Evolutionary Biology

Trophic eggs affect caste determination in the ant *Pogonomyrmex rugosus*

Eléonore Genzoni, **Tanja Schwander**, **Laurent Keller**

Department of Ecology and Evolution, Biophore Building, University of Lausanne, Lausanne, Switzerland • Social Evolution Unit, Chesières, Switzerland

Reviewed Preprint
v3 • June 16, 2025
Revised by authors

Reviewed Preprint
v2 • April 15, 2025

Reviewed Preprint
v1 • September 1, 2023

eLife Assessment

This **important** manuscript by Genzoni et al. reports the striking discovery of a regulatory role for trophic eggs in ant caste determination. Prior to this study, trophic eggs were widely assumed to play only a nutritional role in the colony, but this **compelling** study shows that trophic eggs can suppress queen development, and therefore regulate caste determination in specific social contexts.

https://doi.org/10.7554/eLife.86899.3.sa3

Abstract

Understanding how a single genome creates distinct phenotypes remains a fundamental challenge for biologists. Social insects provide a striking example of polyphenism, with queen and worker castes exhibiting morphological, behavioural, and reproductive differences. Here we show that trophic eggs, which do not contain an embryo and are primarily regarded as a source of food, play a role in the process of caste determination in the harvester ant *Pogonomyrmex rugosus*. When first instar larvae were given access to trophic eggs, they mostly developed into workers. By contrast, larvae without access to trophic eggs developed into queens. We found that trophic eggs differ in many ways from viable eggs, including texture, morphology and their contents of protein, triglycerides, glycogen, sugar and small RNAs. Moreover, comparison of miRNA fragment size distributions suggests differences in the composition of miRNAs between the two egg types. This is the first demonstration of trophic eggs playing a role in caste determination in social insects.

Introduction

Many species of insects, spiders, amphibians, marine invertebrates and sharks produce trophic eggs, a special type of eggs that do not contain an embryo (Levin and Bridges 1995; Blake and Arnofsky 1999; Collin 2004; Kudo and Nakahira 2004; Perry and Roitberg 2006; Strathmann and Strathmann 2006; Gibson et al. 2012; López-Ortega and Williams 2018). It is generally assumed that these non-developing eggs are either a by-product of failed reproduction

or that they serve as nutrition for offspring (Perry and Roitberg 2006). However, the suggestion that trophic eggs solely provide a nutritional function is based on surprisingly little evidence. We here report a direct function of trophic eggs in the determination of alternative phenotypes in ants.

Trophic eggs have been reported in many ant species (**Figure 1** and Supplementary Table 1) and are generally thought to mostly or only serve as food for offspring (Crespi 1977; Hölldobler and Wilson 1990). Trophic eggs play an important role during the time of colony founding (Hölldobler and Wilson 1990). Because in most ant species queens do not forage after the mating flight, they metabolize the alary muscles and fat bodies and convert them into eggs, which serve as food to rear the first batch of larvae (Huber 1905; Keller and Passera 1989). Except for a few species such as *Crematogaster smithi* (Heinze et al. 1995) and *Acanthomyrmex ferox* (Gobin and Ito 2000), the queens generally stop producing trophic eggs after the eclosion of the first workers (see **Figure 1** and Supplementary Table 1). So far the absence of trophic eggs has been reported in only one species (Supplementary Table 1).

In many ant species, workers are capable of producing haploid males but lack a spermatheca and the ability to produce diploid female offspring (Hölldobler and Wilson 1990; Bourke 1988; Hammond and Keller 2004; Wenseleers and Ratnieks 2006). In some species, workers further lay trophic eggs (see supplementary Table 1). These eggs have a trophic function, in particular in genera such as *Pogonomyrmex* where there is no or only little trophallaxis (regurgitative food sharing) among workers. In such species it is has been suggested that trophic eggs may play an important role in food distribution within the colony (Gobin and Ito 2000).

Several studies suggested that the presence of trophic eggs may affect the developmental trajectory of larvae. A study in the Argentine ant *Linepithema humile* showed that the presence of queens in colonies was associated with a drastic decrease in the number of worker-laid trophic eggs as well as a decrease in the proportion of larvae developing into queens (Bartels 1988). Bartels thus proposed that trophic eggs may increase the probability of larvae to develop into queens, which are much larger than workers (Bartels 1988). In some lineages of *Pogonomyrmex barbatus*, queens laid a higher proportion of trophic eggs upon the experimental increase of maternal juvenile hormone (Helms Cahan et al. 2011). Because the treatment also strongly reduced the number of workers produced but triggered a 50% increase in worker body size, trophic eggs were suggested to affect worker size. An increase in the proportion of trophic eggs has also been suggested to be associated with an increase in the proportion of larvae developing into queens (*L. humile*: Bartels 1988; *P. barbatus*: Helms Cahan et al. 2011). Finally, because they observed an increase in nitrogen content with increasing female caste size in the ant *Pogonomyrmex badius*, Smith and Suarez (2010) suggested that the larger castes may consume more trophic (nutritional) eggs than the smaller caste which would feed more on foraged insects. In summary, in at least three species of ants, trophic eggs may play a role in the developmental trajectories of female larvae.

While conducting egg cross fostering experiments in the ant *P. rugosus* to study worker size variation, we observed a sudden increase in the frequency of females developing into queens. During these experiments, we discarded trophic eggs and only cross fostered eggs that would eventually hatch (hereafter viable eggs). This raises the possibility that the absence of trophic eggs influenced the process of caste determination. These observations prompted us to investigate whether trophic eggs play a role in caste determination in *P. rugosus*. Our experiments revealed that the presence of trophic eggs reduces the probability that female larvae develop into reproductive individuals. Metabolomic analyses also revealed profound differences between viable and trophic eggs, including in the composition of miRNAs and content of protein, triglycerides, glycogen, and sugar.

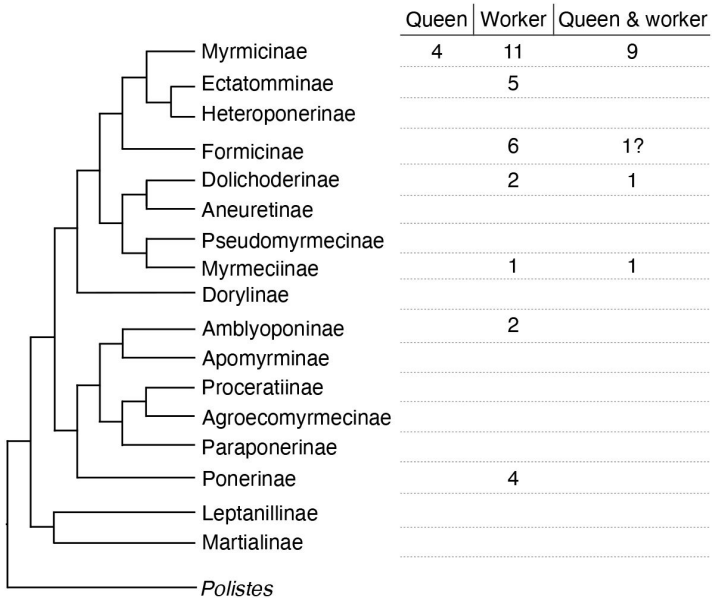


Figure 1.

Trophic egg production is widespread in ants. Simplified phylogenetic tree of ant subfamilies redrawn after Romiguier et al. (2022).

The number of species with documented trophic egg production by queens, workers or both castes is indicated for each subfamily. The question mark indicates that it is unclear whether trophic eggs can be produced by queens (in *Lasius niger* trophic eggs are produced by workers and possibly queens, see supplementary Table 1). Details on the species and related references can be found in Supplementary Table 1.

Materials and methods

Some populations of *Pogonomyrmex rugosus* are characterized by a genetic caste determination system whereby development into queens or workers is determined by whether the eggs are fertilized by males of the same genetic lineage or a different genetic lineage than the queen producing the eggs (Helms Cahan et al. 2002; Julian et al. 2002; Volny and Gordon, 2002; Helms Cahan and Keller 2003). We collected queens from two populations (Bowie and Florence, Arizona, USA) known to harbor only colonies with non-genetic caste determination (Schwander et al. 2007). The queens were collected after mating flights in 2008 (Bowie) and 2023 (Bowie and Florence) to initiate colonies in the laboratory. The colonies were maintained in plastic boxes containing water tubes (glass tubes filled with water and sealed with a cotton plug) at 28°C and 60% humidity, with a 12-h/12-h light:dark cycle. They were fed *ad libitum* once a week with grass seeds, flies and 20% honey water. Eggs were collected in October 2020 for the experiment investigating the effect of trophic eggs on larval caste fate, in November 2021 for estimating the percentage of trophic eggs and from February to December 2021 for the egg content analyses.

All statistical analyses were performed with Rstudio (RStudio Team 2015).

Trophic and viable egg production

To verify that workers do not lay trophic eggs, as previously shown for other *Pogonomyrmex* species (Supplementary Table 1), we created 12 queenless colonies (by removing the queen) and waited approximately three weeks until workers started laying eggs. From each of these colonies, we isolated two groups of five workers for 12 hours every two days for two weeks in November 2020 to obtain eggs. Collected eggs were then placed for 10 days in a petri dish containing a water reservoir to study their development and distinguish whether they were trophic or viable.

To determine whether queens laid variable percentages of trophic eggs over time, we isolated each of 43 *P. rugosus* queens for 8 hours every day for 2 weeks, before and after hibernation, and counted the number of trophic and viable eggs laid (see results for how to discriminate the two types of eggs). We hibernated the queens because we previously showed that hibernation is important to trigger the production of gynes in *P. rugosus* colonies in the laboratory (Schwander et al. 2008; Libbrecht et al. 2013). Hibernation conditions were as described in Libbrecht et al. (2013). The percentage of trophic eggs was compared using a linear mixed effect model with before vs after hibernation as the explanatory variable and colony as a random factor.

To assess whether viable and trophic eggs were laid in a random order, or whether eggs of a given type were laid in clusters, we isolated 11 queens for 10 hours, eight times over three weeks, and collected every hour the eggs laid. To determine whether viable and trophic eggs were laid in a random order, we performed a Wald–Wolfowitz runs test for each queen's egg laying sequence (package *snpar* v.1.0; this non-parametric test calculates the likelihood that a binomial data sequence is random).

Trophic egg influence on the larval caste fate

To determine whether trophic eggs influence the process of caste determination, we compared the development of freshly hatched (first instar) larvae placed in small recipient colonies with and without trophic eggs. From each of 22 donor colonies, we obtained approximately 30 freshly hatched larvae by isolating the queens for 16 hours (from 2pm to 6am) every day for three weeks (in October 2020), with a 24-hour break every three days. Eggs were collected every eight hours and placed during 10 days in a petri-dish with a water reservoir ensuring a high humidity until they hatched. After hatching, for each colony, half of the larvae (i.e., 15 larvae) were then

transferred into a recipient colony containing 20 workers, while the other half were placed in identical recipient colonies, which received in addition 45 0-4 hours-old trophic eggs (i.e., there were 3 trophic eggs per larva). There was no cross-fostering between colonies, so that larvae were always placed in recipient colonies containing workers from the same donor colony. The recipient colonies were maintained at 28°C and 60% humidity, with a 12-h/12-h light:dark cycle and fed *ad libitum* twice a week with grass seeds, flies and 20% honey water. The caste of each newly-produced individual was recorded at the pupal stage. To compare the proportion of queen pupae produced between recipient colonies with and without trophic eggs, we used the package *lme4* (Bates et al. 2015) to perform a binomial generalized linear mixed effects analysis (GLMM/link="logit") fit by maximum likelihood, with caste as response variable (binary categorical factor) and presence/absence of trophic eggs as an explanatory variable. Donor colony was included as a random effect. To test whether the presence of trophic eggs affects survival, we performed a linear mixed effect analysis with mortality as a response variable, presence/absence of trophic eggs as explanatory variable, and colonies as random effects. As we found a significantly higher survival of larvae in recipient colonies with trophic eggs than recipient colonies without trophic eggs (see results), we tested whether the percentage of larvae developing into queens was correlated with survival by performing a linear mixed effects analysis with the percentage of queen pupae as response variable, the survival as an explanatory variable and colonies as a random factor.

Volume and content of trophic and viable eggs

The volumes of trophic (n=11) and viable eggs (n=14) were estimated by using the volume of an ellipse $(\frac{4}{3} \times \pi \times egg\ length \times \left(\frac{egg\ width}{2}\right)^2)$, with egg length and width estimated on images under 10x magnification using ZEN Microscopy Software (v. 1.1.2.0).

To determine the nutritional content of viable and trophic eggs, we quantified the proteins, triglycerides, glycogen, and glucose in both types of eggs. We also quantified long and small RNAs (including miRNAs) as these compounds have been shown to be involved in caste determination in other eusocial species. To obtain the two types of eggs, we isolated 12 queens for 10 hours (7am to 5pm; from March to October 2021) in a dark petri-dish with three workers and a water supply. Eggs were collected every hour (so all eggs were a maximum of one hour old), and trophic and viable eggs were flash-frozen separately in liquid nitrogen. Twenty eggs were pooled for triglycerides-sugar-protein analyses and six eggs for RNA analyses. They were kept at -80°C until the extractions were performed. After the 10 hours of isolation, queens and workers were returned to their colony until the next isolation session. For each of the 12 colonies, we obtained two replicates of viable and trophic egg pools (i.e., 24 replicates in total).

Triglycerides, glycogen and glucose were quantified as described in Tennessen et al. (2014), and protein levels were measured using a Bradford assay (Bradford 1976). The 20 one-hour old eggs per sample were homogenized with beads in 200µl of PBS buffer in a Precellys Evolution tissue homogenizer coupled with a Cryolys Evolution (Bertin Technologies SAS).

For the Bradford assay, 10µl of the homogenate were put in a clear-bottom 96-well plate with 300µl of Coomassie Plus Reagent (Thermo Scientific: 23200) and incubated for 10 minutes at room temperature. Protein standard (Sigma: P5369) was used as standard (ranging from 0-0.5mg/ml) and protein absorbance was read at 595nm on a Hidex Sense Microplate Reader.

For the triglycerides assay, 90µl of homogenate were heat treated at 70°C for 10 minutes, then 40µl were mixed with 40µl of Triglyceride Reagent (Sigma: T2449) for digestion and 40µl were mixed with PBS buffer for free glycerol measurement. After 30 minutes incubation at 37°C, 30µl of each sample and standards were transferred to clear-bottom 96-well plate. 100µl of Free Glycerol Reagent (Sigma: F6428) was added to each sample, mixed well by pipetting, and incubated five minutes at 37°C. Glycerol standard solution (Sigma: G7793) was used as standard (ranging from 0-

1.0mg/ml TAG) and absorbance was read at 540nm on a Hidex Sense Microplate Reader. The triglycerides concentration in each sample was determined by subtracting the absorbance of free glycerol in the corresponding sample.

Glucose and glycogen were quantified as in Tennessen et al. (2014). A 90µl aliquot was heat treated at 70°C for 10min and then diluted 1:2 with PBS. The standard curves for glucose (Sigma, G6918) and glycogen (Sigma: G0885) were made by diluting stocks to 160µg/ml, making 1:1 serial dilution for 160, 80, 40, 20 and 10µg/ml. 40µl of each sample was pipetted in duplicates of a clear microplate, and 30µl of each glucose or glycogen standard was pipetted in duplicates. Amyloglucosidase enzyme (Sigma, A1602) was diluted 3µl into 2000µl of PBS, and 40µl diluted enzyme was pipetted to the glycogen standards and to one well of the sample (for total glucose determination), 40µl PBS was pipetted to the glucose standards and to the other sample well (for free glucose determination). The plate was incubated at 37°C for 60 minutes. 30µl of each standard and samples (in duplicates) were transferred to a UV 96-well plate and 100µl Glucose Assay Reagent (G3293) was pipetted to each well. The plate was incubated at room temperature for 15 minutes and the absorbance was read at 340nm on a Hidex Sense Microplate Reader. The glycogen concentration was quantified by subtracting the free glucose absorbance from the total glycogen + glucose absorbance.

Concentrations of each compound (protein, triglycerides, glycogen, and glucose) were compared between viable and trophic eggs using a linear mixed effects analysis (LMER; package *lme4*), with the concentration as response variable and egg type as explanatory variable. Colony and extraction batch were added as random effects in the model.

Total and small RNA, and DNA

RNA (>200 nt) and small RNA were isolated using the miRNeasy Mini Kit (Qiagen, cat. no. 217004) and RNeasy® MinElute® Cleanup Kit (Qiagen, cat. no. 74204), respectively, following manufacturer instructions. RNA (>200 nt) and small RNA concentrations were measured with a QuantiFluor® RNA System (Promega). RNA (>200 nt) integrity was examined with an Agilent Fragment Analyzer (at the Lausanne Genomic Technologies Facility) using a High Sensitivity Assay and small RNA were examined using the small RNA kit (at the Gene Expression Core Facility at EPFL).

The miRNA and RNA (>200 nt) concentrations were compared between viable and trophic eggs with paired-t-tests (for each type of eggs we used the average of the two replicates per colony). We also compared the fragment size distributions from 18 to 24 nucleotides for miRNAs (Sohel 2016) with a PCA and a Mantel test.

DNA was extracted from pools of six eggs using TRIzol (Life Technologies). DNA concentration was measured with a Nanodrop 3300 (ThermoFisher), and DNA integrity was examined with an Agilent Fragment Analyzer (at the Lausanne Genomic Technologies Facility) using a High Sensitivity Assay. DNA concentrations were compared between viable and trophic eggs using paired-t-tests (sample size is 5 for both types of eggs, each sample being a pool of 6 eggs).

Results

Trophic and viable egg characteristics

P. rugosus queens lay two types of eggs that are morphologically different. Viable eggs are white with a bright surface and have a distinct oval shape, a homogenous content as well as a solid chorion (**Figure 2A**), while trophic eggs are rounder, have a smooth surface and a granular looking content as well as a fragile chorion (**Figure 2D**). Trophic eggs had a significantly larger volume (94.3±4.3nL; n=11) than viable eggs (n=14; 63.3±1.6nL; two-sample t-test, t(23) = -9.54, p =

1.8×10^{-09}). While viable eggs showed embryonic development at 25 and 65 hours (Fig 12B, C) there was no such development for trophic eggs (**Fig. 2E, F**). *P. rugosus* workers only laid viable eggs. They started to lay eggs approximately three weeks after queen removal (n=12 queenless recipient colonies) and approximately 90% of the eggs successfully hatched. However, only approximately 5% successfully developed into pupae which were all males.

The percentage of eggs that were trophic was higher before hibernation (61.6±1.4% mean± SE; n=43 colonies) than after (50.3±2.0%; LMER, t(86)=5.04, $p=9\times10^{-6}$). The production of the two types of eggs was not random (Wald-Wolfowitz runs tests, p-values for the 11 queens in **Table 1**). Instead, each of the 11 queens tended to lay relatively long sequences of either viable (6.1±0.7; mean number per sequence ±SE) or trophic eggs (6.0±0.5; **Figure 3**).

The concentrations of protein, triglycerides, glycogen, and glucose were significantly higher in viable than trophic eggs (LMER, protein: t = -13.11, p <0.0001; triglycerides: t = -11.66, p <0.0001; glycogen: t = -11.98, p <0.0001; glucose: t = -18.60, p <0.0001; **Figure 4**).

The amount of small RNA (<200 nt, including miRNA and tRNA; Nagano and Fraser 2011) was significantly higher in viable eggs (44.3±1.4ng, mean ± SE) than in trophic eggs (22.3±1.1ng; paired-t-test, $t_{(23)} = 15.9$, $p = 6.5*10^{-14}$). The same was true for longer RNAs (>200 nt; viable eggs: 7.6±0.6ng, mean ± SE; trophic eggs: 3.6±0.3ng; paired-t-test, $t_{(23)} = 7.2$, $p = 2.7*10^{-7}$).

The DNA quantification showed that the amount of DNA was about twice higher in viable (15.9±1.9ng/µl) than trophic eggs (8.8±1.9ng/µl; t-test, $t_{(4.7)} = 2.7$, p = 0.045).

There was a significant difference in the miRNA fragment size distribution between viable and trophic eggs (Mantel test, $r_M = 0.26$, p<.0001), as shown on the PCA (**Figure 5A**). There was no difference in the tRNA fragment size distribution between the two types of eggs (Mantel test, $r_M = 0.01$, p=0.30, **Figure 5B**).

Trophic eggs influence caste fate of larvae

The percentage of larvae that developed into queens was significantly lower in recipient colonies that received trophic eggs (27±9% mean±SE; n=22) than in recipient colonies without trophic eggs (83±10%; n=22; binomial GLMM (link="logit"), z = 4.25, $p = 2\times10^{-5}$; **Figure 6A** and Supplementary Table 2). The survival of larvae until the pupal stage was also significantly lower in the colonies without trophic eggs (16.9±3.8%; n=22; LMER, z = 2.66, p = 0.008) than in colonies with trophic eggs (30.2±6.7%; mean±SE; n=22), but the 1.8 fold survival decrease cannot fully account for the 3 fold difference in queen percentage between the two treatments. Furthermore, there was no significant correlation between larval mortality and the percentage of larvae developing into queens (n=44 recipient colonies; LMER, z = 0.97, p = 0.34; **Figure 6B**). These analyses allow us to exclude differential survival between castes as the sole explanation for the higher percentage of queens developing in the recipient colonies without trophic eggs.

Discussion

Our study reveals that *P. rugosus* queens lay a very high proportion (0.6) of trophic eggs. These eggs differ in many ways from viable eggs. First, trophic eggs are larger, rounder, have a smoother surface, a more granular looking content as well as a more fragile chorion than viable eggs. Similar differences between trophic and viable eggs have been reported in other ant species (Wilson 1976; Wardlaw and Elmes 1995; Gobin et al. 1998; Dietemann and Peeters 2000; Dietemann et al. 2002; Perry and Roitberg 2006; Lee et al. 2017). Our analyses also showed

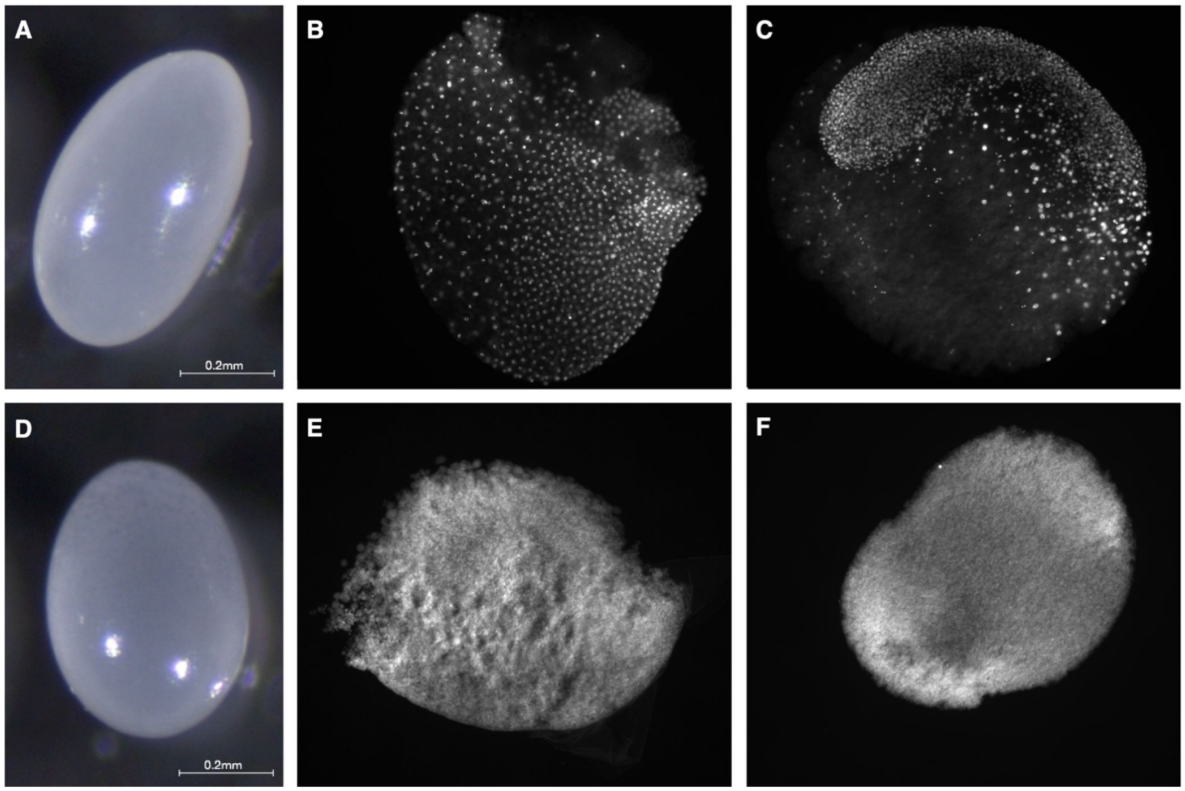


Figure 2.

Morphology and of viable (A) and trophic (D) eggs laid by *P. rugosus* queens.

Fluorescence images with DAPI-counterstained nuclei showing embryonic development of viable eggs at approximately 25 hours (**B**) and 65 hours (**C**). For trophic eggs there was no embryonic development at 25 hours (**E**) nor at 65 hours (**F**).

Table 1.

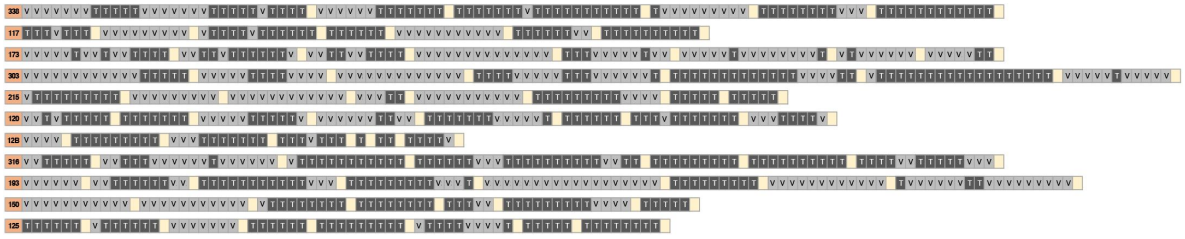
Wald-Wolfowitz runs tests on the queen's egg sequence. Significant p-values (corrected for multiple testing) indicate that queens do not lay viable and trophic eggs in a random sequence.

Queen ID	P-value for egg sequence	P-value of random sequence	Number of eggs per sequence
338	$4.1*10^{-11}$	0.419	94
117	$4.3*10^{-07}$	0.567	63
173	$1.7*10^{-03}$	0.755	92
303	$4.8*10^{-13}$	0.765	110
215	$9.8*10^{-11}$	0.292	70
120	$1.4*10^{-05}$	0.518	75
12B	$1.9*10^{-03}$	0.298	38
316	$3.4*10^{-09}$	0.737	93
193	$4.3*10^{-12}$	0.655	101
150	$1.4*10^{-10}$	0.630	62
125	$1.5*10^{-05}$	0.404	58

Figure 3.

Egg-laying sequences from eleven *P. rugosus* queens.

Every row shows the sequence of viable (V) and trophic (T) eggs laid by a given queen (queen ID in the orange cell). Each egg laying session lasted 10 hours. The yellow squares indicate the intervals (16 hours to several days) between egg-laying sessions.



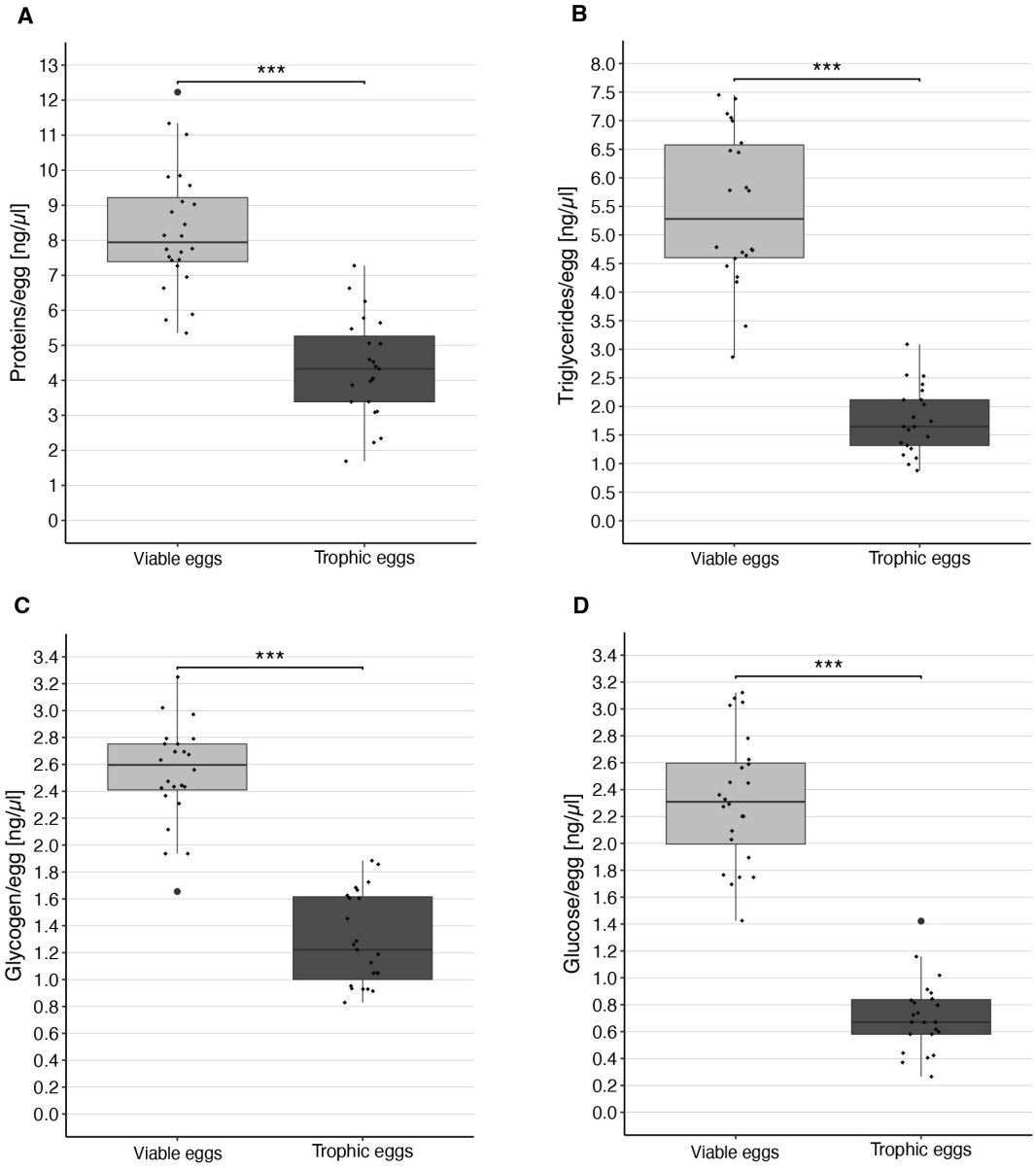


Figure 4.

Concentration (± standard error) of protein (A), triglycerides (B), glycogen (C) and glucose (D) in viable and trophic eggs.

Each dot represents the average of the two replicates per colony.

Figure 5.

First two principal components (PC1 and PC2) explaining size distribution variation for (A) miRNA and (B) tRNA across egg samples, with viable eggs in grey dots and trophic eggs in black triangles.

Ellipses enclose each of the egg type groups.

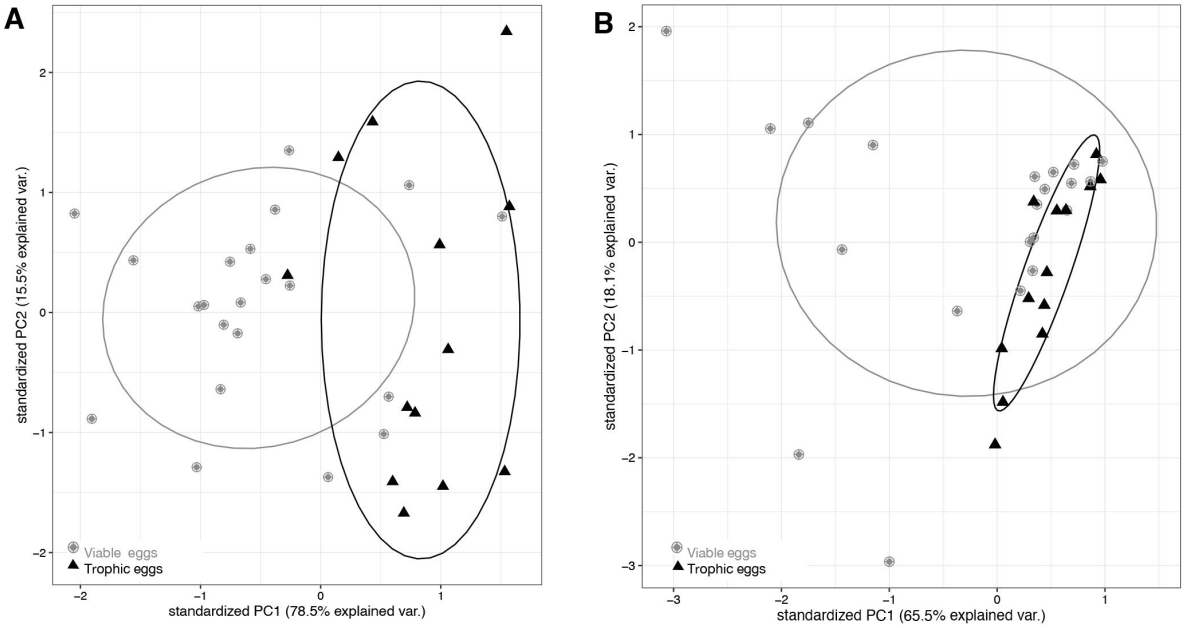
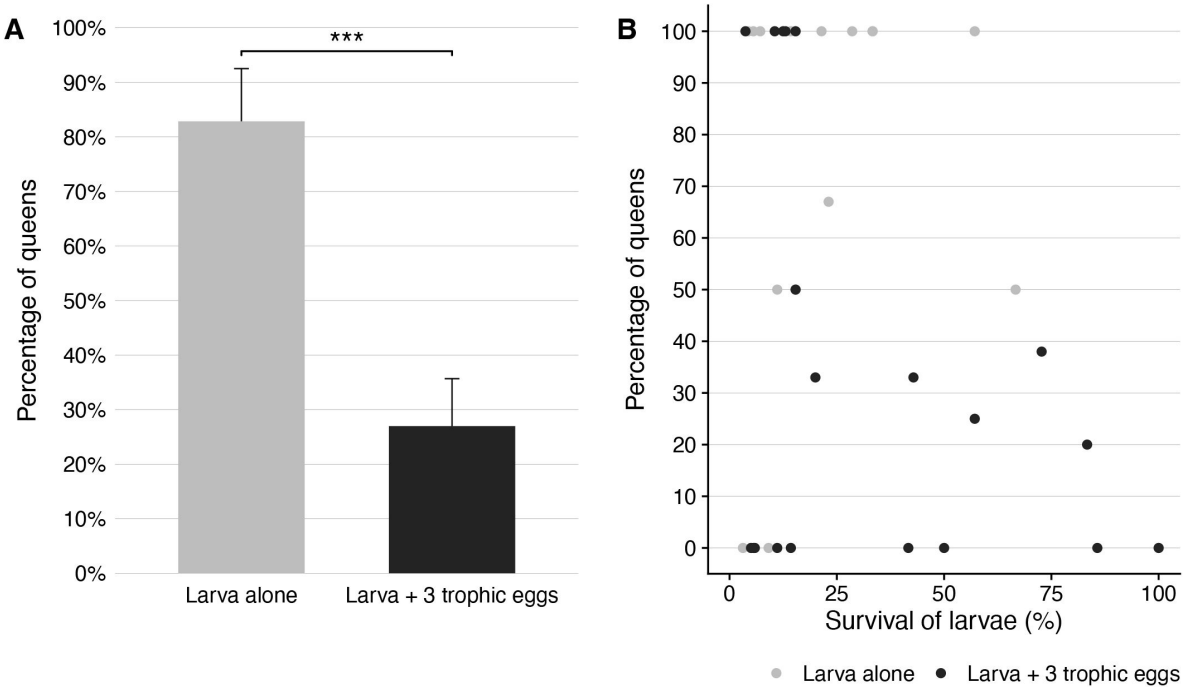


Figure 6.

(**A**) Percentage (± standard error) of queens among the larvae that developed to the pupal stage in colonies without (grey) or with (black) trophic eggs. (**B**) Relationship between the percentage of larvae which developed into queens and the survival of larvae (percentage) between the larval to pupal stages.



that trophic eggs are solely laid by queens; *P. rugosus* workers are able to produce viable eggs which occasionally develop into males, but they do not lay trophic eggs. Moreover, trophic eggs have a reduced DNA content.

Importantly, our experiments showed that the presence of trophic eggs influences the process of caste determination. First instar female larvae fed with trophic eggs were significantly more likely to develop into workers than larvae without access to trophic eggs. This was somewhat surprising because trophic eggs are generally thought to be an important source of nutrients to the colony and, everything else being equal, one would think that eating such eggs should increase the likelihood of females to develop into queens (which are usually larger than workers). Indeed, two earlier studies suggested that an increase in the proportion of trophic eggs might be associated with an increase in the proportion of larvae developing into queens (*L. humile*: Bartels 1988; *P. barbatus*: Helms Cahan et al. 2011). However, in these two studies variation in the availability of trophic eggs was associated with other differences (number of queens in the colony, Bartels 1988; Administration of a JH analogue, Helms Cahan et al. 2011) making it difficult to determine what factor had a causal effect. It would be interesting to experimentally manipulate the quantity of trophic eggs in *L. humile* and *P. barbatus* to determine whether they have a positive or inhibitory effect on the likelihood of larvae to develop into queens.

Our analyses revealed that trophic eggs have a lower content of protein, triglycerides, glycogen, and glucose than viable eggs. A reduced protein content of trophic as compared to viable eggs has also been documented in *Pheidole pallidula* (Lorber and Passera 1981). These findings are in line with the view that trophic eggs do not simply have a nutritional function as it might then be expected that they should at least contain as much nutrients as viable eggs. Interestingly, our analyses also revealed important differences in RNA and miRNA content between the two egg types. miRNAs have already been suggested to influence larval caste determination in the honeybee (Guo et al. 2013) with worker jelly being enriched in miRNAs compared to royal jelly (Guo et al. 2013; Zhu et al. 2017). These studies suggest that it is not the royal jelly that stimulates larval determination into queen, but rather the worker jelly which stimulates the development of larvae into workers. Similarly, our study reveals that compounds found in trophic eggs, perhaps miRNAs, influence larval development towards the worker phenotype. Interestingly, it has also been recently shown that trophallactic fluid in the ant *Camponotus floridanus* contains non-digestive related proteins, microRNAs and juvenile hormone (LeBoeuf et al. 2016). Moreover, comparison of trophallactic fluid proteins across social insect species revealed that many are regulators of growth, development and behavioral maturation (Meurville and LeBoeuf 2021). Finally, a recent study showed that pupae of several ant species produce secretions that play an important role for early larval nutrition with young larvae exhibiting stunted growth and decreased survival without access to the fluid (Snir et al. 2022). This raises the possibility that chemicals delivered in trophic eggs, trophallactic fluids and pupae secretions play previously unsuspected roles in communication and caste development. Given that some ants do not perform trophallaxis, it would be interesting to determine whether there are differences in the content of trophic eggs of species performing trophallaxis and species which do not.

Maternal effects on the process of caste determination have been demonstrated in several social insect species, including *P. rugosus*, either by queen behaviour or content of the eggs being produced (De Menten et al. 2005; Linksvayer 2006; Schwander et al. 2008b; Libbrecht et al. 2013; Wei et al. 2019). This is, to our knowledge the first experimental demonstrations that provisioning of trophic eggs influences caste fate. Since only queens produce trophic eggs in *P. rugosus*, trophic egg provisioning could be the main mechanism underlying the previously documented maternal effects on the process of caste determination. In species where workers produce trophic eggs (Supplementary Table 1), the same mechanism could allow workers to influence colony level caste ratios if their eggs do also affect the process of caste determination. The presence of trophic eggs has been documented in only relatively few ant species (**Table 1**).

However, their presence has been investigated in only few species and it is likely that trophic eggs are produced in most ant species, particularly in those with an independent mode of colony founding,

Finally, our analyses also revealed seasonal differences in the proportion of viable and trophic eggs, with a higher ratio of trophic eggs before hibernation than after. In *Pogonomyrmex* the production of new queens occurs after hibernation (Smith and Tschinkel 2006) or when the queen dies or is removed from the colony (pers. obs). Thus, new queens are typically produced when there are fewer trophic eggs. Our results predict that under natural conditions, a decrease in the proportion of trophic eggs should lead to an increase in the proportion of larvae developing into queens. The same logic applies to species where trophic eggs are laid only by the workers in queenright colonies (Supplementary Table 1). After the queen's death, workers start producing their own male offspring and lay mostly (if not only) viable eggs (*Temnothorax recedens*, Dejean and Passera 1974; *Plagiolepis pygmaea*, Passera 1980; *Myrmecia gulosa*, Dietemann et al. 2002), which again leads to a decrease, or cessation, in trophic egg production. A decrease in trophic egg production and the development of queens were observed simultaneously in freshly orphaned colonies of *Temnothorax recedens* (Dejean and Passera 1974), *Plagiolepis pygmaea* (Passera 1980) and *Myrmecia gulosa* (Dietemann et al. 2002). These examples are consistent with the view that trophic eggs may also play a role in the process of caste determination in other ant species.

In addition to the seasonal differences reported in this study, previous studies in ants have also revealed that young queens produce more trophic eggs than older queens (Hölldobler & Wilson 1990). Interestingly, the production of new queens in ant colonies start only when colonies are several years old and have reached a relatively large size. Thus, this raises the possibility that the decrease of the proportion of trophic eggs laid by queens when they age may contribute to the production of new queens being restricted to colonies having reached a relatively large size.

Egg cannibalism has been reported in a many ant species (Wilson 1971; Sorensen et al. 1983; Bourke 1991; Crespi 1991; Peeters and Tsuji 1993; Aron et al. 1994; Heinze et al.1999; Schultner et al. 2013) and may serve many purposes, including preferential elimination of males, source of energy (Sorensen et al. 1983) and intracolony conflict (Schultner et al. 2014). Our study indicates a new potential role of egg cannibalism as a mechanism to regulate the allocation of energy into the production of new queens versus workers.

In conclusion, this study provides a new striking example of how females can influence the developmental fate of their offspring. Because many ants produce trophic eggs, it is possible that this mechanism of parental manipulation is widespread and plays an important role in the general process of caste determination. It would be interesting to conduct manipulative experiments similar to those of this study in additional species, to determine whether trophic eggs broadly play a role in the process of caste determination. Of interest would also be to determine what chemicals in the egg are responsible for influencing the development of larvae.

Acknowledgements

We thank Dr. C. Berney for technical assistance to develop wet lab protocols. We are grateful to S. McGregor and M. Chapuisat for their helpful comments on the manuscript. This work was supported by an ERC grant and the Swiss NSF (LK) and funding from the University of Lausanne (LK and TS).

Additional files

Supplementary Tables 1 and 2

References

Amor F., Ortega P., Boulay R., Cerdá X. (2017) **Frequent colony orphaning triggers the production of replacement queens via worker thelytoky in a desert-dwelling ant** *Insectes Soc* **64**:373–378 https://doi.org/10.1007/s00040-017-0556-9 | Google Scholar

Aron S., Passera L., Keller L. (1994) **Queen-worker conflict over sex ratio: A comparison of primary and secondary sex ratios in the Argentine ant, Iridomyrmex humilis** *J. Evol. Biol* **7**:403–418 Google Scholar

Augustin J. O., Santos J. F., Elliot S. L. (2011) **A behavioral repertoire of Atta sexdens (Hymenoptera, Formicidae) queens during the claustral founding and ergonomic stages** *Insectes Soc* **58**:197–206 https://doi.org/10.1007/s00040-010-0137-7 | Google Scholar

Azevedo D. O., Zanuncio J. C., Delabie J. H. C., Serrão J.E. (2011) **Temporal variation of vitellogenin synthesis in Ectatomma tuberculatum (Formicidae: Ectatomminae) workers** *J. Insect Physiol* **57**:972–977 https://doi.org/10.1016/j.jinsphys.2011.04.015 | Google Scholar

Baroni Urbani C. B. (1991) **Indiscriminate oophagy by ant larvae: an explanation for brood serial organization?** *Insectes Soc* **38**:229–239 https://doi.org/10.1007/BF01314909 | Google Scholar

Bartels P. J. (1988) **Reproductive caste inhibition by Argentine ant queens: New mechanisms of queen control** *Insectes Soc* **35**:70–81 https://doi.org/10.1007/BF02224139 | Google Scholar

Bates D., Maechler M., Bolker B., Walker S. (2015) **Fitting Linear Mixed-Effects Models Using lme4** *J. Stat. Softw* **67**:1–48 https://doi.org/10.18637/jss.v067.i01 | Google Scholar

Blake J. A., Arnofsky P. L. (1999) **Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny** *Hydrobiologia* **402**:57–106 https://doi.org/10.1023/A | Google Scholar

Bourke A. F. G. (1988) **Worker reproduction in the higher eusocial Hymenoptera** *Quart Rev Biol* **63**:291–311 Google Scholar

Bourke A. F. G. (1991) **Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant Leptothorax acervorum** *Animal Behav* **42**:295–310 Google Scholar

Bradford M. M. (1976) **A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding** *Anal. Biochem* **72**:248–254 https://doi.org/10.1016/0003-2697(76)90527-3 | Google Scholar

Brian M. V, Rigby C. (1978) **The trophic eggs of Myrmica rubra L** *Insect Molecular Biology* **25**:89–110 Google Scholar

CamargoMathias M., Caetano F. (1995) **Trophic eggs in workers of Neoponera villosa ants (Hymenoptera:Ponerinae)** *J. Adv. Zool* **16**:62–66 Google Scholar

Cameron R. C., Duncan E. J., Dearden P. K. (2013) **Biased gene expression in early honeybee larval development** *BMC Genomics* **14**:1–12 https://doi.org/10.1186/1471-2164-14-903 | Google Scholar

Cassill D. (2002) **Brood care strategies by newly mated monogyne Solenopsis invicta (Hymenoptera: Formicidae) queens during colony founding** *Ann. Entomol. Soc. Am* **95**:208–212 Google Scholar

Choe J. C. (1988) **Worker reproduction and social evolution in ants (Hymenoptera: Formicidae)** *Advances in myrmecology* :163–187 Google Scholar

Collin R. (2004) **Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods** *Evolution* **58**:1488–1502 https://doi.org/10.1111/j.0014-3820.2004.tb01729.x | Google Scholar

Collins D. H., Mohorianu I., Beckers M., Moulton V., Dalmay T., Bourke A. F. (2017) **MicroRNAs Associated with Caste Determination and Determination in a Primitively Eusocial Insect** *Sci. Rep* Nature Publishing Group **7**:1–9 https://doi.org/10.1038/srep45674 | Google Scholar

Crespi B. J. (1977) **Cannibalism and trophic eggs in subsocial and eusocial insects** In: Elgar M. A., Crespi B. J., editors. *Cannibalism: Ecology and Evolution Among Diverse Taxa* Oxford: Oxford University Press pp. 176–213 Google Scholar

Dejean A., Passera L. (1974) **Ponte des ouvrières et inhibition royale chez la Fourmi Temnothorax recedens (Nyl.) (Formicidae, Myrmicinae)** *Insectes Soc* **21**:343–355 https://doi.org/10.1007/BF02331564 | Google Scholar

Della Lucia T. M. C., Vilela E. F., Moreira D. D. O., Bento J. M. S., Anjos N. Dos (1990) **Egg-laying in Atta sexdens rubropilosa, under laboratory conditions** In: Vander Meer R. K., Jaffé K., Cedeno A., editors. *Applied Myrmecology: A World Perspective* New York: Westview Press pp. 173–179 Google Scholar

Dietemann V., Hölldobler B., Peeters C. (2002) **Caste specialization and determination in reproductive potential in the phylogenetically primitive ant Myrmecia gulosa** *Insectes Soc* **49**:289–298 https://doi.org/10.1007/s00040-002-8316-9 | Google Scholar

Dietemann V., Peeters C. (2000) **Queen influence on the shift from trophic to reproductive eggs laid by workers of the ponerine ant Pachycondyla apicalis** *Insectes Soc* **47**:223–228 https://doi.org/10.1007/PL00001707 | Google Scholar

Dijkstra M. B., Nash D. R., Boomsma J. J. (2005) **Self-restraint and sterility in workers of Acromyrmex and Atta leafcutter ants** *Insectes Soc* **52**:67–76 https://doi.org/10.1007/s00040-004-0775-8 | Google Scholar

Fletcher D. J. C., Ross K. G. (1985) **Regulation of reproduction in Eusocial hymenoptera** *Annu. Rev. Entomol* **30**:319–343 Google Scholar

Freeland J. (1958) **Biological and social patterns in the Australian bulldog ants of the genus Myrmecia** *Aust. J. Zool* **6**:1–18 https://doi.org/10.1071/ZO9580001 | Google Scholar

Gibson G., Hart C., Coulter C., Xu H. (2012) **Nurse eggs form through an active process of apoptosis in the spionid Polydora cornuta (Annelida)** *Integr. Comp. Biol* **52**:151–160 https://doi.org/10.1093/icb/ics054 | Google Scholar

Gobin B., Billen J., Peeters C. (1999) **Policing behaviour towards virgin egg layers in a polygynous ponerine ant** *Anim. Behav* **58**:1117–1122 https://doi.org/10.1006/anbe.1999.1245 | Google Scholar

Gobin B., Ito F. (2000) **Queens and major workers of Acanthomyrmex ferox redistribute nutrients with trophic eggs** *Naturwissenschaften* **87**:323–326 https://doi.org/10.1007/s001140050731 | Google Scholar

Gobin B., Peeters C., Billen J. (1998) **Production of trophic eggs by virgin workers in the ponerine ant Gnamptogenys menadensis** *Physiol. Entomol* **23**:329–336 https://doi.org/10.1046/j.1365-3032.1998.00102.x | Google Scholar

Guo X., Su S., Skogerboe G., Dai S., Li W., Li Z., Liu F., Ni R., Guo Y., Chen S., Zhang S., Chen R. (2013) **Recipe for a busy bee: MicroRNAs in honey bee caste determination** *PLoS One* **8**:1–10 https://doi.org/10.1371/journal.pone.0081661 | Google Scholar

Hammond R, Keller L. (2004) **Conflict over male parentage in social insects** *PLoS Biology* **2**:e248. Google Scholar

Haskins C.P., Whelden R.M. (1965) **"Queenlessness," Worker Sibship, and Colony Versus Population Structure in the Formicid Genus Rhytidoponera'** *Psyche: A Journal of Entomology* **72**:26 https://doi.org/10.1155/1965/40465 | Google Scholar

Heinze J., Cover S., Hölldobler B. (1995) **Neither worker, nor queen: an ant caste specialized in the production of unfertilized eggs** *Psyche A J. Entomol* **102**:173–185 https://doi.org/10.1155/1995/65249 | Google Scholar

Heinze J., Foitzik S., Oberstadt B., Rüppell O., Hölldobler B. (1999) **A female caste specialized for the production of unfertilized eggs in the ant Crematogaster smithi** *Naturwissenschaften* **86**:93–95 https://doi.org/10.1007/s001140050579 | Google Scholar

Helms Cahan S., Graves C. J., Brent C. S. (2011) **Intergenerational effect of juvenile hormone on offspring in Pogonomyrmex harvester ants** *J. Comp. Physiol. B* **181**:991–999 https://doi.org/10.1007/s00360-011-05Helms | Google Scholar

Helms Cahan S., Keller L. (2003) **Complex hybrid origin of genetic caste determination in harvester ants** *Nature* **424**:306–309 Google Scholar

Helms Cahan S, Parker JD, Rissing SW, Johnson RA, Polony TS, Weiser MD, Smith DR (2002) **Extreme genetic differences between queens and workers in hybridizing Pogonomyrmex harvester ants** *Proc. R. Soc. Lond. B. Biol. Sci* **269**:1871–1877 Google Scholar

Hölldobler B., Carlin N. F. (1989) **Colony founding, queen control and worker reproduction in the ant Aphaenogaster (=Novomessor) cockerelli (Hymenoptera: Formicidae)** *Psyche (Stuttg)* **96**:131–152 Google Scholar

Hölldobler B., Wilson E. O. (1983) **Queen Control in Colonies of Weaver Ants (Hymenoptera: Formicidae)** *Ann. Entomol. Soc. Am* **76**:235–238 https://doi.org/10.1093/aesa/76.2.235 | Google Scholar

Hölldobler B., Wilson E. O. (1990) **The Ants** Cambridge, MA: Belknap Press of Harvard Univ. Press Google Scholar

Hora R. R., Poteaux C., Doums C., Fresneau D. (2007) **Egg cannibalism in a facultative polygynous ant: Conflict for reproduction or strategy to survive?** *Ethology* **113**:909–916 https://doi.org/10.1111/j.1439-0310.2007.01391.x | Google Scholar

Huber J. (1905) **Uber die Koloniegründung bei Atta sexdens** *Biologisches Centralblatt* **25**:625–635 Google Scholar

Hung A. C. F. (1973) **Reproductive biology in dulotic ants: preliminary report (Hymenoptera: Formicidae)** *Entomol. News* **84**:253–259 Google Scholar

Ito F. (1991) **Preliminary report on queenless reproduction in a primitive ponerine ant Amblyopone sp. (reclinata group) in west Java, indonesia** *Psyche (Stuttg)* **98**:319–322 Google Scholar

Ito F. (1993) **Social organization in a primitive ponerine ant: Queenless reproduction, dominance hierarchy and functional polygyny in Amblyopone sp** *(reclinata group) (hymenoptera: Formicidae: Ponerinae). J. Nat. Hist* **27**:1315–1324 https://doi.org/10.1080/00222939300770751 | Google Scholar

Ito F. (2005) **Mechanisms regulating functional monogyny in a Japanese population of Leptothorax acervorum (Hymenoptera, Formicidae): Dominance hierarchy and preferential egg cannibalism** *Belgian J. Zool* **135**:3–8 Google Scholar

Julian GE, Fewell JH, Gadau J, Johnson RA, Larrabee D (2002) **Genetic determination of the queen caste in an ant hybrid zone** *Proc. Natl. Acad. Sci. USA* **99**:8157–8160 Google Scholar

Keller L., Passera L. (1989) **Size and fat content of gynes in relation with the mode of colony founding in ants (Hymenoptera; Formicidae)** *Oecologia* **80**:236–240 Google Scholar

Khila A., Abouheif E. (2008) **Reproductive constraint is a developmental mechanism that maintains social harmony in advanced ant societies** *Proc. Natl. Acad. Sci. U.S.A* **105**:17884–17889 https://doi.org/10.1073/pnas.0807351105 | Google Scholar

Khila A., Abouheif E. (2010) **Evaluating the role of reproductive constraints in ant social evolution** *Philos. Trans. R. Soc. B* **365**:617–630 https://doi.org/10.1098/rstb.2009.0257 | Google Scholar

Klowden M. J. (2013) **Developmental Systems** In: Klowden M. J., editors. *Physiological Systems in Insects (Third Edition)* Academic Press pp. 149–196 Google Scholar

Kudo S., Nakahira T. (2004) **Effects of Trophic-Eggs on Offspring Performance and Rivalry in a Sub-Social Bug** *Oikos* **107**:28–35 Google Scholar

LeBoeuf A. C., Waridel P., Brent C. S., Gonçalves A. N., Menin L., Ortiz D., Riba-Grognuz O., Koto A., Soares Z. G., Privman E., Miska E. A., Benton R., Keller L. (2016) **Oral transfer of chemical cues, growth proteins and hormones in social insects** *eLife* **5**:1–27 https://doi.org/10.7554/eLife.20375 | Google Scholar

Lee C. C., Nakao H., Tseng S. P., Hsu H. W., Lin G. L., Tay J. W., Billen J., Ito F., Lee C. Y., Lin C. C., Yang C. C. S. (2017) **Worker reproduction of the invasive yellow crazy ant Anoplolepis gracilipes** *Front. Zool* **14**:1–12 https://doi.org/10.1186/s12983-017-0210-4 | Google Scholar

Levin L. A., Bridges T. S. (1995) **Pattern and diversity in reproduction and development** In: McEdward L., editors. *Ecology of marine invertebrate larvae* CRC Press pp. 1–48 https://doi.org/10.1201/9780138758950-1 | Google Scholar

Libbrecht R., Corona M., Wende F., Azevedo D. O., Serrão J. E., Keller L. (2013) **Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates maternal effects on polyphenism in ants** *Proc. Natl. Acad. Sci. U.S.A* **110**:11050–11055 https://doi.org/10.1073/pnas.1221781110 | Google Scholar

Linksvayer T. A. (2006) **Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant** *Evolution* **60**:2552 https://doi.org/10.1554/06-011.1 | Google Scholar

López-Ortega M., Williams T. (2018) **Natural enemy defense, provisioning and oviposition site selection as maternal strategies to enhance offspring survival in a sub-social bug** *PLoS One* **13**:1–18 https://doi.org/10.1371/journal.pone.0195665 | Google Scholar

Lorber B., Passera L. (1981) **Étude comparative des protéines solubles des œufs de la fourmi Pheidole pallidula Nyl** *Bull Intérieur SF-UIEIS 97–99* Google Scholar

Masuko K. (2003) **Larval oophagy in the ant Amblyopone silvestrii (Hymenoptera, Formicidae)** *Insectes Soc* **50**:317–322 https://doi.org/10.1007/s00040-003-0688-y | Google Scholar

De Menten L., Fournier D., Brent C., Passera L., Vargo E.L., Aron S. (2005) **Dual Mechanism of Queen Influence over Sex Ratio in the Ant Pheidole Pallidula** *Behavioral Ecology and Sociobiology* **58**:527–33 https://doi.org/10.1007/s00265-005-0964-0 | Google Scholar

Meurville M.-P., LeBoeuf A.C. (2021) **Trophallaxis: The Functions and Evolution of Social Fluid Exchange in Ant Colonies (Hymeno Ptera: Formicidae)** *Myrmecological News* **31**:1–20 https://doi.org/10.25849/myrmecol.news_031:00113 | Google Scholar

Nagano T., Fraser P. (2011) **No-Nonsense Functions for Long Noncoding RNAs** *Cell* **145**:178–181 https://doi.org/10.1016/J.CELL.2011.03.014 | Google Scholar

Passera L. (1978) **Une nouvelle catégorie d'œufs alimentaires: les œufs alimentaires émis par les reines vierges de Pheidole pallidula (Nyl.) (Formicidae, Myrmicinae)** *Insectes Soc* **25**:117–126 Google Scholar

Passera L. (1980) **La fonction inhibitrice des reines de la fourmi Plagiolepis pygmaea Latr.: Rôle des phéromones** *Insectes Soc* **27**:212–225 https://doi.org/10.1007/BF02223665 | Google Scholar

Peeters C. (2017) **Independent colony foundation in Paraponera clavata (hymenoptera: Formicidae): First workers lay trophic eggs to feed queen's larvae** *Sociobiology* **64**:417–422 https://doi.org/10.13102/sociobiology.v64i4.2092 | Google Scholar

Peeters C., Tsuji K. (1993) **Reproductive conflict among ant workers in Diacamma sp. from Japan: dominance and oviposition the absence of gamergate** *Ins. Soc* **40**:119–136 Google Scholar

Perry J. C., Roitberg B. D. (2006) **Trophic egg laying: Hypotheses and tests** *Oikos* **112**:706–714 https://doi.org/10.1111/j.0030-1299.2006.14498.x | Google Scholar

Romiguier J., Borowiec M. L., Weyna A., Helleu Q., Loire E., La Mendola C., Rabeling C., Fisher B. L., Ward P. S., Keller L. (2022) **Ant phylogenomics reveals a natural selection hotspot preceding the origin of complex eusociality** *Current Biology* **32**:2942–2947 https://doi.org/10.1016/j.cub.2022.05.001 | Google Scholar

RStudio Team (2015) **RStudio: Integrated Development Environment for R**

Schultner E., d'Ettorre P., Helanterä H. (2013) **Social conflict in ant larvae: egg cannibalism occurs mainly in males and larvae prefer alien eggs** *Behavioral Ecol* **24**:1306–1311 https://doi.org/10.1093/beheco/art067 | Google Scholar

Schultner E., Gardner A., Karhunen M., Helanterä H. (2014) **Ant larvae as players in social conflict: Relatedness and individual identity mediate cannibalism Intensity** *Am. Nat* **184**:E161–E174 Google Scholar

Schwander T., Cahan S. H., Keller L. (2007) **Characterization and distribution of Pogonomyrmex harvester ant lineages with genetic caste determination** *Molecular Ecology* **16**:367–387 Google Scholar

Schwander T., Humbert J. Y., Brent C. S., Cahan S. H., Chapuis L., Renai E., Keller L. (2008) **Maternal Effect on Female Caste Determination in a Social Insect** *Curr. Biol* **18**:265–269 https://doi.org/10.1016/j.cub.2008.01.024 | Google Scholar

Smeeton L. (1981) **The Source of Males in Myrmica Rubra L. (Hym. Formicidae)** *Insectes Sociaux* **28**:263–78 https://doi.org/10.1007/BF02223628 | Google Scholar

Smith A. A., Hölldobler B., Liebig J. (2008) **Hydrocarbon signals explain the pattern of worker and egg policing in the ant Aphaenogaster cockerelli** *J. Chem. Ecol* **34**:1275–1282 https://doi.org/10.1007/s10886-008-9529-9 | Google Scholar

Smith C. R., Schoenick C., Anderson K. E., Gadau J., Suarez A. V (2007) **Potential and realized reproduction by different worker castes in queen-less and queen-right colonies of Pogonomyrmex badius** *Insect. Soc* **54**:260–267 https://doi.org/10.1007/s00040-007-0940-y | Google Scholar

Smith C. R., Suarez A. V. (2010) **The trophic ecology of castes in harvester ant colonies** *Functional Ecology* **24**:122–130 https://doi.org/10.1111/j.1365-2435.2009.01604.x | Google Scholar

Smith C. R., Tschinkel W. R. (2006) **The sociometry and sociogenesis of reproduction in the Florida harvester ant, Pogonomyrmex badius** *J. Insect Sci* **6**:1–11 https://doi.org/10.1673/2006_06_32.1 | Google Scholar

Snir O., Alwaseem H., Sharma S., Valdés-Rodríguez S., Carroll T.S., Jiang C.S., Razzauti J., Kronauer D.J.C. (2022) **The pupal moulting fluid has evolved social functions in ants** *Nature* **612**:488–494 https://doi.org/10.1038/s41586-022-05480-9 | Google Scholar

Sohel M. H. (2016) **Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges** *Achiev. Life Sci* **10**:175–186 https://doi.org/10.1016/j.als.2016.11.007 | Google Scholar

Sorensen A., Busch T., Vinson S. (1983) **Factors affecting brood cannibalism in laboratory colonies of the imported fire ant, Solenopsis invicta Buren (Hymenoptera: Formicidae)** *J. Kansas Entomol. Soc* **56**:140–150 Google Scholar

Søvik E., Bloch G., Ben-Shahar Y. (2015) **Function and evolution of microRNAs in eusocial Hymenoptera** *Front. Genet* **6**:1–11 https://doi.org/10.3389/fgene.2015.00193 | Google Scholar

Strathmann M. F., Strathmann R. R. (2006) **A vermetid gastropod with complex intracapsular cannibalism of nurse eggs and sibling larvae and a high potential for invasion** *Pacific Sci* **60**:97–108 https://doi.org/10.1353/psc.2005.0062 | Google Scholar

Suzzoni J. P., Passera L., Strambi A. (1979) **Taux des ecdystéroides et déterminisme des castes de la Fourmi** *Pheidole pallidula (Hym. Formicidae)* Google Scholar

Taylor R.W. (1978) **Nothomyrmecia Macrops : A Living-Fossil Ant Rediscovered** *Science* **201**:979–85 https://doi.org/10.1126/science.201.4360.979 | Google Scholar

Tennessen J. aso. M., Barry W. E., Cox J., Thummel C. S. (2014) **Methods for studying metabolism in Drosophila** *Methods* **68**:105–115 https://doi.org/10.1016/j.ymeth.2014.02.034 | Google Scholar

Torossian C. (1968) **Recherches Sur La Biologie et l'éthologie De Dolichoderus Quadripunctatus (L) (Hym. Form. Dolichoderidæ)** *Insectes Sociaux* **15**:375–88 Google Scholar

Volny V. P., Gordon D. M. (2002) **Genetic basis for queen-worker dimorphism in a social insect** *Proc. Natl. Acad. Sci. USA* **99**:6108–6111 Google Scholar

Volny V. P., Greene M. J., Gordon D. M., Volny V. P., Greene M. J., Gordon D. M. (2006) **Brood Production and Lineage Discrimination in the Red Harvester Ant (Pogonomyrmex barbatus)** *Ecology* **87**:2194–2200 Google Scholar

Voss S. H., McDonald J. F., Keith C. H. (1988) **Production and abortive development of fire ant trophic eggs** In: Trager J.C., editors. *Advances in myrmecology* pp. 517–534 Google Scholar

Wardlaw J. C., Elmes G. W. (1995) **Trophic eggs laid by fertile Myrmica queens (Hymenoptera: Formicidae)** *Insectes Soc* **42**:303–308 https://doi.org/10.1007/BF0124042 | Google Scholar

Wardlaw J. C., Elmes G. W. (1998) **Variability in oviposition by workers of six species of Myrmica (hymenoptera, formicidae)** *Insectes Soc* **45**:369–384 https://doi.org/10.1007/s000400050096 | Google Scholar

Wei H., He X.J., Liao C.H., Wu X.B., Jiang W.J., Zhang B., Zhou L.B., Zhang L.Z., Barron A.B., Zeng Z.J. (2019) **A Maternal Effect on Queen Production in Honeybees** *Current Biology* **29**:2208–2213 https://doi.org/10.1016/j.cub.2019.05.059 | Google Scholar

Wenseleers T, Ratnieks F.L.W. (2006) **Comparative analysis supports relatedness theory for worker policing in eusocial Hymenoptera** *Am Nat* **168**:E163–79 https://doi.org/10.1086/508619 | Google Scholar

Wheeler W. M. (1910) **Ants: Their Structure, Development, and Behavior** New York: The Columbia University Press https://doi.org/10.1155/1922/80849 | Google Scholar

Wilson E. O. (1971) **The insect societies** Cambridge, MA: Harvard University Press Google Scholar

Wilson E. O. (1976) **A social ethogram of the neotropical arboreal ant Zacryptocerus varians (Fr. Smith)** *Anim. Behav* **24**:354–363 https://doi.org/10.1016/S0003-3472(76)80043-7 | Google Scholar

Yamada A., Ito F., Hashim R., Eguchi K. (2018) **Queen polymorphism in Acanthomyrmex careoscrobis Moffett, 1986 in Peninsular Malaysia (Hymenoptera: Formicidae: Myrmicinae), with descriptions of hitherto unknown female castes and males** *Asian Myrmecology* **10**:1–19 https://doi.org/10.20362/am.010009 | Google Scholar

Yamauchi K., Furukawa T., Kinomura K., Takamine H., Tsuji K. (1991) **Secondary Polygyny by Inbred Wingless Sexuals in the Dolichoderine Ant Technomyrmex albipes** *Behav. Ecol. Sociobiol* **29**:313–319 Google Scholar

Zhu K., Liu M., Fu Z., Zhou Z., Kong Y., Liang H., Lin Z., Luo J., Zheng H., Wan P., Zhang J., Zen K., Chen J., Hu F., Zhang C. Y., Ren J., Chen X. (2017) **Plant microRNAs in larval food regulate honeybee caste development** *PLoS Genet* **13**:1–23 https://doi.org/10.1371/journal.pgen.1006946 | Google Scholar

Author information

Eléonore Genzoni
Department of Ecology and Evolution, Biophore Building, University of Lausanne, Lausanne, Switzerland
ORCID iD: 0000-0003-3152-992X

For correspondence: eleonore.genzoni@gmail.com

Tanja Schwander
Department of Ecology and Evolution, Biophore Building, University of Lausanne, Lausanne, Switzerland
ORCID iD: 0000-0003-1945-5374

Laurent Keller
Department of Ecology and Evolution, Biophore Building, University of Lausanne, Lausanne, Switzerland, Social Evolution Unit, Chesières, Switzerland
ORCID iD: 0000-0002-5046-9953

Editors

Reviewing Editor
Virginie Courtier-Orgogozo
CNRS - Universite Paris Cite, Paris, France

Senior Editor
Christian Rutz
University of St Andrews, St Andrews, United Kingdom

Reviewer #1 (Public Review):

This manuscript describes a series of experiments documenting trophic egg production in a species of harvester ant, Pogonomyrmex rugosus. In brief, queens are the primary trophic egg producers, there is seasonality and periodicity to trophic egg production, trophic eggs differ in many basic dimensions and contents relative to reproductive eggs, and diets supplemented with trophic eggs had an effect on the queen/worker ratio produced (increasing worker production).

The manuscript is very well prepared and the methods are sufficient. The outcomes are interesting and help fill gaps in knowledge, both on ants as well as insects, more generally.

https://doi.org/10.7554/eLife.86899.3.sa2

Reviewer #2 (Public review):

The revised manuscript by Genzoni et al. reports the striking discovery of a regulatory role for trophic eggs. Prior to this study, trophic eggs were widely assumed to play a nutritional role in the colony, but this study shows that trophic eggs can suppress queen development, and therefore, can play a role in regulating caste determination in specific social contexts. In this revised version of the manuscript, the authors have addressed many of the concerns raised in the first version regarding the lack of sufficient information and context in the Introduction and Discussion.

https://doi.org/10.7554/eLife.86899.3.sa1

Author response:

The following is the authors' response to the previous reviews

> ***Public Reviews:***
>
> ***Reviewer #1 (Public Review):***
>
> *This manuscript describes a series of experiments documenting trophic egg production in a species of harvester ant, Pogonomyrmex rugosus. In brief, queens are the primary trophic egg producers, there is seasonality and periodicity to trophic egg production, trophic eggs differ in many basic dimensions and contents relative to reproductive eggs, and diets supplemented with trophic eggs had an effect on the queen/worker ratio produced (increasing worker production).*
>
> *The manuscript is very well prepared and the methods are sufficient. The outcomes are interesting and help fill gaps in knowledge, both on ants as well as insects, more*

> *generally. More context could enrich the study and flow could be improved.*

We thank the reviewer for these comments. We agree that the paper would benefit from more context. We have therefore greatly extended the introduction.

> ***Reviewer #2 (Public Review):***
>
> *The manuscript by Genzoni et al. provides evidence that trophic eggs laid by the queen in the ant Pogonomyrmex rugosis have an inhibitory effect on queen development. The authors also compare a number of features of trophic eggs, including protein, DNA, RNA, and miRNA content, to reproductive eggs. To support their argument that trophic eggs have an inhibitory effect on queen development, the authors show that trophic eggs have a lower content of protein, triglycerides, glycogen, and glucose than reproductive eggs, and that their miRNA distributions are different relative to reproductive eggs. Although the finding of an inhibitory influence of trophic eggs on queen development is indeed arresting, the egg cross-fostering experiment that supports this finding can be effectively boiled down to a single figure (Figure 6). The rest of the data are supplementary and correlative in nature (and can be combined), especially the miRNA differences shown between trophic and reproductive eggs. This means that the authors have not yet identified the mechanism through which the inhibitory effect on queen development is occurring. To this reviewer, this finding is more appropriate as a short report and not a research article. A full research article would be warranted if the authors had identified the mechanism underlying the inhibitory effect on queen development. Furthermore, the article is written poorly and lacks much background information necessary for the general reader to properly evaluate the robustness of the conclusions and to appreciate the significance of the findings.*

We thank the reviewer for these comments. We agree that the paper would benefit by having more background information and more discussion. We have followed this advice in the revision.

> ***Reviewer #3 (Public Review):***
>
> *In "Trophic eggs affect caste determination in the ant Pogonomyrmex rugosus" Genzoni et al. probe a fundamental question in sociobiology, what are the molecular and developmental processes governing caste determination? In many social insect lineages, caste determination is a major ontogenetic milestone that establishes the discrete queen and worker life histories that make up the fundamental units of their colonies. Over the last century, mechanisms of caste determination, particularly regulators of caste during development, have remained relatively elusive. Here, Genzoni et al. discovered an unexpected role for trophic eggs in suppressing queen development - where bi-potential larvae fed trophic eggs become significantly more likely to develop into workers instead of gynes (new queens). These results are unexpected, and potentially paradigm-shifting, given that previously trophic eggs have been hypothesized to evolve to act as an additional intracolony resource for colonies in potentially competitive environments or during specific times in colony ontogeny (colony foundation), where additional food sources independent of foraging would be beneficial. While the evidence and methods used are compelling (e.g., the sequence of reproductive vs. trophic egg deposition by single queens, which highlights that the production of trophic eggs is tightly regulated), the connective tissue linking many experiments is missing and the downstream mechanism is speculative (e.g., whether miRNA, proteins, triglycerides, glycogen levels in trophic eggs is what suppresses queen development). Overall, this research elevates the importance of trophic eggs in regulating queen and worker development but how this is achieved remains unknown.*

We thank the reviewer for these comments and agree that future work should focus on identifying the substances in trophic eggs that are responsible for caste determination.

> ***Recommendations For The Authors:***
>
> ***Reviewer #1 (Recommendations For The Authors):***
>
> *Introduction:*
>
> *The context for this study is insufficiently developed in the introduction - it would be nice to have a more detailed survey of what is known about trophic eggs in insects, especially social insects. The end of the introduction nicely sets up the hypothesis through the prior work described by Helms Cahan et al. (2011) where they found JH supplementation increased trophic egg production and also increased worker size. I think that the introduction could give more context about egg production in Pogonomyrmex and other ants, including what is known about worker reproduction. For example, Suni et al. 2007 and Smith et al. 2007 both describe the absence of male production by workers in two different harvester ants. Workers tend to have underdeveloped ovaries when in the presence of the queen. Other species of ants are known to have worker reproduction seemingly for the purpose of nutrition (see Heinze and Hölldober 1995 and subsequent studies on Crematogaster smithi). Because some ants, including Pogonomyrmex, lack trophallaxis, it has been hypothesized that they distribute nutrients throughout the nest via trophic eggs as is seen in at least one other ant (Gobin and Ito 2000). Interestingly, Smith and Suarez (2009) speculated that the difference in nutrition of developing sexual versus worker larvae (as seen in their pupal stable isotope values) was due to trophic egg provisioning - they predicted the opposite as was found in this study, but their prediction was in line with that of Helms Cahan et al. (2011). This is all to say that there is a lot of context that could go into developing the ideas tested in this paper that is completely overlooked. The inclusion of more of what is known already would greatly enrich the introduction.*

We agree that it would be useful to provide a larger context to the study. We now provide more information on the life-history of ants and explained under what situations queens and workers may produce trophic eggs. We also mentioned that some ants such as *Crematogaster smithi* have a special caste of “large workers” which are morphologically intermediate between winged queens and small workers and appear to be specialized in the production of unfertilized eggs. We now also mention the study of Goby and Ito (200) where the authors show that trophic eggs may play an important role in food distribution withing the colony, in particular in species where trophallaxis is rare or absent.

> *Methods:*
>
> *L49: What lineage is represented in the colonies used? The collection location is near where both dependent-lineage (genetic caste determining) P. rugosus and "H" lineage exist. This is important to know. Further, depending on what these are, the authors should note whether this has relevance to the study. Not mentioning genetic caste determination in a paper that examines caste determination is problematic.*

This is a good point. We have now provided information at the very beginning of the material and method section that the queens had been collected in populations known not to have dependentlineage (genetic caste determining) mechanisms of caste determination.

> *L63 and throughout: It would be more efficient to have a paragraph that cites R (must be done) and RStudio once as the tool for all analyses. It also seems that most model*

> *construction and testing was done using lme4 - so just lay this out once instead of over and over.*

We agree and have updated the manuscript accordingly.

> *L95: 'lenght' needs to be 'length' in the formula.*

Thanks, corrected.

> *L151: A PCA was used but not described in the methods. This should be covered here. And while a Mantel test is used, I might consider a permANOVA as this more intuitively (for me, at least) goes along with the PCA.*

We added the PCA description in the Material and Method section.

> *Results:*
>
> *I love Fig. 3! Super cool.*

Thanks for this positive comment.

> *Discussion:*
>
> *It would be good to have more on egg cannibalism. This is reasonably well-studied and could be good extra context.*

We have added a paragraph in the discussion to mention that egg cannibalism is ubiquitous in ants.

> *Supp Table 1: P. badius is missing and citations are incorrectly attributed to P. barbatus.*

P. badius was present in the Table but not with the other *Pogonomyrmex* species. For some genera the species were also not listed in alphabetic order. This has been corrected.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *Comments on introduction:*
>
> *The introduction is missing information about caste determination in ants generally and Pogonomyrmex rugosis specifically. This is important because some colonies of Pogonomyrmex rugosis have been shown to undergo genetic caste determination, in which case the main result would be rendered insignificant. What is the evidence that caste determination in the lineages/colonies used is largely environmentally influenced and in what contexts/environmental factors? All of this should be made clear.*

This is a good point. We have expanded the introduction to discuss previous work on caste determination in *Pogonomyrmex* species with environmental caste determination and now also provide evidence at the beginning of the Material and Method section that the two populations studied do not have a system of genetic caste determination.

> *Line 32 and throughout the paper: What is meant exactly by 'reproductive eggs'? Are these eggs that develop specifically into reproductives (i.e., queens/males) or all eggs that are non-trophic? If the latter, then it is best to refer to these eggs as 'viable' in order to prevent confusion.*

We agree and have updated the manuscript accordingly.

> *Figure 1/Supp Table 1: It is surprising how few species are known to lay trophic eggs. Do the authors think this is an informative representation of the distribution of trophic egg production across subfamilies, or due to lack of study? Furthermore, the branches show ant subfamilies, not families. What does the question mark indicate? Also, the information in the table next to the phylogeny is not easy to understand. Having in the branches that information, in categories, shown in color for example, could be better and more informative. Finally, having the 'none' column with only one entry is confusing - discuss that only one species has been shown to definitely not lay trophic eggs in the text, but it does not add much to the figure.*

Trophic eggs are probably very common in ants, but this has not been very well studied. We added a sentence in the manuscript to make this clear.

Thanks for noticing the error family/subfamily error. This has been corrected in Figure 1 and Supplementary Table 1.

The question mark indicates uncertainty about whether queens also contribute to the production of trophic eggs in one species (*Lasius niger*). We have now added information on that in the Figure legend.

We agree with the reviewer that it would be easier to have the information on whether queens and workers produce trophic on the branches of the Tree. However, having the information on the branches would suggest that the “trait” evolved on this part of the tree. As we do not know when worker or queen production of trophic eggs exactly evolved, we prefer to keep the figure as it is.

Finally, we have also removed the none in the figure as suggested by the reviewer and discussed in the manuscript the fact that the absence of trophic eggs has been reported in only one ant species (*Amblyopone silvestrii*: Masuko 2003_)._

> *Comments on materials and methods:*
>
> *Why did they settle on three trophic eggs per larva for their experimental setup?*

We used three trophic eggs because under natural conditions 50-65% of the eggs are trophic. The ratio of trophic eggs to viable eggs (larvae) was thus similar natural condition.

> *Line 50: In what kind of setup were the ants kept? Plaster nests? Plastic boxes? Tubes? Was the setup dry or moist? I think this information is important to know in the context of trophic eggs.*

We now explain that colonies were maintained in plastic boxes with water tubes.

> *Line 60: Were all the 43 queens isolated only once, or multiple times?*

Each of the 43 queens were isolated for 8 hours every day for 2 weeks, once before and once after hibernation (so they were isolated multiple times). We have changed the text to make clear that this was done for each of the 43 queens.

> *Could isolating the queen away from workers/brood have had an effect on the type of eggs laid?*

This cannot be completely ruled out. However, it is possible to reliably determine the proportion of viable and trophic eggs only by isolating queens. And importantly the main aim of these experiments was not to precisely determine the proportion viable and trophic eggs, but to show that this proportion changes before and after hibernation and that queens do not lay viable and trophic eggs in a random sequence.

> *Since it was established that only queens lay trophic eggs why was the isolation necessary?*

Yes this was necessary because eggs are fragile and very difficult to collect in colonies with workers (as soon as eggs are laid they are piled up and as soon as we disturb the nest, a worker takes them all and runs away with them). Moreover, it is possible that workers preferentially eat one type of eggs thus requiring to remove eggs as soon as queens would have laid them. This would have been a huge disturbance for the colonies.

> *Line 61: Is this hibernation natural or lab induced? What is the purpose of it? How long was the hibernation and at what temperature? Where are the references for the requirement of a diapause and its length?*

The hibernation was lab induced. We hibernated the queens because we previously showed that hibernation is important to trigger the production of gynes in *P. rugosus* colonies in the laboratory (Schwander et al 2008; Libbrecht et al 2013). Hibernation conditions were as described in Libbrecht et al (2013).

> *Line 73: If the queen is disturbed several times for three weeks, which effect does it have on its egg-laying rate and on the eggs laid? Were the eggs equally distributed in time in the recipient colonies with and without trophic eggs to avoid possible effects?*

It is difficult to respond what was the effect of disturbance on the number and type of eggs laid. But again our aim was not to precisely determine these values but determine whether there was an effect of hibernation on the proportion of trophic eggs. The recipient colonies with and without trophic eggs were formed in exactly the same way. No viable eggs were introduced in these colonies, but all first instar larvae have been introduced in the same way, at the same time, and with random assignment. We have clarified this in the Material and Method section.

> *Line 77: Before placing the freshly hatched larvae in recipient colonies, how long were the recipient colonies kept without eggs and how long were they fed before giving the eggs? Were they kept long enough without the queen to avoid possible effects of trophic eggs, or too long so that their behavior changed?*

The recipient colonies were created 7 to 10 days before receiving the first larvae and were fed ad libitum with grass seeds, flies and honey water from the beginning. Trophic eggs that would have been left over from the source colony should have been eaten within the first few days after creating the recipient colonies. However, even if some trophic eggs would have remained, this would not influence our conclusion that trophic eggs influence caste fate, given the fully randomized nature of our treatments and the considerable number of independent replicates. The same applies to potential changes in worker behavior following their isolation from the queen.

> *Line 77: Is it known at what stage caste determination occurs in this species? Here first instar larvae were given trophic eggs or not. Does caste-determination occur at the first*

> *instar stage? If not, what effect could providing trophic eggs at other stages have on caste-determination?*

A previous study showed that there is a maternal effect on caste determination in the focal species (Schwander et al 2008). The mechanism underlying this maternal effect was hypothesized to be differential maternal provisioning of viable eggs. However, as we detail in the discussion, the new data presented in our study suggests that the mechanism is in fact a different abundance of trophic eggs laid by queens. There is currently no information when exactly caste determination occurs during development

> *Comments on results:*
>
> *Line 65: How does investigating the order of eggs laid help to "inform on the mechanisms of oogenesis"?*

We agree that the aim was not to study the mechanism of oogenesis. We have changed this sentence accordingly: “To assess whether viable and trophic eggs were laid in a random order, or whether eggs of a given type were laid in clusters, we isolated 11 queens for 10 hours, eight times over three weeks, and collected every hour the eggs laid”

> *Figure 2: There is no description/discussion of data shown in panels B, C, E, and F in the main text.*

We have added information in the main text that while viable eggs showed embryonic development at 25 and 65 hours (Fig 12 B, C) there was no such development for trophic eggs (Fig. 2 E,F).

> *Line 172: Please explain hibernation details and its significance on colony development/life cycle.*

We have added this information in the Material and Method section.

> *Figure 6: How is B plotted? How could 0% of gynes have 100% survival?*

The survival is given for the larvae without considering caste. We have changed the de X axis of panel B and reworded the Figure legend to clarify this.

> *Is reduced DNA content just an outcome of reduced cell number within trophic eggs, i.e., was this a difference in cell type or cell number? Or is it some other adaptive reason?*

It is likely to be due to a reduction in cell number (trophic eggs have maternal DNA in the chorion, while viable eggs have in addition the cells from the developing zygote) but we do not have data to make this point.

> *Is there a logical sequence to the sequence of egg production? The authors showed that the sequence is non-random, but can they identify in what way? What would the biological significance be?*

We could not identify a logical sequence. Plausibly, the production of the two types of eggs implies some changes in the metabolic processes during egg production resulting in queens producing batches of either viable or trophic eggs. This would be an interesting question to study, but this is beyond the scope of this paper.

> *Figure 6b is difficult to follow, and more generally, legends for all figures can be made clearer and more easy to follow.*

We agree. We have now improved the legends of Fig 6B and the other figures.

> *Lines 172-174: "The percentage of eggs that were trophic was higher before hibernation…than after. This higher percentage was due to a reduced number of reproductive eggs, the number of trophic eggs laid remained stable" - are these data shown? It would be nice to see how the total egglaying rate changes after hibernation. Also, is the proportion of trophic eggs laid similar between individual queens?*

No the data were not shown and we do not have excellent data to make this point. We have therefore removed the sentence “This higher percentage was due to a reduced number of reproductive eggs, the number of trophic eggs laid remained stable” from the manuscript.

> *Figure 6B: Do several colonies produce 100% gynes despite receiving trophic eggs? It would be interesting if the authors discussed why this might occur (e.g., the larvae are already fully determined to be queens and not responsive to whatever signal is in the trophic eggs).*

The reviewer is correct that 4 colonies produced 100% gynes despite receiving trophic eggs. However, the number of individuals produced in these four colonies was small (2,1,2,1, see supplementary Table 2). So, it is likely that it is just by chance that these colonies produced only gynes.

> *Figure 5: Why a separation by "size distribution variation of miRNA"? What is the relevance of looking at size distributions as opposed to levels?*

We did that because there many different miRNA species, reflected by the fact that there is not just one size peak but multiple one. This is why we looked at size distribution

> *Figure 2: The image of the viable embryo is not clear. If possible, redo the viable to show better quality images.*

Unfortunately, we do not anymore have colonies in the laboratory so this is not possible.

> *Comments on discussion:*
>
> *Lines 236-247: Can an explanation be provided as to why the effect of trophic eggs in P. rugosus is the opposite of those observed by studies referenced in this section? Could P. rugosus have any life history traits that might explain this observation?*

In the two mentioned studies there were other factors that co-varied with variation in the quantity of trophic eggs. We mentioned that and suggested that it would be useful to conduct experimental manipulation of the quantity of trophic eggs in the Argentine ant and *P. barbatus* (the two species where an effect of trophic eggs had been suggested).

> *The discussion should include implications and future research of the discovery.*

We made some suggestions of experiments that should be performed in the future

> *The conclusion paragraph is too short and does not represent what was discussed.*

We added two sentences at the end of the paragraph to make suggestions of future studies that could be performed.

> *Lines 231 to 247: Drastically reduce and move this whole part to the introduction to substantiate the assumption that trophic eggs play a nutritional role.*

We moved most of this paragraph to the introduction, as suggested by the reviewer.

> ***Reviewer #3 (Recommendations For The Authors):***
>
> *I would like to commend the authors on their study. The main findings of the paper are individually solid and provide novel insight into caste determination and the nature of trophic eggs. However, the inferences made from much of the data and connections between independent lines of evidence often extend too far and are unsubstantiated.*

We thank the reviewer for the positive comment. We made many changes in the manuscript to improve the discussion of our results.

https://doi.org/10.7554/eLife.86899.3.sa0