has twice as many queens per colony (> 70) as the species with the second most queens. Excluding it from the analysis renders the interaction non-significant.

Our finding that queen and male sizes are phylogenetically correlated is consistent with our model if α, which affects male and queen fitness alike, is a more important driver of size variation than β, which only affects queen fitness. However, we expect β to be influenced by ecological factors to a much greater extent than α, and thus our baseline expectation in relation to the model is that β should be the major driver of variation. There are both adaptive (correlated selection) and non-adaptive (genetic constraints) explanations for the phylogenetic correlation of male and queen sizes.

First, large size may be favoured in both queens and males, but via different mechanisms. There is evidence that larger males achieve more matings in mating swarms and males in species with female calling can survive for several weeks, which could influence their body size evolution (Boomsma et al. 2005; Shik et al. 2013). As dispersal tends to be male biased in ants, especially in species with multiple queens (Hakala et al. 2019), larger size may also be favoured to facilitate active directed flight. It is also possible that large male sizes are favoured in species with large colonies, if such colonies place demands on large testes or accessory glands to facilitate colony founding and egg laying. Our finding that males have larger thorax volumes than queens in 28% of the species we sampled suggests that male size can be under positive selection.

Second, the queen-male phylogenetic correlation could be due to a genetic correlation between queen and male sizes that constrains dimorphism. However, worker and queen sizes can be vastly different, and workers themselves can vary considerably from caste to caste (Hölldobler and Wilson 1990; Bell-Roberts et al. 2024), suggesting that genetic constraints can be overcome if optimal sizes are divergent enough. The fact that workers, who rear the brood, control the size of developing individuals also suggests a lack of genetic constraints. We need more data on the determinants of colony success and variation in male behaviour to better understand the interplay between divergent selection, genetic correlations and correlated evolution.

Applying the same theory, with appropriate modifications, to different major evolutionary transitions is a rare undertaking. While broad correlations between queen size and colony size and complexity in social insects have been discussed in light of social evolution theory (e.g. Bourke 1999; Matte & LeBoeuf 2025; Bell-Roberts et al 2024), we provide a case study where we directly apply similar models across levels, in order to uncover similarities and differences in social evolution across life's hierarchical levels.

DATA AVAILABILITY

Data, code, and supplementary information currently available here:

https://github.com/pipdowning/SuperOrganismal_Anisogamy/tree/main

AUTHOR CONTRIBUTIONS

PAD: methodology, formal analysis, investigation, data curation, visualisation, writing – original draft, writing – review and editing; JL: conceptualization, methodology, formal analysis, investigation, visualisation, writing – original draft, writing – review and editing; LB-R: data curation, writing – original draft, writing – review and editing; HH:

conceptualization, project administration, data curation,, writing – original draft, writing – review and editing

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CONFLICT OF INTEREST

We declare no conflict of interest.

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