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 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.

Keyword: Trophic eggs, *Pogonomyrmex rugosus* ant, caste determination, miRNA, triglycerides, glycogen, proteins

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.

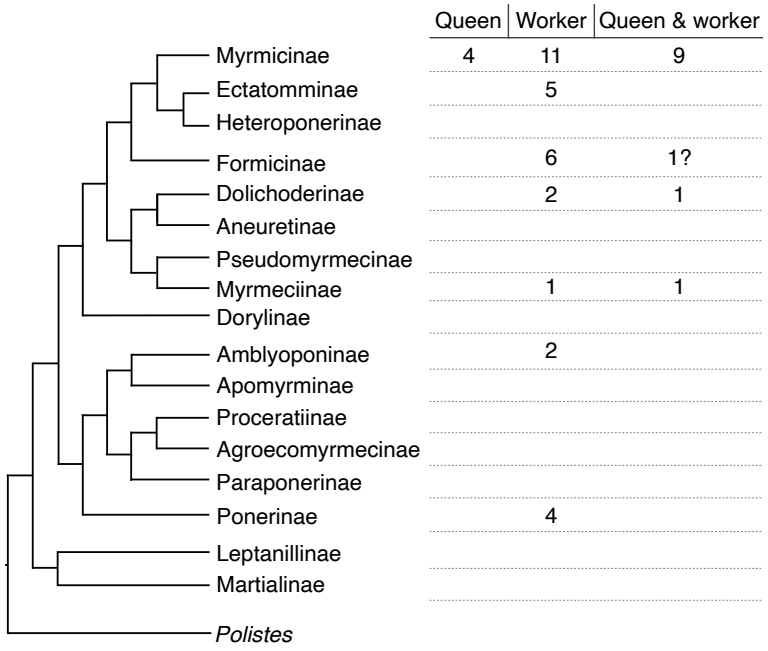


Figure 1. Trophic egg production is widespread in ants. Simplified phylogenetic tree of ant subfamilies redrawn after Romiguier *et al.* (2022). The number of species with documented trophic egg production by queens, workers or both castes is indicated for each subfamily. The question mark indicates 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).

105 Trophic and viable egg production

106 To verify that workers do not lay trophic eggs, as previously shown for other *Pogonomyrmex* species
107 (Supplementary Table 1), we created 12 queenless colonies (by removing the queen) and waited
108 approximately three weeks until workers started laying eggs. From each of these colonies, we isolated
109 two groups of five workers for 12 hours every two days for two weeks in November 2020 to obtain eggs.
110 Collected eggs were then placed for 10 days in a petri dish containing a water reservoir to study their
111 development and distinguish whether they were trophic or viable.
112
113 To determine whether queens laid variable percentages of trophic eggs over time, we isolated each of 43
114 *P. rugosus* queens for 8 hours every day for 2 weeks, before and after hibernation, and counted the
115 number of trophic and viable eggs laid (see results for how to discriminate the two types of eggs). We
116 hibernated the queens because we previously showed that hibernation is important to trigger the
117 production of gynes in *P. rugosus* colonies in the laboratory (Schwander *et al.* 2008; Libbrecht *et al.*
118 2013). Hibernation conditions were as described in Libbrecht *et al*. (2013). The percentage of trophic
119 eggs was compared using a linear mixed effect model with before vs after hibernation as the explanatory
120 variable and colony as a random factor.
121
122 To assess whether viable and trophic eggs were laid in a random order, or whether eggs of a given type
123 were laid in clusters, we isolated 11 queens for 10 hours, eight times over three weeks, and collected
124 every hour the eggs laid. To determine whether viable and trophic eggs were laid in a random order, we
125 performed a Wald–Wolfowitz runs test for each queen's egg laying sequence (package *snpar* v.1.0; this
126 non-parametric test calculates the likelihood that a binomial data sequence is random).
127

128 Trophic egg influence on the larval caste fate

129 To determine whether trophic eggs influence the process of caste determination, we compared the
130 development of freshly hatched (first instar) larvae placed in small recipient colonies with and without
131 trophic eggs. From each of 22 donor colonies, we obtained approximately 30 freshly hatched larvae by
132 isolating the queens for 16 hours (from 2pm to 6am) every day for three weeks (in October 2020), with
133 a 24-hour break every three days. Eggs were collected every eight hours and placed during 10 days in a
134 petri-dish with a water reservoir ensuring a high humidity until they hatched. After hatching, for each
135 colony, half of the larvae (i.e., 15 larvae) were then transferred into a recipient colony containing 20
136 workers, while the other half were placed in identical recipient colonies, which received in addition 45
137 0-4 hours-old trophic eggs (i.e., there were 3 trophic eggs per larva). There was no cross-fostering

138 between colonies, so that larvae were always placed in recipient colonies containing workers from the
139 same donor colony. The recipient colonies were maintained at 28°C and 60% humidity, with a 12-h/12-
140 h light:dark cycle and fed *ad libitum* twice a week with grass seeds, flies and 20% honey water. The caste
141 of each newly-produced individual was recorded at the pupal stage. To compare the proportion of queen
142 pupae produced between recipient colonies with and without trophic eggs, we used the package *lme4*
143 (Bates *et al.* 2015) to perform a binomial generalized linear mixed effects analysis (GLMM/link="logit")
144 fit by maximum likelihood, with caste as response variable (binary categorical factor) and
145 presence/absence of trophic eggs as an explanatory variable. Donor colony was included as a random
146 effect. To test whether the presence of trophic eggs affects survival, we performed a linear mixed effect
147 analysis with mortality as a response variable, presence/absence of trophic eggs as explanatory variable,
148 and colonies as random effects. As we found a significantly higher survival of larvae in recipient colonies
149 with trophic eggs than recipient colonies without trophic eggs (see results), we tested whether the
150 percentage of larvae developing into queens was correlated with survival by performing a linear mixed
151 effects analysis with the percentage of queen pupae as response variable, the survival as an explanatory
152 variable and colonies as a random factor.
153

154 Volume and content of trophic and viable eggs

155 The volumes of trophic (n=11) and viable eggs (n=14) were estimated by using the volume of an ellipse
156 ($\frac{4}{3} \times \pi \times egg\ length \times \left(\frac{egg\ width}{2}\right)^2$), with egg length and width estimated on images under 10x
157 magnification using ZEN Microscopy Software (v. 1.1.2.0).
158

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

169 Triglycerides, glycogen and glucose were quantified as described in Tennessen *et al.* (2014), and protein
170 levels were measured using a Bradford assay (Bradford 1976). The 20 one-hour old eggs per sample
171 were homogenized with beads in 200µl of PBS buffer in a Precellys Evolution tissue homogenizer
172 coupled with a Cryolys Evolution (Bertin Technologies SAS).
173
174 For the Bradford assay, 10µl of the homogenate were put in a clear-bottom 96-well plate with 300µl of
175 Coomassie Plus Reagent (Thermo Scientific: 23200) and incubated for 10 minutes at room temperature.
176 Protein standard (Sigma: P5369) was used as standard (ranging from 0-0.5mg/ml) and protein absorbance
177 was read at 595nm on a Hidex Sense Microplate Reader.
178
179 For the triglycerides assay, 90µl of homogenate were heat treated at 70°C for 10 minutes, then 40µl were
180 mixed with 40µl of Triglyceride Reagent (Sigma: T2449) for digestion and 40µl were mixed with PBS
181 buffer for free glycerol measurement. After 30 minutes incubation at 37°C, 30µl of each sample and
182 standards were transferred to clear-bottom 96-well plate. 100µl of Free Glycerol Reagent (Sigma: F6428)
183 was added to each sample, mixed well by pipetting, and incubated five minutes at 37°C. Glycerol
184 standard solution (Sigma: G7793) was used as standard (ranging from 0-1.0mg/ml TAG) and absorbance
185 was read at 540nm on a Hidex Sense Microplate Reader. The triglycerides concentration in each sample
186 was determined by subtracting the absorbance of free glycerol in the corresponding sample.
187
188 Glucose and glycogen were quantified as in Tennessen *et al.* (2014). A 90µl aliquot was heat treated at
189 70°C for 10min and then diluted 1:2 with PBS. The standard curves for glucose (Sigma, G6918) and
190 glycogen (Sigma: G0885) were made by diluting stocks to 160µg/ml, making 1:1 serial dilution for 160,
191 80, 40, 20 and 10µg/ml. 40µl of each sample was pipetted in duplicates of a clear microplate, and 30µl
192 of each glucose or glycogen standard was pipetted in duplicates. Amyloglucosidase enzyme (Sigma,
193 A1602) was diluted 3µl into 2000µl of PBS, and 40µl diluted enzyme was pipetted to the glycogen
194 standards and to one well of the sample (for total glucose determination), 40µl PBS was pipetted to the
195 glucose standards and to the other sample well (for free glucose determination). The plate was incubated
196 at 37°C for 60 minutes. 30µl of each standard and samples (in duplicates) were transferred to a UV 96-
197 well plate and 100µl Glucose Assay Reagent (G3293) was pipetted to each well. The plate was incubated
198 at room temperature for 15 minutes and the absorbance was read at 340nm on a Hidex Sense Microplate
199 Reader. The glycogen concentration was quantified by subtracting the free glucose absorbance from the
200 total glycogen + glucose absorbance.
201

202 Concentrations of each compound (protein, triglycerides, glycogen, and glucose) were compared
203 between viable and trophic eggs using a linear mixed effects analysis (LMER; package *lme4*), with the
204 concentration as response variable and egg type as explanatory variable. Colony and extraction batch
205 were added as random effects in the model.
206

207 Total and small RNA, and DNA

208 RNA (>200 nt) and small RNA were isolated using the miRNeasy Mini Kit (Qiagen, cat. no. 217004)
209 and RNeasy® MinElute® Cleanup Kit (Qiagen, cat. no. 74204), respectively, following manufacturer
210 instructions. RNA (>200 nt) and small RNA concentrations were measured with a QuantiFluor® RNA
211 System (Promega). RNA (>200 nt) integrity was examined with an Agilent Fragment Analyzer (at the
212 Lausanne Genomic Technologies Facility) using a High Sensitivity Assay and small RNA were
213 examined using the small RNA kit (at the Gene Expression Core Facility at EPFL).
214 The miRNA and RNA (>200 nt) concentrations were compared between viable and trophic eggs with
215 paired-t-tests (for each type of eggs we used the average of the two replicates per colony). We also
216 compared the fragment size distributions from 18 to 24 nucleotides for miRNAs (Sohel 2016) with a
217 PCA and a Mantel test.
218
219 DNA was extracted from pools of six eggs using TRIzol (Life Technologies). DNA concentration was
220 measured with a Nanodrop 3300 (ThermoFisher), and DNA integrity was examined with an Agilent
221 Fragment Analyzer (at the Lausanne Genomic Technologies Facility) using a High Sensitivity Assay.
222 DNA concentrations were compared between viable and trophic eggs using paired-t-tests (sample size is
223 5 for both types of eggs, each sample being a pool of 6 eggs).
224

225 Results

226 Trophic and viable egg characteristics

227 *P. rugosus* queens lay two types of eggs that are morphologically different. Viable eggs are white with a
228 bright surface and have a distinct oval shape, a homogenous content as well as a solid chorion (Figure
229 2A), while trophic eggs are rounder, have a smooth surface and a granular looking content as well as a
230 fragile chorion (Figure 2D). Trophic eggs had a significantly larger volume (94.3$\pm$4.3nL; n=11) than
231 viable eggs (n=14; 63.3$\pm$1.6nL; two-sample t-test, $t(23) = -9.54$, $p = 1.8\times10^{-09}$). While viable eggs
232 showed embryonic development at 25 and 65 hours (Fig 12B, C) there was no such development for
233 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.

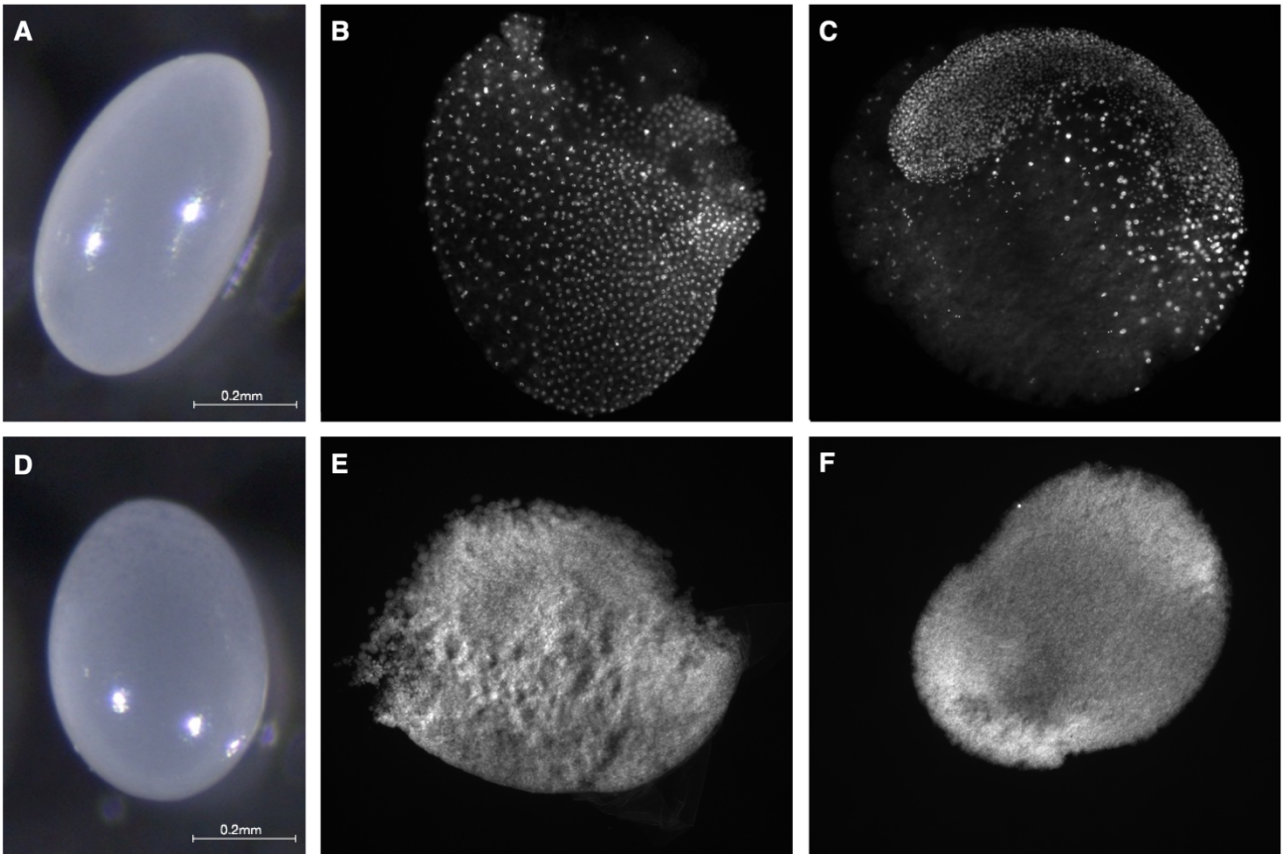
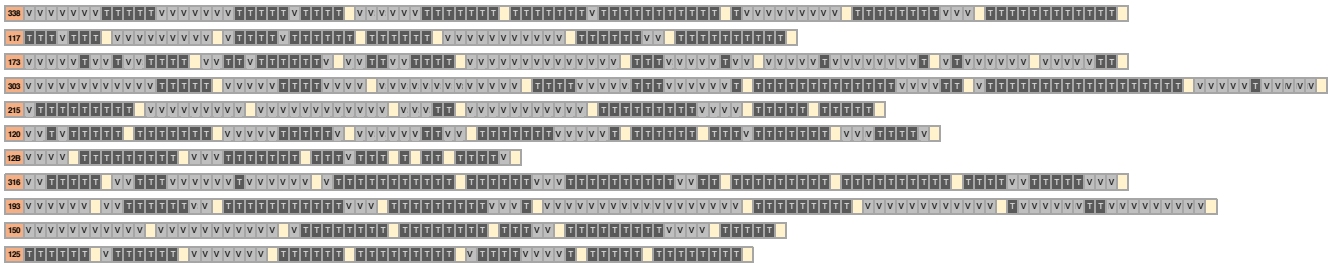


Figure 2. Morphology and of viable (**A**) and trophic (**D**) eggs laid by *P. rugosus* queens. Fluorescence images with DAPI-counterstained nuclei showing embryonic development of viable eggs at approximately 25 hours (**B**) and 65 hours (**C**). For trophic eggs there was no embryonic development at 25 hours (**E**) nor at 65 hours (**F**).

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=9x10^{-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).



249 **Figure 3.** Egg-laying sequences from eleven *P. rugosus* queens. Every row shows the sequence of viable (V) and trophic (T)
250 eggs laid by a given queen (queen ID in the orange cell). Each egg laying session lasted 10 hours. The yellow squares indicate
251 the intervals (16 hours to several days) between egg-laying sessions.
252
253 **Table 1.** Wald-Wolfowitz runs tests on the queen's egg sequence. Significant p-values (corrected for multiple testing) indicate
254 that queens do not lay viable and trophic eggs in a random sequence.
255

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

256
257 The concentrations of protein, triglycerides, glycogen, and glucose were significantly higher in viable
258 than trophic eggs (LMER, protein: $t = -13.11$, $p < 0.0001$; triglycerides: $t = -11.66$, $p < 0.0001$; glycogen:
259 $t = -11.98$, $p < 0.0001$; glucose: $t = -18.60$, $p < 0.0001$; Figure 4).

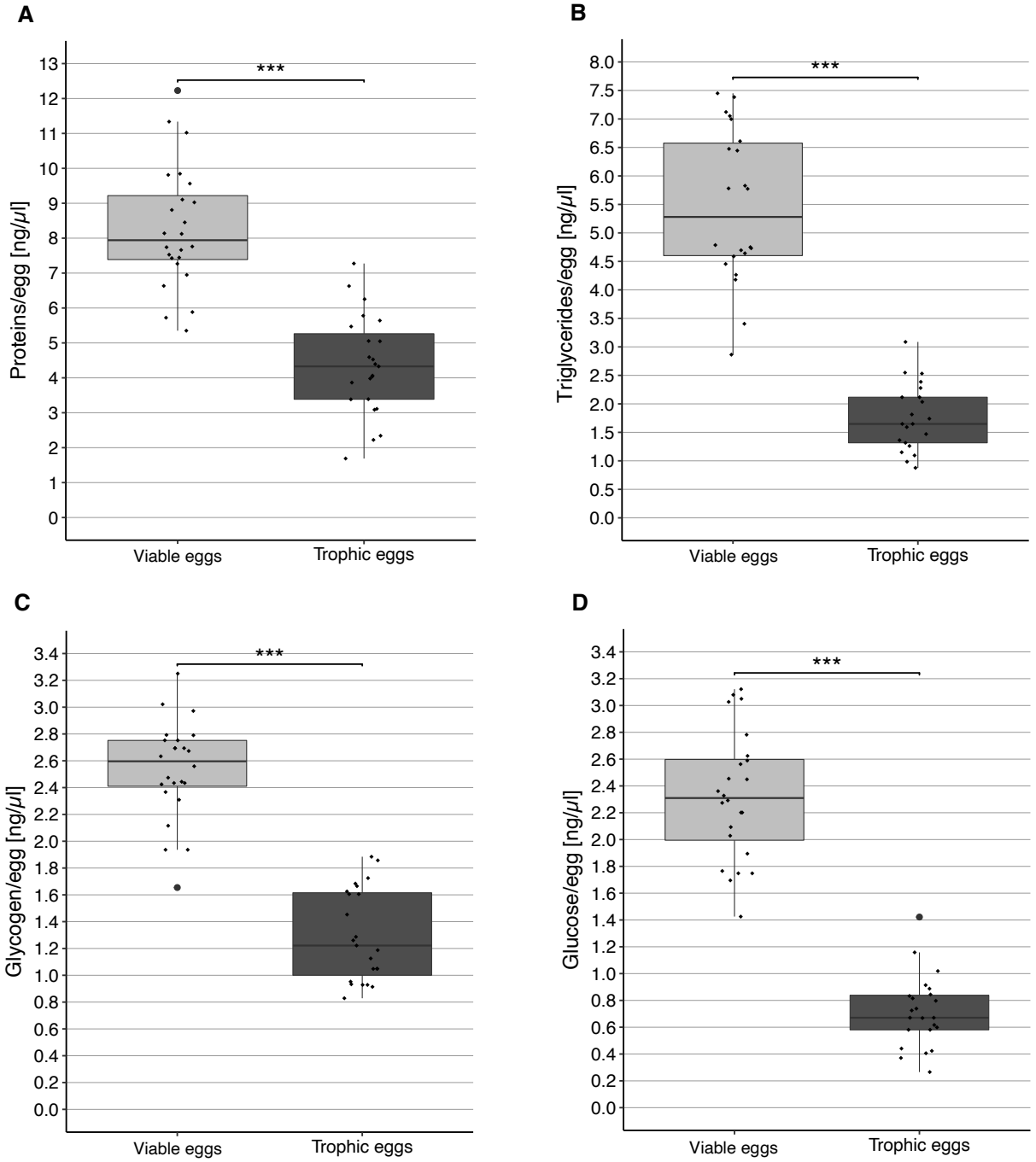


Figure 4. Concentration (± standard error) of protein (**A**), triglycerides (**B**), glycogen (**C**) and glucose (**D**) in viable and trophic eggs. Each dot represents the average of the two replicates per colony.

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).

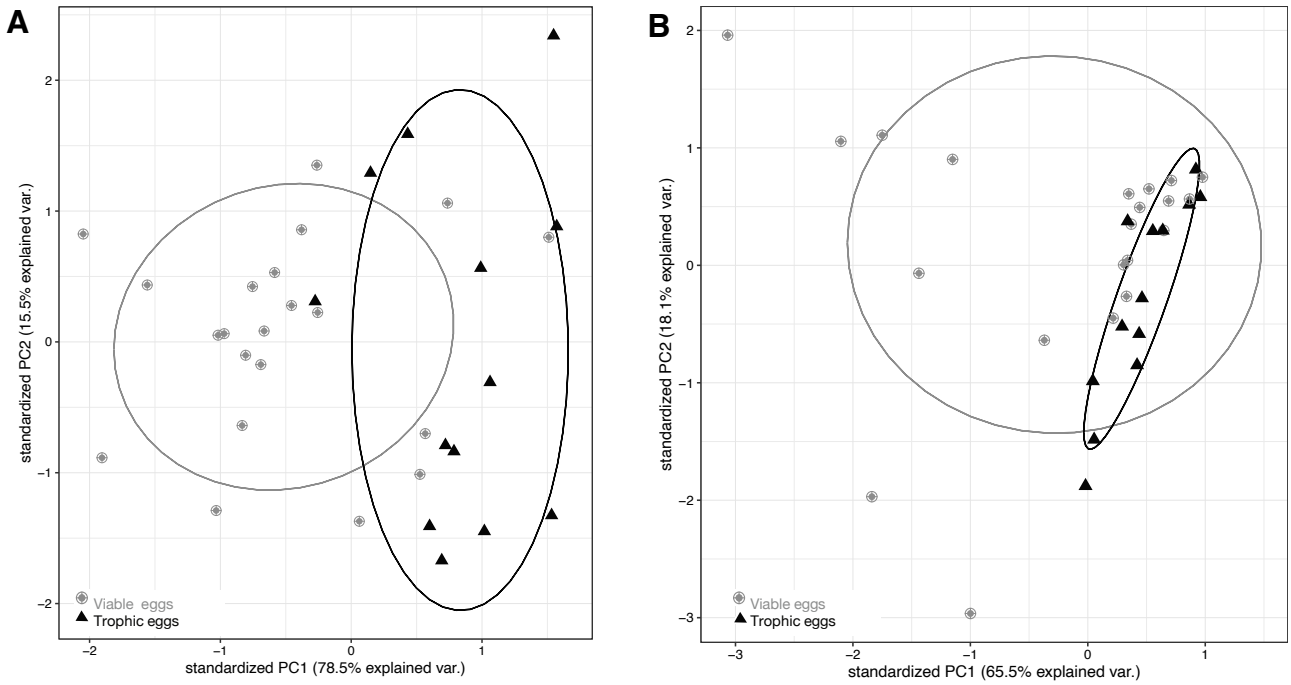


Figure 5. First two principal components (PC1 and PC2) explaining size distribution variation for (**A**) miRNA and (**B**) tRNA across egg samples, with viable eggs in grey dots and trophic eggs in black triangles. Ellipses enclose each of the egg type groups.

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"), $z = 4.25$, $p = 2x10^{-5}$; 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.

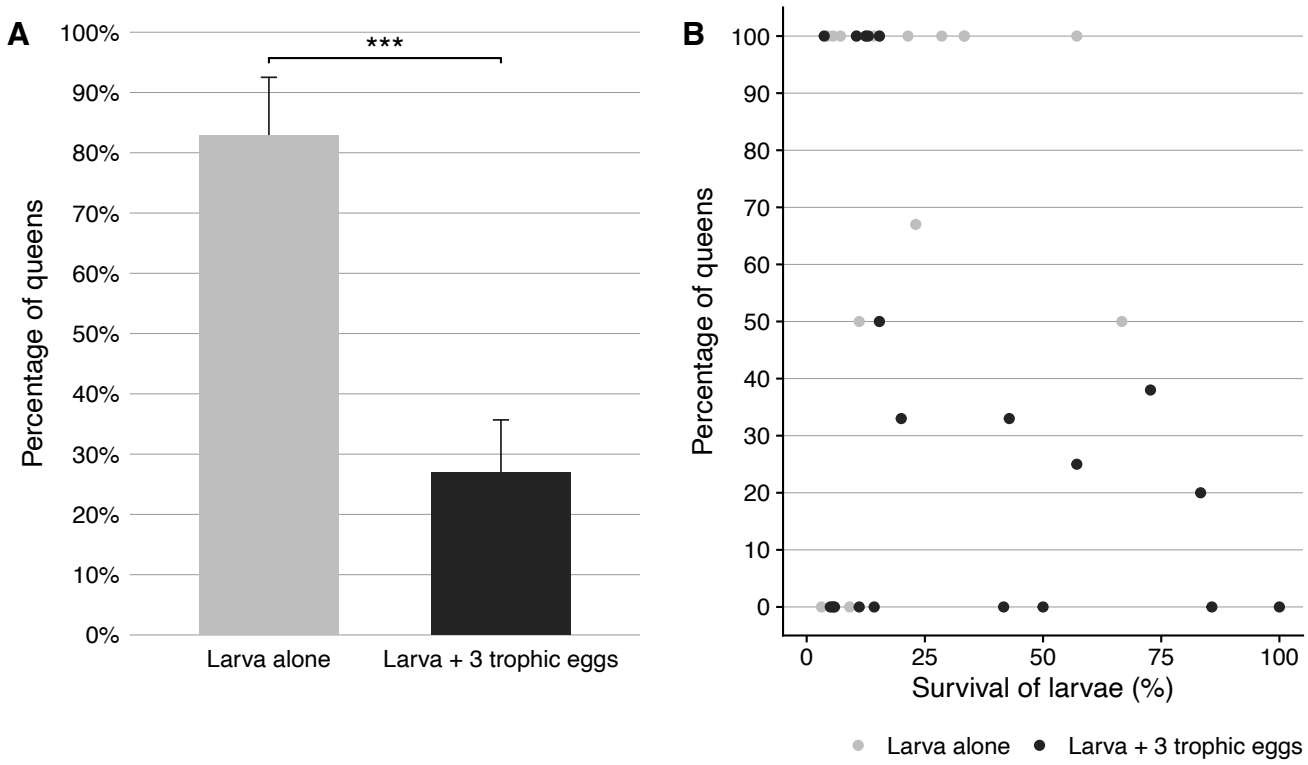


Figure 6. (**A**) Percentage (± standard error) of queens among the larvae that developed to the pupal stage in colonies without (grey) or with (black) trophic eggs. (**B**) Relationship between the percentage of larvae which developed into queens and the survival of larvae (percentage) between the larval to pupal stages.

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 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

309 into workers than larvae without access to trophic eggs. This was somewhat surprising because trophic
310 eggs are generally thought to be an important source of nutrients to the colony and, everything else being
311 equal, one would think that eating such eggs should increase the likelihood of females to develop into
312 queens (which are usually larger than workers). Indeed, two earlier studies suggested that an increase in
313 the proportion of trophic eggs might be associated with an increase in the proportion of larvae developing
314 into queens (*L. humile*: Bartels 1988; *P. barbatus*: Helms Cahan *et al.* 2011). However, in these two
315 studies variation in the availability of trophic eggs was associated with other differences (number of
316 queens in the colony, Bartels 1988; Administration of a JH analogue, Helms Cahan *et al.* 2011) making
317 it difficult to determine what factor had a causal effect. It would be interesting to experimentally
318 manipulate the quantity of trophic eggs in *L. humile* and *P. barbatus* to determine whether they have a
319 positive or inhibitory effect on the likelihood of larvae to develop into queens.
320
321 Our analyses revealed that trophic eggs have a lower content of protein, triglycerides, glycogen, and
322 glucose than viable eggs. A reduced protein content of trophic as compared to viable eggs has also been
323 documented in *Pheidole pallidula* (Lorber and Passera 1981). These findings are in line with the view
324 that trophic eggs do not simply have a nutritional function as it might then be expected that they should
325 at least contain as much nutrients as viable eggs. Interestingly, our analyses also revealed important
326 differences in RNA and miRNA content between the two egg types. miRNAs have already been
327 suggested to influence larval caste determination in the honeybee (Guo *et al.* 2013) with worker jelly
328 being enriched in miRNAs compared to royal jelly (Guo *et al.* 2013; Zhu *et al.* 2017). These studies
329 suggest that it is not the royal jelly that stimulates larval determination into queen, but rather the worker
330 jelly which stimulates the development of larvae into workers. Similarly, our study reveals that
331 compounds found in trophic eggs, perhaps miRNAs, influence larval development towards the worker
332 phenotype. Interestingly, it has also been recently shown that trophallactic fluid in the ant *Camponotus*
333 *floridanus* contains non-digestive related proteins, microRNAs and juvenile hormone (LeBoeuf *et al.*
334 2016). Moreover, comparison of trophallactic fluid proteins across social insect species revealed that
335 many are regulators of growth, development and behavioral maturation (Meurville and LeBoeuf 2021).
336 Finally, a recent study showed that pupae of several ant species produce secretions that play an important
337 role for early larval nutrition with young larvae exhibiting stunted growth and decreased survival without
338 access to the fluid (Snir *et al.* 2022). This raises the possibility that chemicals delivered in trophic eggs,
339 trophallactic fluids and pupae secretions play previously unsuspected roles in communication and caste
340 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.

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398 # References

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

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

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

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

410 Baroni Urbani, C. B. 1991. Indiscriminate oophagy by ant larvae: an explanation for brood serial
411 organization? *Insectes Soc.* **38**:229–239. doi.org/10.1007/BF01314909

412 Bartels, P. J. 1988. Reproductive caste inhibition by Argentine ant queens: New mechanisms of queen
413 control. *Insectes Soc.* **35**:70–81. doi.org/10.1007/BF02224139

414 Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using
415 lme4. *J. Stat. Softw.* **67**:1–48. doi.org/doi:10.18637/jss.v067.i01

416 Blake, J. A., and P. L. Arnofsky. 1999. Reproduction and larval development of the spioniform
417 Polychaeta with application to systematics and phylogeny. *Hydrobiologia*. **402**:57-106.
418 doi.org/10.1023/A

419 Bourke, A. F. G. 1988. Worker reproduction in the higher eusocial Hymenoptera. *Quart Rev Biol* **63:**
420 291–311.

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

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

426 Brian, M. V, Rigby, C. 1978. The trophic eggs of *Myrmica rubra* L. *Insect Molecular Biology.* **25**(1):89-
427 110.

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

430 Cameron, R. C., E. J. Duncan, and P. K. Dearden. 2013. Biased gene expression in early honeybee larval
431 development. *BMC Genomics*. **14**:1–12. BioMed Central. doi.org/10.1186/1471-2164-14-903

432 Cassill, D. 2002. Brood care strategies by newly mated monogyne *Solenopsis invicta* (Hymenoptera:
433 Formicidae) queens during colony founding. *Ann. Entomol. Soc. Am.* **95**:208–212.
434 doi.org/10.1603/0013-8746(2002)095[0208:BCSBNM]2.0.CO;2

435 Choe, J. C. 1988. Worker reproduction and social evolution in ants (Hymenoptera: Formicidae).
436 Advances in myrmecology. 163-187

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

439 Collins, D. H., I. Mohorianu, M. Beckers, V. Moulton, T. Dalmay, and Bourke A. F. 2017. MicroRNAs
440 Associated with Caste Determination and Determination in a Primitively Eusocial Insect. *Sci. Rep.*
441 **7**:1–9. Nature Publishing Group. doi.org/10.1038/srep45674

442 Crespi, B. J. 1977. Cannibalism and trophic eggs in subsocial and eusocial insects. Pp. 176–213 in M. A.
443 Elgar and B. J. Crespi, eds. Cannibalism: Ecology and Evolution Among Diverse Taxa.

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

447 Della Lucia, T. M. C., E. F. Vilela, D. D. O. Moreira, J. M. S. Bento, and N. Dos Anjos. 1990. Egg-
448 laying in Atta sexdens rubropilosa, under laboratory conditions. Pp. 173–179 in R. K. Vander Meer,
449 K. Jaffé, and A. Cedeno, eds. Applied Myrmecology: A World Perspective. Westview Press, New
450 York.

451 Dietemann, V., B. Hölldobler, and C. Peeters. 2002. Caste specialization and determination in
452 reproductive potential in the phylogenetically primitive ant *Myrmecia gulosa. Insectes Soc*.
453 **49**:289–298. doi.org/10.1007/s00040-002-8316-9

454 Dietemann, V., and C. Peeters. 2000. Queen influence on the shift from trophic to reproductive eggs laid
455 by workers of the ponerine ant *Pachycondyla apicalis*. *Insectes Soc.* **47**:223–228.
456 doi.org/10.1007/PL00001707

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

459 Fletcher, D. J. C., and K. G. Ross. 1985. Regulation of reproduction in Eusocial hymenoptera. *Annu.*
460 *Rev. Entomol.* **30**:319–343.

461 Freeland, J. 1958. Biological and social patterns in the Australian bulldog ants of the genus *Myrmecia*.
462 *Aust. J. Zool.* **6**:1–18. doi.org/https://doi.org/10.1071/ZO9580001

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

466 Gobin, B., J. Billen, and C. Peeters. 1999. Policing behaviour towards virgin egg layers in a polygynous
467 ponerine ant. *Anim. Behav.* **58**:1117–1122. doi.org/10.1006/anbe.1999.1245

468 Gobin, B., and F. Ito. 2000. Queens and major workers of *Acanthomyrmex ferox* redistribute nutrients
469 with trophic eggs. *Naturwissenschaften* **87**:323–326. doi.org/10.1007/s001140050731

470 Gobin, B., C. Peeters, and J. Billen. 1998. Production of trophic eggs by virgin workers in the ponerine
471 ant *Gnamptogenys menadensis. Physiol. Entomol*. **23**:329–336. doi.org/10.1046/j.1365-
472 3032.1998.00102.x

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

476 Hammond, R and L. Keller 2004. Conflict over male parentage in social insects. *PLoS Biology*, 2(9):
477 e248, 1472-1482.

478 Haskins, C.P., and Whelden R.M. 1965. "Queenlessness," Worker Sibship, and Colony Versus
479 Population Structure in the Formicid Genus Rhytidoponera'. *Psyche: A Journal of Entomology*.
480 **72**(ID 040465): 26 pages. doi.org/10.1155/1965/40465

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

483 Heinze, J., S. Foitzik, B. Oberstadt, O. Rüppell, and B. Hölldobler. 1999. A female caste specialized for
484 the production of unfertilized eggs in the ant *Crematogaster smithi*. *Naturwissenschaften*. **86**:93–
485 95. doi.org/10.1007/s001140050579

486 Helms Cahan, S., C. J. Graves, and C. S. Brent. 2011. Intergenerational effect of juvenile hormone on
487 offspring in *Pogonomyrmex harvester* ants. *J. Comp. Physiol. B*. **181**:991–999.
488 doi.org/10.1007/s00360-011-05Helms

Helms Cahan, S. and L. Keller L. 2003 Complex hybrid origin of genetic caste determination in harvester ants. *Nature.* **424**:306-309

Helms Cahan S., J.D. Parker, S.W. Rissing, R.A. Johnson, T.S. Polony, M.D. Weiser and D. R. Smith D.R. 2002. Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants**.** *Proc. R. Soc. Lond. B. Biol. Sci.* **269**:1871-1877

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

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

Hölldobler, B., and E. O. Wilson. 1990 The Ants. Cambridge, MA: Belknap Press of Harvard Univ. Press.

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

Huber, J. 1905. Uber die Koloniegründung bei *Atta sexdens*. *Biologisches Centralblatt* **25**:625–635.

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

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.

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. doi.org/10.1080/00222939300770751

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.

Julian, G.E. J.H., Fewell, J., Gadau J., R.A., Johnson R.A. and D. Larrabee D. 2002. Genetic determination of the queen caste in an ant hybrid zone. *Proc. Natl. Acad. Sci. USA.* **99**: 8157-8160

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

519 Khila, A., and E. Abouheif. 2008. Reproductive constraint is a developmental mechanism that maintains
520 social harmony in advanced ant societies. *Proc. Natl. Acad. Sci. U.S.A.* **105**:17884-17889.
521 doi.org/10.1073/pnas.0807351105

522 Khila, A., and E. Abouheif. 2010. Evaluating the role of reproductive constraints in ant social evolution.
523 *Philos. Trans. R. Soc. B.* **365**:617–630. doi.org/10.1098/rstb.2009.0257

524 Klowden, M. J. 2013. Developmental Systems. Pp. 149–196 in M. J. Klowden, ed. Physiological Systems
525 in Insects (Third Edition). Academic Press.

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

528 LeBoeuf, A. C., P. Waridel, C. S. Brent, A. N. Gonçalves, L. Menin, D. Ortiz, O. Riba-Grognuz, A.
529 Koto, Z. G. Soares, E. Privman, E. A. Miska, R. Benton, and L. Keller. 2016. Oral transfer of
530 chemical cues, growth proteins and hormones in social insects. *Elife*. **5:e20375**:1-27.
531 doi.org/10.7554/eLife.20375

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

535 Levin, L. A., and T. S. Bridges. 1995. Pattern and diversity in reproduction and development. Pp. 1–48
536 in L. McEdward, ed. Ecology of marine invertebrate larvae. CRC Press.
537 doi.org/10.1201/9780138758950-1

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

541 Linksvayer, T. A. 2006. Direct, maternal, and sibsocial genetic effects on individual and colony traits in
542 an ant. *Evolution.* **60**(12): 2552. doi.org/10.1554/06-011.1

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

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

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

De Menten, L., Fournier, D., Brent, C., Passera, L., Vargo, E.L., and Aron. S. 2005. Dual Mechanism of Queen Influence over Sex Ratio in the Ant *Pheidole Pallidula*. *Behavioral Ecology and Sociobiology.* **58**(6): 527-33. doi.org/10.1007/s00265-005-0964-0.

Meurville, M.-P., and LeBoeuf, A.C. 2021. Trophallaxis: The Functions and Evolution of Social Fluid Exchange in Ant Colonies (Hymeno Ptera: Formicidae). *Myrmecological News.* **31**:1-20. doi.org/doi: 10.25849/myrmecol.news_031:001 13

Nagano, T., and Fraser, P. 2011. No-Nonsense Functions for Long Noncoding RNAs. *Cell* **145**:178-181. doi.org/10.1016/J.CELL.2011.03.014

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.

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

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. doi.org/10.13102/sociobiology.v64i4.2092

Peeters, C. and 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

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

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**(13):2942-2947.e4. doi.org/10.1016/j.cub.2022.05.001

RStudio Team. 2015. RStudio: Integrated Development Environment for R. Boston, MA.

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. doi.org/10.1093/beheco/art067.

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.

Schwander, T., Cahan, S. H. & Keller, L. 200). Characterization and distribution of *Pogonomyrmex* harvester ant lineages with genetic caste determination. *Molecular Ecology* **16**:367–387.

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

584 Smeeton, L. 1981. The Source of Males in *Myrmica Rubra* L. (Hym. Formicidae). *Insectes Sociaux.*
585 **28**(3):263-78. doi.org/10.1007/BF02223628.

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

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

592 Smith, C. R., & Suarez, A. V. (**2010**). The trophic ecology of castes in harvester ant colonies. Functional
593 Ecology, **24**(1), 122-130. doi.org/10.1111/j.1365-2435.2009.01604.x

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

596 Snir, O., Alwaseem, H., Heissel, S. Sharma, A., Valdés-Rodríguez, S., Carroll, T.S., Jiang, C.S.,
597 Razzauti, J., Kronauer, D.J.C. 2022. The pupal moulting fluid has evolved social functions in ants.
598 *Nature*. **612**(7940):488-494. doi.org/10.1038/s41586-022-05480-9

599 Sohel, M. H. 2016. Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and
600 Challenges. *Achiev. Life Sci.* **10**:175–186. doi.org/10.1016/j.als.2016.11.007

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

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

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

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

611 Taylor, R.W. 1978. *Nothomyrmecia Macrops*: A Living-Fossil Ant Rediscovered. *Science.*
612 **201**(4360):979-85. doi.org/10.1126/science.201.4360.979

613 Tennessen, J. aso. M., W. E. Barry, J. Cox, and C. S. Thummel. 2014. Methods for studying metabolism
614 in *Drosophila*. *Methods* **68**:105–115. doi.org/10.1016/j.ymeth.2014.02.034

615 Torossian, C. 1968. Recherches Sur La Biologie et l'éthologie De *Dolichoderus Quadripunctatus* (L)
616 (Hym. Form. Dolichoderidæ). *Insectes Sociaux.* **15**(4):375-88

617 Volny, V. P., and D. M. Gordon. 2002. Genetic basis for queen-worker dimorphism in a social insect.
618 *Proc. Natl. Acad. Sci. USA.* **99**: 6108-6111

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

622 Voss, S. H., J. F. McDonald, and C. H. Keith. 1988. Production and abortive development of fire ant
623 trophic eggs. *Advances in myrmecology*. Pp. 517-534. ed. J. C. Trager. New York : E. J. Brill.

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

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

628 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.,
629 Zeng, Z.J. 2019. A Maternal Effect on Queen Production in Honeybees. *Current Biology.*
630 **29**(13):2208-2213.e3. doi.org/10.1016/j.cub.2019.05.059.

631 Wenseleers, T and F.L.W. Ratnieks. 2006 Comparative analysis supports relatedness theory for worker
632 policing in eusocial Hymenoptera. Am Nat. 168 :E163-79. doi: 10.1086/508619

633 Wheeler, W. M. 1910. Ants: Their Structure, Development, and Behavior. The Columbia University
634 Press, New York. doi.org/10.1155/1922/80849

635 Wilson, E. O. 1971. The insect societies. Harvard University Press, Cambridge, MA.

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

638 Yamada, A., F. Ito, R. Hashim, and K. Eguchi. 2018. Queen polymorphism in Acanthomyrmex
639 careoscrobis Moffett, 1986 in Peninsular Malaysia (Hymenoptera: Formicidae: Myrmicinae), with
640 descriptions of hitherto unknown female castes and males. *Asian Myrmecology.* **10**(e010009):1-
641 19. doi.org/10.20362/am.010009

642 Yamauchi, K., T. Furukawa, K. Kinomura, H. Takamine, and K. Tsuji. 1991. Secondary Polygyny by
643 Inbred Wingless Sexuals in the Dolichoderine Ant *Technomyrmex albipes*. *Behav. Ecol. Sociobiol*.
644 **29**:313–319.

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