Journal of Insect Physiology 70 (2014) 134–142

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

# The cost of being queen: Investment across *Pogonomyrmex* harvester ant gynes that differ in degree of claustrality

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ARTICLE INFO

*Article history:*
Received 3 March 2014
Received in revised form 28 July 2014
Accepted 31 July 2014
Available online 8 August 2014

*Keywords:*
*Pogonomyrmex*
Harvester ant
Parental investment
Colony founding
Claustral
Semi-claustral

ABSTRACT

The role of the ant colony largely consists of non-reproductive tasks, such as foraging, tending brood, and defense. However, workers are vitally linked to reproduction through their provisioning of sexual offspring, which are produced annually to mate and initiate new colonies. Gynes (future queens) have size-associated variation in colony founding strategy (claustrality), with each strategy requiring different energetic investments from their natal colony. We compared the per capita production cost required for semi-claustral, facultative, and claustral gynes across four species of *Pogonomyrmex* harvester ants. We found that the claustral founding strategy is markedly expensive, costing approximately 70% more energy than that of the semi-claustral strategy. Relative to males, claustral gynes also had the largest differential investment and smallest size variation. We applied these investment costs to a model by Brown and Bonhoeffer (2003) that predicts founding strategy based on investment cost and foraging survivorship. The model predicts that non-claustral foundresses must survive the foraging period with a probability of 30–36% in order for a foraging strategy to be selectively favored. These results highlight the importance of incorporating resource investment at the colony level when investigating the evolution of colony founding strategies in ants.



## 1. Introduction

Social insects form decentralized systems where a concordance between individual and colonial behavior must translate to fitness gain. Ant colonies are composed of physically independent somatic (worker) and germ (queen and male) components that perform different tasks. However, during annual reproduction collective worker behavior is vitally linked to the development of sexual offspring. Resource investment by workers into gynes (future queens) is likely to affect their success in colony founding. Given the interdependence of both individual and colonial components, cost-benefit analyses at both levels are integral to understanding how reproductive strategies evolve in ants.

Colony founding is an extremely vulnerable period for harvester ant gynes. Individuals of independently founding species first leave their natal colonies during a nuptial flight to mate. They then disperse and initiate new colonies, usually solitarily. The mortality rate during this stage is extremely high (Pfennig, 1995) and may reach ~99 percent (Billick et al., 2001; Gordon and Kulig, 1996; Wiernasz and Cole, 1995), but surviving colonies experience high longevity (15 to >30 years, Gordon, 1991; Johnson, 2001). Although survival is often a matter of stochastic good luck of a foundress happening to land in a suitable and unoccupied area, there is, however, likely to be strong selection pressure upon even these fortunate females to behave optimally relative to claustrality. Thus, every mature, reproductive colony has been 'lucky' at its initiation, but has also simultaneously survived a strong selective filter.

Several colony founding strategies correlate with gyne morphology and physiology in ants (Johnson et al., 1996; Johnson, 2006; Keller and Passera, 1988, 1989; Peeters and Ito, 2001; Rüppell and Heinze, 1999; Rüppell et al., 1998, 2001; Stille, 1996). One such tactic is degree of claustrality, which describes the extent to which gynes rely on their internal reserves for raising their first brood of workers (termed nanitics or minims). Claustral (C) gynes depend entirely on their internal body reserves and do not forage (Hölldobler and Wilson, 1990). They have a large body size with sufficient lipid and storage protein stores (Hahn et al., 2004). Alternatively, semi-claustral (SC) gynes are smaller in size with fewer reserves, necessitating obligate foraging during colony initiation. Finally, some species are facultative foragers (F), meaning that some gynes forage while others do not. For these species,

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http://dx.doi.org/10.1016/j.jinsphys.2014.07.006
0022-1910/

foraging variation exists among gynes within colonies (Anderson and Keyel, 2006; Brown and Bonhoeffer, 2003; Enzmann and Nonacs, pers. obs.; Johnson, 2002, 2006), and likely exists across populations and across years due to variation in resource availability (Brown and Bonhoeffer, 2003; Johnson, 2006). Gynes of facultative species are generally intermediate in size and contain higher lipid and storage protein reserves (Hahn et al., 2004; Johnson, 2002, 2006) than obligately semi-claustral species.

Given the disparity of when resources for reproduction are obtained, variation in claustrality has been equated to different breeding strategies in life history theory (Johnson, 2006). Claustral gynes are capital breeders because they use only stored energy for reproduction, while semi-claustral gynes are income breeders because they use energy that is acquired during the reproductive period (Stearns, 1992).

In the harvester ant genus, *Pogonomyrmex*, a full range of claustrality (C, F, and SC) is employed across species (Brown and Bonhoeffer, 2003; Hahn et al., 2004; Johnson, 2006). This variability is an unusual case in the Myrmicinae, since fully claustral founding is a derived condition and largely predominant in more derived ant subfamilies (Myrmicinae, Formicinae, Dolichoderinae). Although the semi-claustral strategy is a basal trait (Brown and Bonhoeffer, 2003; Peeters, 1997; Peeters and Ito, 2001) and dominant in older subfamilies (Ponerinae, Myrmeciinae, Northomyrmecinae), its secondary reoccurrence in *Pogonomyrmex* and thirteen other more derived ant genera (Brown and Bonhoeffer, 2003) suggests that it has current adaptive value (Johnson, 2002). The full continuum of claustrality within *Pogonomyrmex* makes it an excellent study genus for cost-benefit analysis of this trait.

Given that gynes are almost always larger than workers in ants, their production cost is an expensive investment on the part of the colony (Keller and Passera, 1989). Gynes that vary in degree of claustrality have different resource needs, such that greater individual quality (more lipid and protein) trades off with greater numbers. Workers are vitally linked to gyne investment in that they are responsible for providing adequate nutrition to gynes during the larval (Wheeler, 1986) and post-eclosal (Boomsma and Issaks, 1985; Nielsen et al., 1985) stages of development. The behavior of the colony thus plays an integral role in the maintenance of different colony founding strategies.

While it is presumed that colonies vary in their provisioning regimes, a comprehensive study of gyne investment across different degrees of claustrality has not been conducted. The difference in production cost between claustral, facultative and semi-claustral gynes is unknown, though it is accepted that claustral gynes are more expensive. Even though production cost can be roughly estimated by adult mass, this value is not always accurate because it doesn't account for respiration throughout development. Metabolic costs can be very high in certain developmental stages, such as in *Lasius flavus* where gyne pupal metamorphosis consumes more than a third of the biomass accumulated as larvae (Peakin et al., 1989). Also, while absolute body size of large gynes may render them more costly to produce initially, small gynes have higher mass-specific metabolic rates (Johnson, 1998), such that their developmental costs may in part balance out the investments required by large size. Thus it is important to determine both, reserve and metabolic costs across strategies. Here we investigate the total energetic costs of gynes that differ in degree of claustrality in terms of:

(1) The average per capita energetic cost, size, and lipid reserve.
(2) The comparative cost of gynes relative to males.
(3) When each strategy may be selectively advantageous (Brown and Bonhoeffer, 2003).

## 2. Methods

### 2.1. Study species

Four species of *Pogonomyrmex* harvester ants that differ in degree of claustrality were collected in California and Florida during their reproductive seasons (spring and summer) over four years (Table 1). Colonies of *Pogonomyrmex californicus* were monogynous in the collection localities. It should be noted that having only one claustral representative, *P. badius* may limit the generality of this study, as this species may turn out to be atypical relative to all other claustral species. However, there is no *a priori* reason as to why the demands of colony founding relative to claustrality would be fundamentally different in *P. badius* and therefore make it inappropriate for comparison. Gynes and males were collected in the following developmental stages: larvae (late instar), early pupae, late pupae, and virgin imagos. Imagos (both sexes) were present approximately a month after larvae and pupae were found in nests of all species, with some overlap of imago and pupae presence. Only imagos whose cuticle was darkened in color were used in measurements, since mature individuals and callows are significantly different in lean mass (dry mass – lipids) (Enzmann pers. obs.; Tschinkel, 1998). Gynes typically accumulate reserves after eclosion up to a limit. Because we could not determine the eclosion dates of the gynes that were collected, the average lipid and protein reserves may be underestimated.

### 2.2. Size measurements

For all species, late-instar larvae were grouped as having the largest wet masses out of all larvae collected ($\geqslant$20.0 mg for *P. californicus, P. occidentalis,* and *P. salinus* and $\geqslant$50 mg for *P. badius*). Larvae of this size were not found in *P. occidentalis* colonies, presumably because all the sexual larvae had pupated by the time of collection. Therefore, size and mass measurements for late instar *P. occidentalis* larvae were extrapolated by calculating the percent difference of larvae in the next smallest size class (13–19 mg) between *P. occidentalis* and *P. salinus* (also a facultative species).

**Table 1**
Location, degree of claustrality, and dates of collection of the *Pogonomyrmex* study species.

| Species | Degree of claustrality | Location | Dates |
|---|---|---|---|
| *P. californicus* | Semi-claustral[a] | Independence, CA Motte Rimrock Reserve, Perris, CA | June–July 2006, 2007, 2008 |
| *P. salinus* | Facultative[b,c] | Sierra Nevada Aquatic Research Laboratory, Mammoth Lakes, CA | July–August 2006, 2008 |
| *P. occidentalis* | Facultative[a,d] | Hallelujah Junction, CA | July–August 2006, 2007, 2008 |
| *P. badius* | Claustral[e] | Apalachicola National Forest, near Tallahassee, FL | May–June 2009, 2010 |

[a] Johnson (2002).
[b] Anderson and Keyel (2006).
[c] Enzmann and Nonacs (unpub data).
[d] Billick et al. (2001).
[e] Smith (pers. comm).

Wet mass, dry mass, length, and width measurements were taken for late-star larvae, early and late pupae, and imagos. Widths of larvae were taken at mid-length of each individual. Widths of pupae were taken at the wing buds. Mass was taken using a Cahn C-33 microbalance to the nearest hundredth of a milligram, and the other measurements were taken using a digital caliper to the nearest hundredth of a millimeter.

### 2.3. Respiration

Metabolic rates ($VCO_2$) were measured using single individuals at all developmental stages for the four species using a Sable Systems respirometry system (Sable Systems, Las Vegas NV) at 26 °C at the University of Nevada, Las Vegas. Dry, $CO_2$-free air was pumped through respirometry chambers at a flow rate of 50 ml $min^{-1}$ for larvae and pupae and 100 ml $min^{-1}$ for adults to a Li-Cor LI-6262 infrared gas analyzer (Li-Cor Inc., Lincoln NE) calibrated with 100 ppm span gas.

Because there are several behavioral and environmental factors that affect metabolic rate (Waters and Harrison, 2012), all specimens were treated as consistently as possible. The variation in ground temperature at each field site was unknown, so respiration temperature was kept consistent across species. *Pogonomyrmex* ants tend to bring brood to the surface to warm in the morning when ambient temperatures are about ~26–30 °C (Enzmann and Nonacs pers. obs., for *P. occidentalis* see Cole, 1994), so we considered 26 °C an appropriate temperature for respiration measurements. Larvae were kept with groups of workers, and Kentucky bluegrass (*Poa pratensis*) seeds were given as food. All specimens were allowed to acclimatize for at least 10 min before measurement. Adult gynes and males were unrestrained to allow movement during measurements. Mass-specific metabolic rates were calculated by dividing by wet mass. Metabolic rates of late-instar larvae of *P. occidentalis* were extrapolated using the slope of metabolic rates vs. mass across other larval size classes (ranging 4.3–16.57 mg) in this species. Sample sizes across species and developmental stages ranged from 3 to 19 individuals from 1 to 3 colonies (see Table 3).

### 2.4. Lipid and lean mass measurements

For lipid quantification, wet mass was assessed for early pupae, late pupae, and imago gynes and males. Samples were then dried for at least 48 h. at 60 °C and re-weighed. Lipids were then extracted by submersing samples in petroleum ether (bp 40–60 °C) for three days, with the solvent being replaced each day. *Pogonomyrmex badius* gynes were submerged for an extra 3 days because they were much larger. Samples were then re-dried at 60 °C for at least 48 h and re-weighed. Lipid quantity was determined by calculating the difference between dry weights before and after lipid extraction. Sample sizes across species and developmental stages ranged from 3 to 29 individuals from 2 to 5 colonies.

### 2.5. Total investment costs

Average energetic content (J) contained in lipids and lean mass (carbohydrates and protein) was calculated using the following values: lipid: 39.33 J $mg^{-1}$, lean mass: 18.87 J $mg^{-1}$ (Peakin, 1972). These values have been used to calculate energetic contents of other ant species (Smith and Tschinkel, 2006; Tschinkel, 1993). For respiration costs, it was unknown which resources were being utilized during the time when metabolic rates were measured. We assumed that lipids were the primary source of energy, as they are in larvae and pupae of other insects (Downer, 1985). We used the conversion factor $2.80 \times 10^{-2}$ J µl $CO_2^{-1}$ for lipid metabolism (Withers, 1992).

We tracked pupation time of all study species (except *P. badius*) under laboratory conditions (approx. 25 °C). These pupation times were about 2/3 longer than field estimates of the same or similar harvester ant species (Cole, 1934; MacKay, 1981; Smith and Tschinkel, 2006). We therefore decreased the pupation time by 2/3 in order to better match field development time. In order to estimate larval development times for *P. californicus, P. salinus,* and *P. occidentalis*, we applied the modified pupal times to a ratio developed by Porter (1988): 1(egg): 2.4(larva): 1.3(pupa). This ratio was shown to be consistent independent of temperature in *Solenopsis invicta* (Porter, 1988). For these three species the times of 15 days for larval and 8 days for pupal development were used. For *P. badius,* 25 days for larval and 14 days for pupal development were used based field estimates of total development time (Smith and Tschinkel, 2006).

The time in the imagal stage (between eclosion and the nuptial flight) is somewhat unpredictable across years. For three species (*P. salinus, P. occidentalis,* and *P. badius*), nuptial flights are triggered by rain. In our collection localities, the window for monsoonal rains is approximately 30 days for *P. salinus* (Enzmann and Nonacs, pers. obs.) and *P. occidentalis* (based on thunderstorm probability from 1974 to 2012, http://weatherspark.com) and about 49 days for *P. badius* (Smith and Tschinkel, 2006). The photoperiod-triggered flights of *P. californicus* are estimated to occur within a 30-day window given that mature alates were found in late June–July in our collection localities, and flights are recorded to only occur from May to July (Johnson, 2000).

Given the potential variability of nuptial flights, post-eclosal time was kept constant at 14 days for all the species. However, since later flights would make sexuals more costly to the colony (due to added metabolic costs), we calculated the additional number of post-eclosal days it would take for a less costly gyne to equal a more costly one based on respiration. While it is possible that gynes could also accumulate (or lose) reserves during longer post-eclosal periods, we could not account for this variation since the eclosion times of wild-caught gynes were unknown.

Total investment costs of gynes and males were calculated using the following equation:

$$\sum(R_l * \text{time}_l) + E_{p(\text{early})} + (R_p * \text{time}_p) + E_i - E_{p(late)} + (R_i * \text{time}_i) = \text{investment cost}$$

$E$ = energy content (lipid and lean), $R$ = respiration cost, time = developmental time, l = larva, p = pupa, i = imago, early = early pupal development, late = late pupal development

This equation includes the energy invested into larval growth (resources contained in the early pupal stage), as well as energy accumulated post eclosion (the difference between late pupal and mature adult stages). The respiration costs are included for larval (late instar), pupal, and imagal stages. Respiration costs of the egg stage were not included given their difficulty of collection from wild colonies.

### 2.6. Statistics and model

The effects of species, collection year, and colony (independent variables) on gyne wet mass (dependent variable) were analyzed for the three smaller species (*P. californicus, P. salinus, and P. occidentalis*) using a mixed variance model with a random effect of colony and a fixed effect of collection year. Gynes of the largest species, *P. badius,* were first left out of the model because they were only collected for one year (which produced collinearity between species and collection year). However, collection year had no significant affect on wet mass comparisons, so the model was

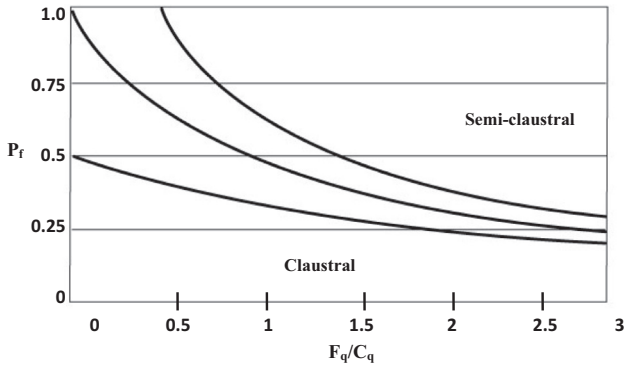


**Fig. 1.** The relationship between claustral gyne provisioning costs (*x*-axis: $F_q$ = the additional resource cost for provisioning a gyne to be fully claustral, $C_q$ = the cost of producing a gyne), and probability of surviving foraging in semi-claustral or facultative gynes (*y*-axis, $P_f$). The boundaries delimit where claustral and semi-claustral gynes are assumed to have equal fitness, when claustral founding is twice as fit (2, upper curve), and when it is half as fit (0.5, lower curve) as semi-claustral founding. Generally, as mortality during foraging increases or the relative cost of provisioning claustral gynes decreases, the claustral strategy is favored. Redrawn from Brown and Bonhoeffer (2003).

re-run with *P. badius* included. The same model was used for males except that *P. badius* was included since they were closer in size to males of the other species (species-year collinearity was not present for male comparisons).

The effects of species and colony (independent variables) on lipid content and dry mass (dependent variables) were assessed across all four species using a mixed variance model with a random effect of colony. Year was omitted in both cases because lipid data were collected from a subset of samples collected during a single year for each species.

A model by Brown and Bonhoeffer, (2003) was used to estimate when a claustral vs. non-claustral (foraging) strategy would be evolutionarily favored. The model plots claustral and semi-claustral strategies in parameter space based on the relative cost of making a non-clasutral gyne into a claustral one ($f_q/C_q$) and the survivorship probability of the foraging period after nest construction ($P_f$) (Fig. 1). Although initially included in the model, the resources available for gyne production and survivorship between mating and nest construction were not key determinants of strategy selection. The cost of making a claustral gyne was calculated for *P. californicus* (SC), *P. salinus* (F), and *P. occidentalis* (F) using the difference in energetic cost between *P. badius* (C) and these species. Claustral founding is selected for when the following inequality is met:

$$1/(1 + f_q/C_q) > P_f$$

Thus, a minimum value of $P_f$ foraging survivorship is necessary in order for a foraging strategy (SC or F) to remain evolutionarily stable. When calculating $P_f$, we assumed equal fitness between claustral and semi-claustral strategies

## 3. Results

### 3.1. Size measurements

Gynes of the claustral species, *P. badius*, averaged approximately 73% greater in wet mass than gynes of the facultative *P. salinus*, which had the smallest wet mass. They were about 77% larger in dry mass than semi-claustral gynes of *P. californicus*. Wet and dry mass significantly differed between all gyne comparisons except for between *P. californicus* and *P. salinus*, and dry mass between *P. salinus* and *P. occidentalis* (Mixed Variance Model, *P. cal* & *P. occ* wet: $z = -0.11$, $p < 0.01$, dry: $z = 2.95$, $p < 0.05$; *P. sal* & *P occ* wet: $\chi^2 = 141.2$, $p < 0.01$, dry: $\chi^2 = 3.16$, $p = 0.07$; *P. sal & P. bad* wet: $\chi^2 = 1019.9$, $p < 0.01$ dry: $\chi^2 = 246.6$, $p < 0.01$; *P. occ & P. bad* wet: $\chi^2 = 492.3$, $p < 0.01$ dry: $\chi^2 = 185.3$, $p < 0.01$, Table 2). There were no significant differences in wet or dry masses of males across all four species, including the claustral *P. badius.*

Coefficients of variation (CV) for gyne wet mass were greatest in the smallest species, *P. salinus* and *P. californicus*. Gyne CV was smaller in the mid-sized *P. occidentalis* and was smallest in *P. badius*. Wet mass variation was greater in gynes relative to males in only the semi-claustral species (*P. californicus*). Males had greater variation in the other species, which became more pronounced with increase in degree of claustrality (Fig. 2).

### 3.2. Respiration

Three types of respiration found in harvester ants (continuous, cyclic, and discontinuous; Johnson and Gibbs, 2004) were exhibited by all species. All larvae and pupae exhibited continuous respiration. Adult gynes and males exhibited all three types of respiration (Table 3), but only discontinuous and cyclic types were used in energy investment calculations because they were the most common and present across all four species. In species where both discontinuous and cyclic data were available, they were averaged. Whole-body metabolic rates for the claustral species (*P. badius*) in all developmental stages were higher than those of the three smaller species (e.g., adult gynes were 60% higher). However mass-specific values were either smaller or very similar to those of the smaller species, indicating a cheap metabolic cost per milligram mass for the larger-sized species. The difference between gyne and male metabolic rates (in all developmental stages) increased with the general increase in size and degree of claustrality. The smallest difference was apparent in *P. californicus* and the largest in *P. badius* (8% and 57% higher in gynes relative to males, respectively, Table 3).

### 3.3. Investment costs

Total per capita investment costs of gynes were positively associated with degree of claustrality, with the claustral species costing approximately 70% more energy to produce relative to the cheapest species (*P. californicus*). The semi-claustral and facultative species were modestly different from each other (Fig. 3).

In the energy investment calculations, a 14-day post-eclosion period was assumed across all species because the time of the nuptial flights is variable across years. The number of post-eclosal days necessary to equalize the cost of a non-claustral gyne (*P. californicus*, *P. salinus*, and *P. occidentalis*) and a claustral gyne (*P. badius*) with a 14-day post eclosal period would be 130, 195, and 183 days, respectively. Therefore, even allowing for a 30-day variation in nuptial flights, the non-claustral species would never be as costly as the claustral species. However, it would be possible for the costs of non-claustal species to equalize across each other, as additional post-eclosal days necessary ranged from 17 to 28 days. Thus, flight time variation in these species could match costs in some years and exaggerate differences in others. We argue that a 14-day post eclosal period is a good estimate of the average costs of sexuals across years.

Lipid contents (mg) of gyne pupae and imagos were both positively correlated with degree of claustrality, though not significantly different between *P. salinus* and *P. occidentalis* imagos (Mixed Variance Model, *P. cal* & *P. sal* $z = 2.71$, $p < 0.01$; *P. cal* & *P. occ* $z = 3.23$, $p < 0.01$; *P. cal* & *P. bad* $z = 11.63$, $p < 0.001$; *P. sal* & *P. bad* $\chi^2 = 98.6$, $p < 0.001$; *P. occ* & *P. bad* $\chi^2 = 85.4$, $p < 0.001$, Fig. 4). Most of the lipids in the claustral *P. badius* were

**Table 2**
Wet mass, dry mass, length, width, and headwidth (Avg ± SE) of gyne and male larval, pupal, and imagal developmental stages. Sample size and number of colonies, respectively, are in parentheses.

| Species & developmental stage | Wet mass (mg) | Dry mass (mg) | Length (mm) | Width (mm) | Headwidth (mm) |
|---|---|---|---|---|---|
| *P. californicus* | | | | | |
| Larvae | 23.74 ± 0.25 (60,5) | | 6.49 ± 0.06 | 2.62 ± 0.04 | |
| Gyne pupae (early) | 20.89 ± 0.31 (22,4) | 5.27 ± 0.16 (21,3) | 6.52 ± 0.09 | 2.67 ± 0.03 | 2.27 ± 0.02 |
| Gyne pupae (late) | 19.51 ± 0.69 (8,3) | 4.86 ± 0.14 (9,3) | 6.30 ± 0.07 | 2.71 ± 0.05 | 2.34 ± 0.03 |
| Male pupae (early) | 21.72 ± 0.33 (30,4) | 5.39 ± 0.12 (20,2) | 6.70 ± 0.04 | 2.68 ± 0.04 | 2.00 ± 0.03 |
| Male pupae (late) | 19.45 ± 0.72 (5,3) | 4.48 ± 0.16 (5,3) | 6.49 ± 0.20 | 2.64 ± 0.14 | 2.03 ± 0.16 |
| Gyne | 16.30 ± 0.45 (15,2) | 6.06 ± 0.21 (26,3) | | | |
| Male | 15.27 ± 0.27 (22,2) | 4.41 ± 0.11(25,2) | | | |
| *P. salinus* | | | | | |
| Larvae | 21.91 ± 0.14 (49,5) | | 6.69 ± 0.04 | 2.29 ± 0.02 | |
| Gyne pupae (early) | 21.98 ± 0.23 (26,4) | 5.58 ± 0.18 (23,5) | 6.68 ± 0.05 | 2.82 ± 0.02 | 2.09 ± 0.01 |
| Gyne pupae (late) | 18.86 ± 0.63 (17,3) | 5.12 ± 0.36 (13,4) | 6.38 ± 0.06 | 2.75 ± 0.05 | 2.03 ± 0.03 |
| Male pupae (early) | 20.82 ± 0.29 (30,2) | 5.19 ± 0.10 (14,2) | 6.61 ± 0.04 | 2.63 ± 0.02 | 1.86 ± 0.02 |
| Male pupae (late) | 18.35 ± 0.45 (16,2) | 4.67 ± 0.13 (28,3) | 6.37 ± 0.06 | 2.62 ± 0.03 | 1.90 ± 0.03 |
| Gyne | 14.57 ± 0.19 (125,11) | 7.43 ± 0.27 (29,5) | | | |
| Male | 10.68 ± 0.236 (88,7) | 3.38 ± 0.13 (16,3) | | | |
| *P. occidentalis* | | | | | |
| Larvae (extrapolated) | 21.30 | | | | |
| Gyne pupae (early) | 25.73 ± 0.21 (56,6) | 6.76 ± 0.11 (20,3) | 7.19 ± 0.02 | 2.99 ± 0.02 | 2.24 ± 0.02 |
| Gyne pupae (late) | 25.24 ± 0.22 (53,6) | 6.70 ± 0.12 (22,3) | 7.07 ± 0.04 | 3.04 ± 0.03 | 2.27 ± 0.02 |
| Male pupae (early) | 23.50 ± 0.40 (46,5) | 5.14 ± 0.24 (18,3) | 6.88 ± 0.05 | 2.78 ± 0.03 | 1.95 ± 0.03 |
| Male pupae (late) | 22.78 ± 0.24 (50,6) | 5.09 ± 0.18 (22,3) | 6.83 ± 0.04 | 2.85 ± 0.02 | 2.05 ± 0.02 |
| Gyne | 22.29 ± 0.27 (50,4) | 9.88 ± 0.37 (23,4) | | | |
| Male | 16.19 ± 0.45 (45,4) | 5.17 ± 0.21 (22,3) | | | |
| *P. badius* | | | | | |
| Larvae | 61.16 ± 1.19 (26,3) | | 8.66 ± 0.10 | 3.41 ± 0.04 | |
| Gyne pupae (early) | 63.68 ± 1.28 (11,4) | 13.86 ± 0.58 (10,4) | 9.60 ± 0.09 | 3.94 ± 0.4 | 3.59 ± 0.07 |
| Gyne pupae (late) | 58.00 (1,1) | 14.07 ± 0.39 (2,2) | 9.39 | 3.91 | 3.65 |
| Male pupae (early) | 33.51 ± 0.82 (9,3) | 7.14 ± 0.21 (15,3) | 7.91 ± 0.09 | 3.21 ± 0.03 | 2.52 ± 0.04 |
| Male pupae (late) | 29.58 ± 1.05 (5,1) | 7.03 ± 0.38 (10,2) | 7.85 ± 0.13 | 3.34 ± 0.05 | 2.61 ± 0.05 |
| Gyne | 54.78 ± 1.44 (4,4) | 26.86 ± 1.65 (6,4) | | | |
| Male | 20.93 ± 1.59 (4,1) | 5.48 ± 0.13 (28,4) | | | |

**Table 3**
Metabolic rates (MR) and mass-specific metabolic rates (Avg ± SE) of late instar larvae, pupae, and imago of sexuals at 26 °C. Respiration types: C = continuous, Cyc = cyclic D = discontinuous.

| Species & developmental stage | MR ± SE (μl $CO_2$ $h^{-1}$) | Mass Specific MR ± SE (μl $CO_2$ $h^{-1}$ $mg^{-1}$) | *N* | # Colonies sampled | Respiration type |
|---|---|---|---|---|---|
| *P. californicus* | | | | | |
| Larvae | 2.81 ± 0.28 | 0.11 ± 0.13 | 7 | 1 | C |
| Gyne pupae | 2.34 ± 0.25 | 0.12 ± 0.01 | 8 | 2 | C |
| Male pupae | 3.18 ± 0.14 | 0.15 ± 0.01 | 6 | 2 | C |
| Gyne | 4.94 ± 0.15 | 0.30 ± 0.02 | 4 | 2 | D |
| | 9.81 ± 0.87 | 0.58 ± 0.03 | 5 | 2 | Cyc |
| Male | 4.54 | 0.26 | 1 | 1 | D |
| | 9.80 ± 2.80 | 0.70 ± 0.20 | 2 | 1 | Cyc |
| *P. salinus* | | | | | |
| Larvae | 1.78 ± 0.28 | 0.08 ± 0.01 | 4 | 1 | C |
| Gyne pupae | 3.29 ± 0.13 | 0.16 ± 0.01 | 14 | 1 | C |
| Male pupae | 2.91 ± 1.08 | 0.20 ± 0.10 | 2 | 1 | C |
| Gyne | 4.73 ± 0.37 | 0.34 ± 0.03 | 5 | 2 | D |
| | 7.63 | 0.48 | 1 | 1 | Cyc |
| Male | 4.57 ± 0.57 | 0.40 ± 0.06 | 6 | 2 | D |
| | 7.35 | 1.10 | 1 | 1 | Cyc |
| *P. occidentalis* | | | | | |
| Larvae (extrapolated)* | 1.89 | 0.09 | | | |
| Gyne pupae | 2.10 ± 0.28 | 0.08 ± 0.01 | 9 | 2 | C |
| Male pupae | 3.23 ± 0.10 | 0.15 ± 0.01 | 10 | 3 | C |
| Gyne | 4.82 ± 0.34 | 0.22 ± 0.02 | 14 | 3 | D |
| | 5.35 ± 1.29 | 0.25 ± 0.06 | 4 | 1 | Cyc |
| Male | 4.80 ± 0.37 | 0.31 ± 0.03 | 7 | 3 | D |
| | 8.21 ± 0.97 | 0.58 ± 0.08 | 4 | 2 | Cyc |
| *P. badius* | | | | | |
| Larvae | 5.38 ± 0.31 | 0.09 ± 0.01 | 15 | 3 | C |
| Gyne pupae | 5.54 ± 0.42 | 0.10 ± 0.01 | 12 | 3 | C |
| Male pupae | 4.57 ± 0.41 | 0.13 ± 0.01 | 10 | 2 | C |
| Gyne | 11.91 ± 1.27 | 0.23 ± 0.02 | 3 | 3 | D |
| Male | 5.11 ± 0.31 | 0.23 ± 0.02 | 3 | 1 | D |
| | 8.12 ± 0.86 | 0.55 ± 0.09 | 2 | 1 | Cyc |

* Metabolic rate was extrapolated using an equation: MR = 0.0527 (mass) + 0.766, constructed from the slope of MR and mass of smaller larvae (*N* = 5).

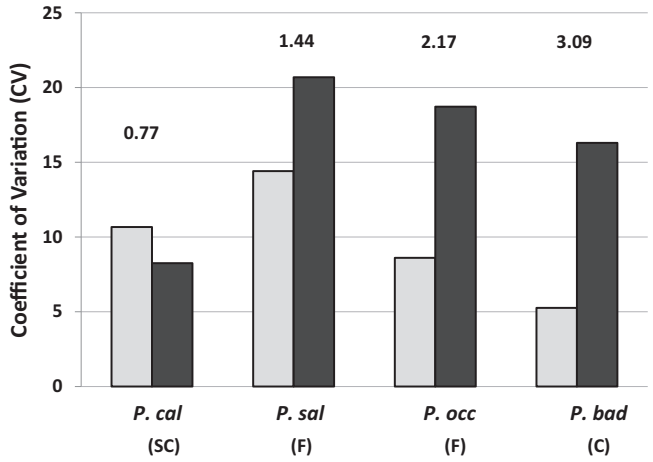


**Fig. 2.** Coefficient of variation of gyne (grey) and male (black) wet mass across study species *P. cal* = *P. californicus* (SC); *P. sal* = *P. salinus*, (F); *P. occ* = *P. occidentalis* (F); *P. bad* = *P. badius* (C). Numbers above bars are the ratios of male: gyne wet mass.

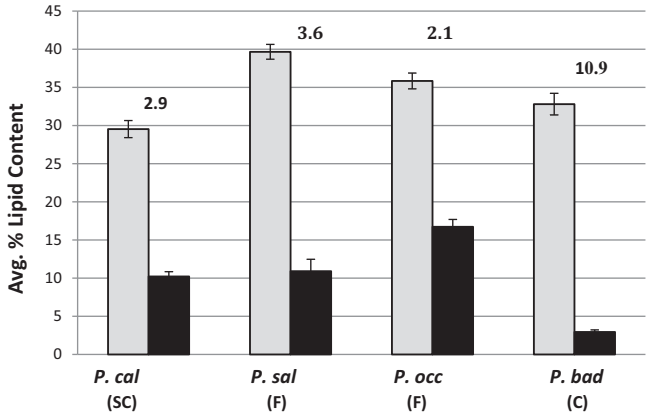


**Fig. 5.** Percent lipid content of dry mass (Avg ± SE) of gynes (grey) and males (black) across species that differ in degree of claustrality (increasing from left to right). *P. cal* = *P. californicus* (SC); *P. sal* = *P. salinus* (F); *P. occ* = *P. occidentalis* (F); *P. bad* = *P. badius* (C). Numbers above bars are the ratios of gyne: male % lipid content.

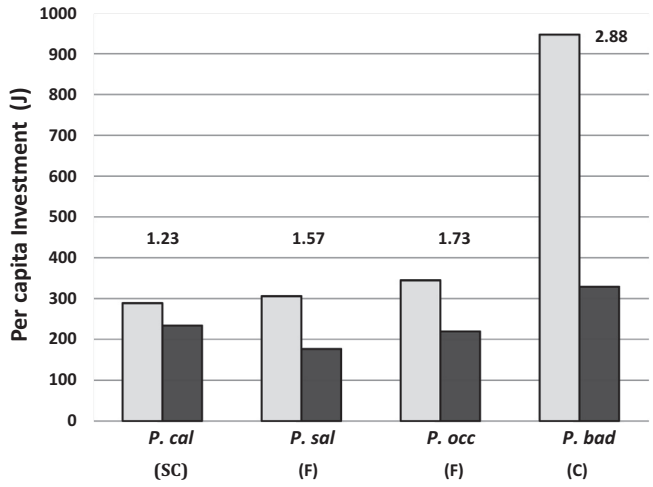


**Fig. 3.** Per capita investment of gynes (grey) and males (black) across species that differ in degree of claustrality (increasing from left to right). *P. cal* = *P. californicus* (SC); *P. sal* = *P. salinus*, (F); *P. occ* = *P. occidentalis* (F); *P. bad* = *P. badius* (C). Numbers above bars are the ratios of gyne: male investment.

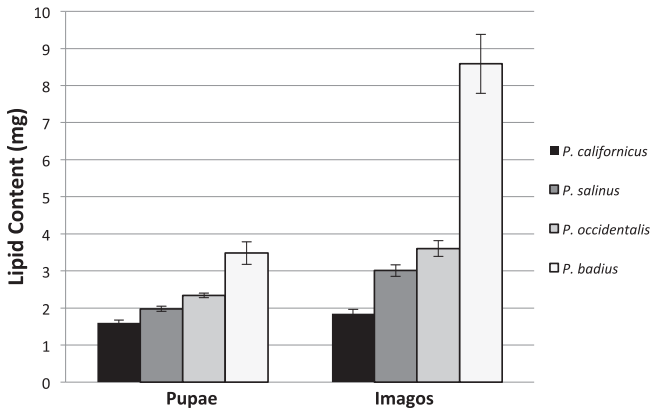


**Fig. 4.** Lipid content (Avg ± SE) in gyne pupae and imagos of *P. californicus* (SC), *P. salinus* (F), *P. occidentalis* (F), and *P. badius* (C).

accumulated after eclosion, as is evident by the large increase in lipids in imagos relative to pupae (Fig. 4). When compared to overall dry mass, the percent lipid content was largest in the two facultative species (*P. salinus* and *P. occidentalis*), followed by the claustral species (*P. badius*), and then the semi-claustral species (*P. californicus*) (Fig. 5). Male lipid content differed significantly across all species except for between *P. californicus* and *P. salinus* and between *P. salinus* and *P. badius* imagos (Mixed Variance Model, *P. cal* & *P. occ* $z = 3.52$, $p < 0.01$; *P. cal* & *P. bad* $z = -2.63$, $p < 0.01$; *P. sal* & *P. occ* $\chi^2 = 22.1$, $p < 0.001$; *P. occ* & *P. bad* $\chi^2 = 46.2$, $p < 0.001$). Gyne investment relative to males differed the most in the claustral *P. badius*, with gynes requiring 65% more per capita energy (Fig. 4). Even more drastic was the 91% of lipids invested differentially into gynes over males in this species (Fig. 5).

### 3.4. Mass as an estimator of investment

In order to compare whether mass was a good indicator of parental investment in *Pogonomyrmex*, ratios of adult gyne and male masses (wet and dry) of the three smaller species (*P. californicus*, *P. salinus*, *and P. occidentalis*) over the largest species (*P. badius*) were compared. For gynes, both wet and dry mass slightly underestimated production cost, with the mass ratios deviating from production cost ratios by 0.007–0.05. The wet mass ratio of *P. occidentalis* over *P. badius* slightly overestimated cost while dry mass was an accurate proxy. For males, wet and dry masses tended to marginally overestimate production cost, with differences in ratios ranging from 0.01 to 0.1. Only the wet mass ratio of *P. salinus* over *P. badius* slightly underestimated the production cost ratio.

### 3.5. Evolution of claustrality

According to the model by Brown and Bonhoeffer (2003), in order for *P. californicus* to maintain a semi-claustral strategy, foundresses would need to survive foraging with a probability of at least 30%. Because of their slightly larger investment, *P. salinus* and *P. occidentalis* would require a probability of 32% and 36%, respectively, in order to maintain foraging in their facultative strategy. These percentages represent the survivorship required only during the foraging period (between nest construction and raising the first brood) and do not include mortality associated with the nuptial flight, dispersal, and nest construction. Given that a large percentage of gynes often die prior to the foraging period (Gordon and Kulig, 1996), it is important to note that this model assumes 30–36% survivorship after this significant period of mortality.

## 4. Discussion

### 4.1. Resource investment

A fully claustral strategy of gynes involves a substantially greater energetic cost relative to semi-claustral and facultative congeners. The greater mass, development time, and whole-body metabolic rates of the claustral *P. badius* gynes rendered them about 70% more costly to produce on a per capita basis. *P. badius* gynes also contained the most lipid reserves and significantly more percent lipid relative to dry mass than the obligately semi-claustral *P. californicus*, supporting the idea that lipid reserves are important for claustral founding (Johnson, 2002; Keller and Passera, 1989). *P. badius* gynes also likely use storage protein reserves in order start colonies claustrally (Hahn et al., 2004; Martinez and Wheeler, 1994; Wheeler and Buck, 1995; Wheeler and Martinez, 1995), which would be reflected in their much larger dry mass.

The investment costs of semi-claustral and facultative gynes had greater similarity to each other, suggesting that the facultative *P. salinus* and *P. occidentalis* are closer to the semi-claustral side of the continuum than the claustral side. However, the greatest amounts of lipid relative to dry mass were found in these two facultative species, which is concurrent with other reports (*P. salinus* ~ 40%, *P. occidentalis* gynes ~ 43–48%, Johnson, 2002). Although *P. salinus* gynes were not significantly different from those of *P. californicus* in dry mass (and smaller in wet mass), they had the highest percent lipid. Because *P. salinus* is facultative, some gynes may use lipid reserves to start colonies claustrally. However, this is likely uncommon, as almost all *P. salinus* foundresses observed in the lab needed to forage to produce brood (Anderson and Keyel, 2006; Enzmann and Nonacs, unpub. data). Alternatively, *P. salinus* may have high lipid contents for other energetic needs, such as nest construction and foraging. For instance, *P. salinus* foundresses build larger and deeper nests than *P. californicus* and dig for longer times than semi-claustral and claustral congeners (Enzmann and Nonacs, 2010). Larger lipid stores may also allow foundresses to embark on more or longer foraging trips.

Wet and dry masses are often used as proxies for production costs in insects (e.g., Danforth, 1990; Frohlich and Tepedino, 1986; Trivers and Hare, 1976) and the question arises about whether mass comparisons accurately reflect investment differences (Bosch and Vicens, 2002). When adult gyne wet and dry masses of the three smaller species (*P. californicus*, *P. salinus*, and *P. occidentalis*) were compared to the largest species (*P. badius*), most mass ratios were modestly smaller than production cost ratios, indicating that mass slightly underestimates investment. On the other hand, wet and dry mass tended to marginally overestimate production cost on males. While these mass comparisons did not precisely match investment comparisons, the differences were very small. This suggests that in *Pogonomyrmex*, wet and dry mass both serve as close approximations to the production costs of sexuals.

### 4.2. Investment in gynes versus males

The per capita investment of gynes relative to males positively correlated with degree of claustrality. This indicates that gyne quality is prioritized over male quality as the dependence on stored reserves for colony founding increases. The difference between sexes was the most apparent in the claustral species (*P. badius*), where it is evident that gyne quality, in both total investment and percent lipid content, is favored over male quality. The coefficient of variation of gynes relative to males was negatively correlated to degree of claustrality. The advantages that a large body size bestows during colony founding likely contribute to the reduced size variation of gynes relative to males. Wiernasz and Cole, (2003) attribute the small size variation in *P. occidentalis* to their finding that large foundresses were more likely to survive the early stages of colony initiation than small foundresses. Our results not only corroborate these finding but also suggest that selection for narrow gyne size variation becomes increasingly stronger as the degree of claustrality increases.

### 4.3. Evolution of claustrality

Resource allocation in ants is shaped by selection at both the individual and colony levels (Bourke and Franks, 1995; Hahn, 2006), and is a principle influential factor in the evolution of colony founding strategies. At the individual level, large gyne size confers greater survivorship (Wiernasz and Cole, 2003), increased oviposition rate (Wagner and Gordon, 1999), reduced desiccation rate (Lighton et al., 1993), and reduced predation risk associated with foraging (Hölldobler and Wilson, 1990). Small body size associated with the semi-claustral strategy also affords benefits to gynes, but in later stages of colony founding. Given that these gynes forage for external resources, they are not limited by energy to raise their minims. This permits release from a size vs. quantity tradeoff resulting in a greater number of minims, as is apparent in the obligately semi-claustral *P. californicus* (Johnson, 2002) and the facultative *P. desertorum* (Johnson, 2006). Having a larger initial workforce can jump-start colony growth (Porter and Tschinkel, 1986; Tschinkel, 1992; Vargo, 1988).

At the colony level, workers face the trade-off of producing few large versus many small gynes. Given that multiple size-associated strategies exist, it is likely that different colony-level investment patterns are adaptive. Gyne size and its level of plasticity may be influenced by genetic factors (Smith et al., 2008) and geographic conditions related to temperature (e.g., latitude and altitude; Blanckenhorn and Demont, 2004; Chown and Klok, 2003). The availability and predictability of food before and during colony founding is also likely key to shaping these reproductive strategies (Brown and Bonhoeffer, 2003; Gilboa and Nonacs, 2006; Johnson, 2006). Employing a claustral strategy theoretically assumes a predictable environment where resources are stable during gyne development, yet unpredictable during colony founding. Alternatively, an obligately semi-claustral strategy assumes predictable food availability after colony initiation when foundresses forage. A facultative strategy, however, may be most advantageous in highly variable environments, since the production of both small and large gynes may serve as a bet-hedging tactic. (Brown and Bonhoeffer, 2003; Johnson, 2006). It is also possible that colony founding strategies vary intraspecifically across populations, such as in cases where the average gyne mass differs across geographic localities (Anderson and Keyel, 2006; Johnson, 2002, 2006).

The model by Brown and Bonhoeffer (2003) predicts selection for claustral vs. semi-claustral strategies based on the relative per capita cost of gyne production (the colony level) and probability of gyne survivorship during foraging (the individual level). It is notable that this model uses both, a colony-level variable and an individual-level variable for the prediction of founding strategy evolution. Our data show that non-claustral gynes must survive foraging with a probability of at least 30–36% in order for their strategy to be favored. In the model, facultative species are predicted to fall on the line that demarcates claustral vs. semi-claustral strategies or vary in each parameter space from year to year. While it is difficult to measure foundress survivorship in the field, Wiernasz and Cole (2003) found that 29% (117/611) of *P. occidentalis* foundresses (facultative) survived for the first 18 days after the nuptial flight. This period likely included time when foundresses were foraging and is a close percentage to what the model predicts (however, because this species is facultative, some

foundresses may not have foraged). It would be interesting to test this model on other non-claustral species to determine how well its two variables predict degree of claustrality.

## 5. Conclusions

Colony founding strategies in ants are diverse and often correlated with body size. While colony founding behavior is subject to selection at the individual level (the foundress), selection is also likely significant at the level of the parental colony. This study highlights the large difference in per capita investment cost between foraging (SC and F) and non-foraging (C) strategies, which reflects the difference in energy allocation strategies that parental colonies likely employ (e.g., investment in few large vs. many small gynes, or a mixture of sizes, Gilboa and Nonacs, 2006). Though the evolution of different levels of claustrality is likely influenced by multiple factors, the model by Brown and Bonhoeffer (2003) is useful in predicting strategies based on both individual and colony-level variables. Measurements of investment cost and foraging survivorship across more species will be useful in testing its accuracy.

## Acknowledgements

We thank Deborah Hoshizaki for her assistance with metabolism analyses, as well as Walter Tschinkel and his lab for their help in obtaining *P. badius* specimens. We also appreciate advice from Robert Johnson about colony founding in harvester ants and for his remarks on the manuscript. This work involved collecting at the University of California Valentine Eastern Sierra Reserve and was supported by two Grants from the University of California Valentine Eastern Sierra Reserve, a Mildred Mathias Grant, a California Desert Research Fund Grant from the Community Foundation and NSF Award IOS-0719591 to D.K. Hoshizaki and A.G. Gibbs.

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