

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

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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 reproductive 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.

eLife assessment

This work is a **valuable** advance in our understanding of a very interesting biological phenomenon: the role of trophic eggs in ant caste determination. The evidence supporting the unexpected finding that feeding larvae trophic eggs promotes the development of the non-reproductive caste is **compelling**. However, even though there is a description of how trophic and fertile eggs differ in composition, the evidence of its significance for caste determination is **incomplete**.

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), where they are also thought to mostly or only serve as food for the offspring (Crespi 1977). Although they are believed to be generally laid by workers, they can also be laid by the queens (see **Figure 1** and Supplementary Table 1). While conducting egg cross fostering experiments in the ant *Pogonomyrmex rugosus* to study worker size variation, we observed a sudden increase in the frequency of females developing into queens. During these experiments, we only cross fostered reproductive eggs, raising the possibility that the absence of trophic eggs influenced the process of caste determination. Similarly, changes in the ratio of trophic and reproductive eggs has been proposed as a possible explanation for changes in worker size in another *Pogonomyrmex* species following treatments with a JH analogue (Helms Cahan *et al.* 2011). Because these treatments resulted in an increased proportion of trophic eggs, the authors proposed that the presence of trophic eggs might have altered the nutritional environment of developing larvae, thereby leading to increased worker size. These observations prompted us to investigate whether trophic eggs play a role in caste determination in *P. rugosus*. Our experiments show that the presence of trophic eggs reduces the probability that female larvae develop into reproductive individuals. Metabolomic analyses also revealed profound differences between reproductive and trophic eggs, including in the composition of miRNAs and content of protein, triglycerides, glycogen, and sugar.

Materials and methods

Pogonomyrmex rugosus colonies were initiated by queens collected after mating flights in 2008 (Bowie, Arizona, USA) or 2013 (Bowie and Florence, Arizona, USA). The colonies were maintained at 28°C and 60% humidity, with a 12-h/12-h light:dark cycle and fed 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 the percentage of trophic eggs and from February to December 2021 for the egg content analysis.

Trophic and reproductive 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 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 reproductive.

To determine whether queens lay variable percentages of trophic eggs over time, we isolated 43 *P. rugosus* queens for 8 hours every day for 2 weeks, before and after hibernation, and counted the number of trophic and reproductive eggs laid (see results for how to discriminate the two types of eggs). The percentage of trophic eggs was compared using a linear mixed effect model within Rstudio (RStudio Team 2015), with before vs after hibernation as the explanatory variable and colony as a random factor.

To inform on the mechanisms of oogenesis, we assessed whether reproductive and trophic eggs were laid in a random order, or whether eggs of a given type were laid in clusters. To this end, we isolated 11 queens for 10 hours, eight times over three weeks, and collected every hour the eggs laid. To determine whether reproductive and trophic eggs were laid in a random order, we used

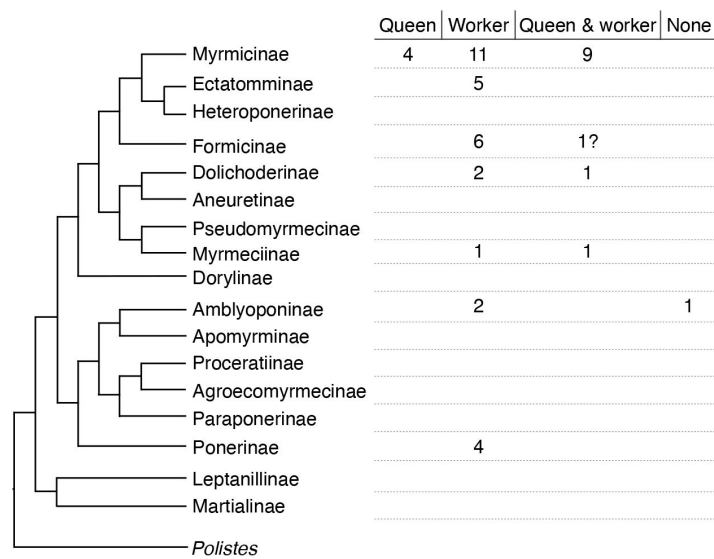


Figure 1.

Trophic egg production is widespread in ants. Simplified phylogenetic tree of ant families redrawn after Romiguier *et al.* (2022) <https://doi.org/10.1093/aegres/taab011>. The number of species with documented trophic egg production by queens, workers or both castes, as well as absence of trophic eggs, is indicated for each family. Details on the species and related references can be found in Supplementary Table 1.

Rstudio (package *snpar* v.1.0; [RStudio Team 2015](#)) to perform a Wald–Wolfowitz runs test for each queen’s egg laying sequence (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, half of the larvae were then transferred into a recipient colony containing 20 workers, while the other half of the larvae were placed in identical recipient colonies, which received in addition three 0–4 hours-old trophic eggs. 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 twice a week with grass seeds, flies and 20% honey water. The caste of each 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 Rstudio ([RStudio Team 2015](#)) and the package *lme4* ([Bates et al. 2015](#)) to perform a binomial generalized linear mixed effects analysis (GLMM) 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 reproductive eggs

The volumes of trophic (n=11) and reproductive eggs (n=14) were estimated by using the volume of an ellipse ($\frac{4}{3} \times \pi \times \text{egg length} \times (\frac{\text{egg width}}{2})^2$), 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 reproductive 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 ([Suzzoni et al. 1979](#); [Schwander et al. 2008a](#); [Helms Cahan et al. 2011](#); [Cameron et al. 2013](#); [Guo et al. 2013](#); [Libbrecht et al. 2013](#); [Søvik et al. 2015](#); [Collins et al. 2017](#)). 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 reproductive 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 reproductive and trophic egg pools (i.e., 24 replicates in total).

Triglycerides, glycogen and glucose were quantified as described in [Tennesen 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 reproductive and trophic eggs with Rstudio ([RStudio Team 2015](#)) and the package *lme4* ([Bates *et al.* 2015](#)) using a linear mixed effects analysis (LMER), 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 (>200nt) concentrations were compared between reproductive and trophic eggs with paired-t-tests in Rstudio ([RStudio Team 2015](#)); 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 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 reproductive 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

H2>Trophic and reproductive egg characteristics

P. rugosus queens lay two types of eggs that are morphologically different. Reproductive 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 ($94.3 \pm 4.3 \text{ nL}$; $n=11$) than reproductive eggs ($n=14$; $63.3 \pm 1.6 \text{ nL}$; two-sample t-test, $t(23) = -9.54$, $p = 1.8 \times 10^{-09}$). *P. rugosus* workers only laid reproductive 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 ($61.6 \pm 1.4\%$ mean $\pm$ SE; $n=43$ colonies) than after ($50.3 \pm 2.0\%$; LMER, $t(86)=5.04$, $p=9 \times 10^{-6}$). This higher percentage was due to a reduced number of reproductive eggs, the number of trophic eggs laid remained stable. 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 reproductive (6.1 ± 0.7 ; mean number per sequence $\pm$ SE) or trophic eggs (6.0 ± 0.5 ; **Figure 3** [↗](#)).

The concentrations of protein, triglycerides, glycogen, and glucose were significantly higher in reproductive 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 reproductive eggs ($44.3 \pm 1.4 \text{ ng}$, mean $\pm$ SE) than in trophic eggs ($22.3 \pm 1.1 \text{ ng}$; paired-t-test, $t_{(23)} = 15.9$, $p = 6.5 \times 10^{-14}$). The same was true for longer RNAs (>200 nt; reproductive eggs: $7.6 \pm 0.6 \text{ ng}$, mean $\pm$ SE; trophic eggs: $3.6 \pm 0.3 \text{ ng}$; paired-t-test, $t_{(23)} = 7.2$, $p = 2.7 \times 10^{-7}$).

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

There was a significant difference in the miRNA fragment size distribution between reproductive and trophic eggs (Mantel test, $r_M = 0.26$, $p < 0.0001$), 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 ($27 \pm 9\%$ mean $\pm$ SE; $n=22$) than in recipient colonies without trophic eggs ($83 \pm 10\%$; $n=22$; binomial GLMM, $z = 4.25$, $p = 2 \times 10^{-5}$; **Figure 6A** [↗](#) and Supplementary Table 2). The survival of larvae until the pupal stage was also significantly lower in the colonies without trophic eggs ($16.9 \pm 3.8\%$; $n=22$; LMER, $z = 2.66$, $p = 0.008$) than in colonies with trophic eggs

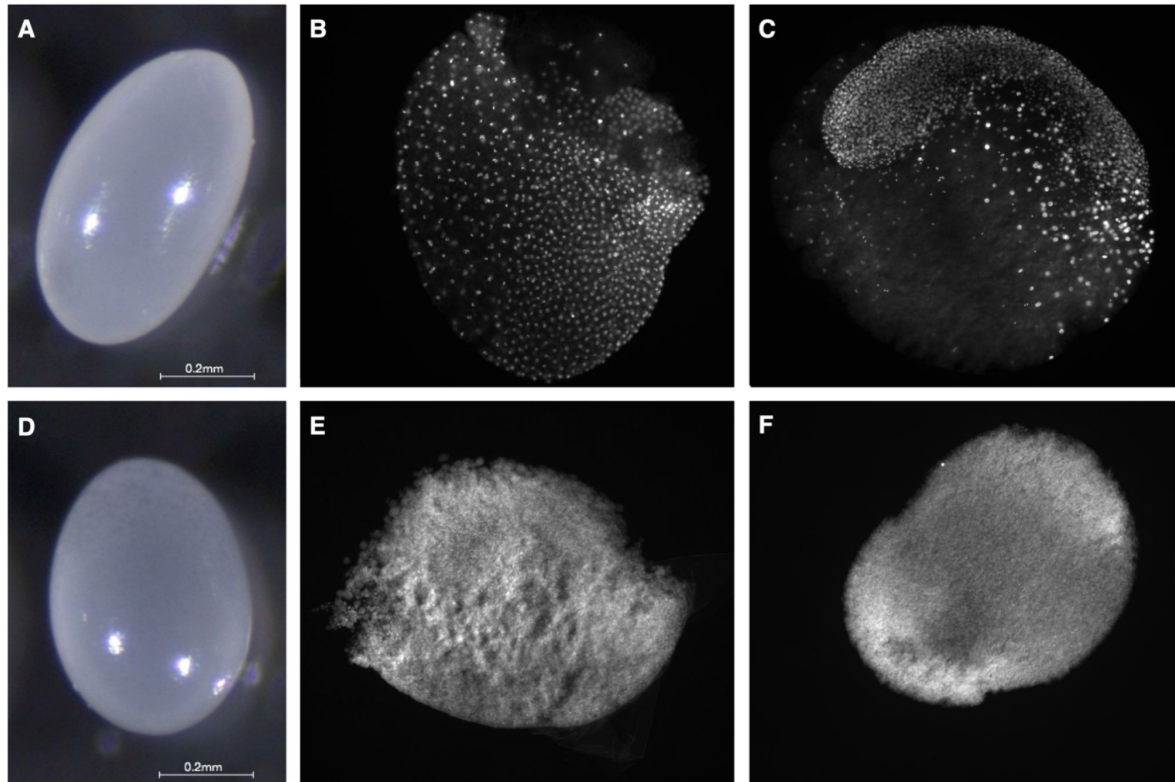


Figure 2.

Morphology and development of eggs laid by *P. rugosus* queens. Reproductive egg general morphology (**A**), with embryonic development at approximately 25 hours (**B**) and 65 hours (**C**). In trophic eggs (**D**), there is no embryonic development at 25 hours (**E**) nor at 65 hours (**F**). Panels B, C, E, F represent fluorescence images with DAPI-counterstained nuclei.

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

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 reproductive and trophic eggs in a random sequence.

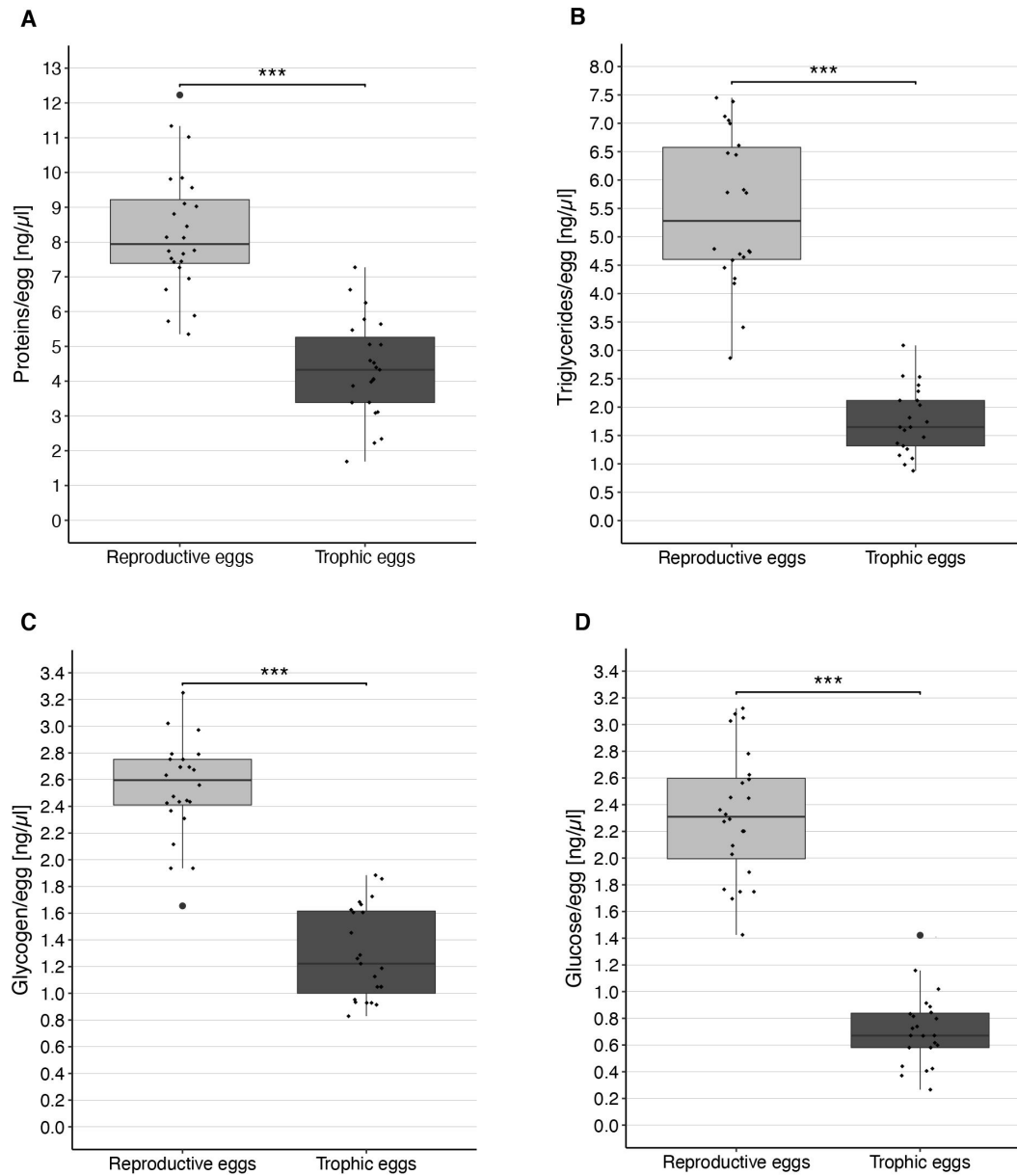


Figure 4.

Concentration of protein (A), triglycerides (B), glycogen (C) and glucose (D) in reproductive and trophic eggs. Each dot represents the average of the two replicates per colony.

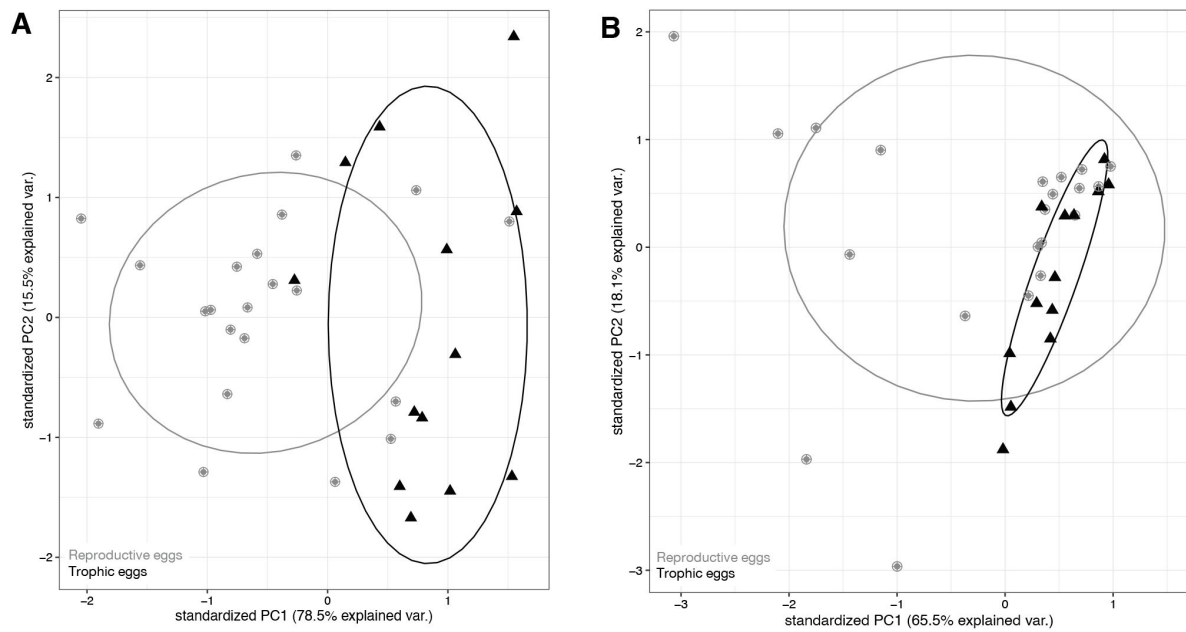


Figure 5.

First two principal components (PC1 and PC2) explaining size distribution variation for **(A)** miRNA and **(B)** tRNA across egg samples, with reproductive eggs in grey and trophic eggs in black. There is a separation of the samples by egg types for miRNAs, but not for tRNA. Ellipses enclose each of the egg type groups.

($30.2 \pm 6.7\%$; $\text{mean} \pm \text{SE}$; $n=22$), 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 an 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 reproductive eggs. First, trophic eggs are larger, rounder, have a smoother surface, a more granular looking content as well as a more fragile chorion than reproductive eggs. Similar differences between trophic and reproductive 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 that trophic eggs are solely laid by queens; *P. rugosus* workers are able to produce reproductive 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, 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 trophic eggs (laid by workers) fed to the larvae as well as a decrease in the proportion of larvae developing into queens (Bartels 1988). Bartels thus proposed that the deprivation of trophic eggs may have an inhibitory effect on the probability of larvae to develop into queens (Bartels 1988), but no experiment was performed to show a causal effect of trophic eggs. In some lineages of *P. barbatus*, the experimental increase of maternal juvenile hormone resulted in a 50% increase in worker body size, as well as a sharp reduction in total number of progeny reared and a higher proportion of trophic eggs laid by queens (Helms Cahan *et al.* 2011). This was interpreted as an effect of trophic egg availability or brood/worker ratio on the nutritional environment. Importantly, in these two studies the consumption of trophic eggs was suggested to either increase the size of the individuals produced or the likelihood to develop into queens (which are larger than workers). By contrast, our study reveals that the consumption of trophic eggs reduces the likelihood of developing into queens.

Our analyses revealed that trophic eggs have a lower content of protein, triglycerides, glycogen, and glucose than reproductive eggs. A reduced protein content of trophic as compared to reproductive 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 nutritive function as it might then be expected that they should at least contain as much nutrients as reproductive 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 differentiation into queen, but rather the worker jelly which stimulates the development of larva into worker. 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

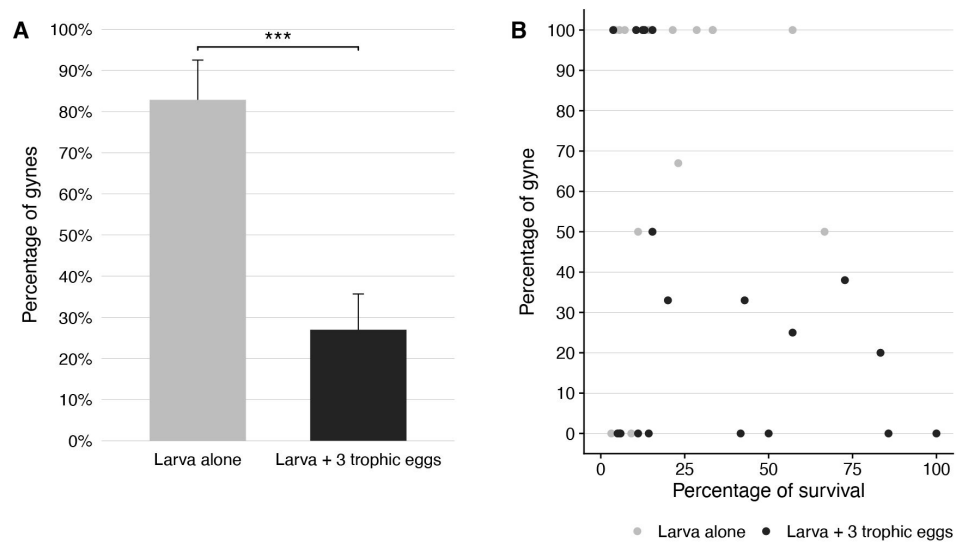


Figure 6.

(A) Percentage of larvae which developed into queens in recipient colonies without (grey) or with (black) trophic eggs. (B) Scatterplot of the relationship between the percentage of larvae which developed into queens and the percentage of survival from larvae to pupae.

Camponotus floridanus contains non-digestion 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.

Finally, our analyses also revealed seasonal differences in the proportion of reproductive 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 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) reproductive 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 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 play an important role in the general process of caste determination.

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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.

- <https://doi.org/10.7554/eLife.86899.1.sa2>

Reviewer #2 (Public Review):

The manuscript by Genzoni et al. provides evidence that trophic eggs laid by the queen in the ant *Pogonomyrmex rugosus* 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.

- <https://doi.org/10.7554/eLife.86899.1.sa1>

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 intra-colony 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.

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