



Colony founding by pleometrosis in the semiclaustral seed-harvester ant *Pogonomyrmex californicus* (Hymenoptera: Formicidae)

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Pleometrosis, or colony founding via multiple queens, occurs in a localized population of the seed-harvester ant *Pogonomyrmex californicus*. In an apparently unusual secondary modification, queens of *P. californicus* are also obligate foragers; that is, queens must forage to garner the resources necessary to rear their first brood. Laboratory experiments measured the costs and benefits of pleometrosis and queen foraging in *P. californicus* in terms of queen survival, mass loss by queens and brood production. In all experiments, queen survival was positively associated with number of queens. Queen survival also varied with food level: survival was higher in fed treatments compared with unfed treatments at low queen numbers, whereas survival of unfed queens increased to the level of fed queens at higher queen numbers. Total mass loss of queens varied by food level, but not queen number, with fed queens losing about 50% less mass than unfed queens. Brood production also varied with queen number and food level. Total number of brood was positively associated with number of queens; at each queen number, fed queens produced more brood than unfed queens. The number of brood produced per queen, however, was similar across queen numbers. Fed queens also produced workers that were heavier than those produced by unfed queens, whereas head width of these minors was similar. Longer-term experiments revealed that these queen associations do not undergo queen reduction upon emergence of the first workers, but rather exhibit primary polygyny.

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Cooperative breeding involves a conflict between the advantages of reproducing in groups and the risks of being exploited by other group members. Despite these risks, cooperative breeding has evolved numerous times across a wide variety of taxa that includes birds, mammals, social insects and other arthropods (e.g. beetles, aphids, thrips, social shrimp; for reviews see Brockman 1997; Cockburn 1998; Queller & Strassman 1998; Cahan et al. 2002). Kin selection is often invoked to explain such cooperation, but this hypothesis has been challenged by studies that have shown cooperative breeding among nonrelatives (e.g. Cockburn 1998; Clutton-Brock 2003). The general consensus for vertebrates is that cooperative breeding is favoured by constraining ecological factors such as nest site limitation, inability of two parents to collect sufficient food to rear brood, and high predation

pressure (Brockman 1997). Empirical evidence also suggests that cooperation can be maintained via group augmentation, whereby fitness of all group members increases with size of the group (Clutton-Brock 2003).

In ants, colony founding via multiple queens (i.e. pleometrosis) has attracted the attention of biologists because all known cases of such associations involve cooperation among nonrelatives (see Bernasconi & Strassman 1999). Thus, kin selection cannot operate in these associations, so the prediction is that these groups confer direct or indirect benefits to all group members. The benefits of pleometrosis include at least one of the following: (1) earlier emergence of the first workers, (2) an increased number of workers, (3) decreased mass loss by queens, and (4) increased defence against workers from neighbouring mature colonies (Waloff 1957; Taki 1976; Bartz & Hölldobler 1982; Tschinkel & Howard 1983; Rissing & Pollock 1987, 1991; Sommer & Hölldobler 1995; Jerome et al. 1998). Moreover, pleometrosis provides the proximate benefit of speeding the colony through its early growth stages, which in turn, provides the ultimate

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benefit of enhanced colony survival and earlier production of sexual offspring (Vargo 1988; Brown 2000). Pleometrosis can also enhance queen survival during the founding stage, but this benefit soon disappears because colonies typically reduce to one queen after the first workers emerge (Waloff 1957; Bartz & Hölldobler 1982; Tschinkel & Howard 1983; Rissing & Pollock 1987; Sommer & Hölldobler 1995). For pleometrosis to persist in the presence of queen reduction requires that the survival probability for a queen in an association of n queens is more than n times greater than that of a solitary queen (Adams & Tschinkel 1995). Overall, pleometrosis is viewed as advantageous when intercolony competition is intense (both by adult and incipient colonies) (Bartz & Hölldobler 1982; Rissing & Pollock 1991; Tschinkel 1992b; Adams & Tschinkel 1995; Jerome et al. 1998; Bernasconi & Strassman 1999) or in harsh abiotic environments (Pfennig 1995; Cahan 2001b).

A powerful approach to dissect factors important in the evolution and maintenance of cooperative breeding involves examining species that display intraspecific variation in this behaviour (for vertebrate examples see Emlen 1984; Hatchwell & Komdeur 2000). Ants have also proven valuable in this regard because several species display geographical variation in queen number during nest founding or within mature nests (Herbers 1986; Keller 1995; Cahan et al. 1998; Pedersen & Boomsma 1999; Cahan 2001a).

The seed-harvester ant *Pogonomyrmex californicus* (Buckley) (subfamily Myrmicinae), displays geographical variation in nest founding (Rissing et al. 2000), and thus represents an ideal organism for dissecting factors related to the evolution of pleometrosis. Single queens found nests in most populations of *P. californicus*, but an apparently localized population in San Diego County, California, U.S.A., exhibits pleometrosis; this population represents the only known occurrence of pleometrosis within *Pogonomyrmex*. Queens of *P. californicus* are also obligate foragers (i.e. single queens cannot survive and rear their first brood without an external food source; Johnson 2002). Consequently, multiple queens cannot pool body reserves to produce additional workers (as occurs in most pleometrotic ants) but rather must obtain resources to rear their brood by exposing themselves to the risks associated with foraging.

This study examined the effects of group size and food level on survival and brood production by queens of *P. californicus*. Using laboratory experiments, I tested the hypothesis that increased group size provides significant benefits to individuals in terms of survival and brood production, and that these benefits are enhanced by food. I also tested the hypothesis that queen foraging provides an energy benefit to both queens and the first brood of workers. In this regard, I tested two predictions: first, that foraging has a positive effect on queen mass (i.e. fed queens lose a lower percentage of their body mass than do unfed queens); second, that the first brood of workers reared by fed queens are heavier in mass, but not size, compared with those reared by unfed queens. The latter prediction was derived from data showing that single, fed queens from a haplometrotic population (i.e. nests started

by one queen) of *P. californicus* produced minim workers that were heavier than predicted by their head width (Johnson 2002); this increased mass probably resulted from feeding upon seeds harvested by the queen. Lastly, I determined the fate of pleometrotic queens following emergence of the first workers.

METHODS

Effect of Queen Number and Food Level on Queen Survival and Brood Production

I conducted laboratory experiments in each of 3 years (1997–1999) to determine the effect of queen number and food level on queen survival and brood production (number of larvae, pupae and minim workers). The experiments used 8-ounce (237-ml) glass bottles that contained 250 ml of soil and 30 ml of water; bottles were covered with a plastic petri dish that contained several holes. Soil was collected at the Salt River Recreation Area, north of Mesa, Arizona, U.S.A., in 1997, and from northeast of Casa Grande, Arizona, in 1998 and 1999; *P. californicus* occurred at both sites. Soil was passed through a 2-mm sieve, mixed into a composite sample, and placed in the bottles.

Queens were collected during the mating flight season (late June through mid-July) in Cameron Valley, San Diego County, California (32°43'N, 116°28'W; 1015 m elevation). In this area, *P. californicus* only occurs in open valleys that consist of grasslands and scattered sagebrush, *Artemisia tridentata* (Beauchamp 1986); the seed-harvester ant *Messor andrei* (Mayr) is a common associate in this habitat. Large numbers of queens could not be located on the day of their mating flights, so I also excavated queens from starting nests and collected those that were foraging. Queens were placed in a closed container with moistened paper towels for more than 2 h to facilitate hydration, then marked with Tamiya paint (Aliso Viejo, California) and weighed. One to five queens were placed in each bottle. The bottles were then positioned randomly in a darkened room maintained at 25–30°C. Water was added to bottles at the rate of 10 ml every 10 days beginning on day 20.

Food level (fed and unfed) was the second treatment (see Table 1); the fed treatment involved adding Kentucky bluegrass, *Poa pratensis*, seeds ad libitum to the bottles beginning on day 5, whereas bottles in the unfed treatment remained without seeds throughout the experiment. In 1999, an error resulted in only two bottles being placed in the three-queen fed treatment; these data are included in the figures, but they are excluded from statistical analyses.

Approximately 7–10 days after workers began to forage (day 44 in 1997, day 54 in 1998, and day 50 in 1999), I emptied all bottles, weighed live queens and counted larvae, pupae and workers (only workers were counted in 1997).

I set up an additional bottle experiment, as above, to assess the effect of queen number and food level on size of minim workers. I emptied the bottles after 50 days, and

Table 1. Summary of treatments for *Pogonomyrmex californicus* queens in the bottle experiments, 1997–1999

Year	Treatment	Number of bottles	Number of queens	Initial wet mass (mg ± SE)*
1997	1-Unfed	34	34	No data
	3-Unfed	19	57	No data
	5-Unfed	20	100	No data
	Total	73	191	
1998	1-Unfed	92	92	12.62 ± 0.13 ^a
	1-Fed	92	92	12.62 ± 0.17 ^a
	2-Unfed	50	100	12.40 ± 0.17 ^a
	2-Fed	54	108	11.90 ± 0.14 ^b
	Total	288	392	
1999	1-Unfed	101	101	14.57 ± 0.13 ^a
	1-Fed	101	101	14.41 ± 0.17 ^a
	2-Unfed	50	100	13.46 ± 0.12 ^{bc}
	2-Fed	50	100	13.87 ± 0.14 ^{ab}
	3-Unfed	61	183	14.03 ± 0.10 ^{ab}
	3-Fed†	2	6	13.10 ± 0.51 ^c
	5-Unfed	30	150	13.37 ± 0.10 ^{bc}
	5-Fed	30	150	13.05 ± 0.13 ^c
	Total	425	891	

Treatment gives the number of queens placed in each bottle and whether or not seeds were provided.

*One-way ANOVA: 1998: $F_{3,388(\text{mass})} = 5.2$, $P = 0.002$; 1999: $F_{7,883(\text{mass})} = 16.9$, $P < 0.001$. Significant differences across treatments in initial wet mass of queens are indicated within each year by the superscript letters a–c: a > b > c. Groupings are based on an a posteriori Duncan's multiple range test.

†The small sample size resulted from an error during set-up.

removed minims that had acquired mature coloration, then dried them at 50–55°C for more than 72 h, and weighed them. Head width was measured by projecting a 10× image from a binocular microscope to a video monitor; the image on the monitor was measured to 0.01 mm using NIH Image (available at <http://rsb.info.nih.gov/nih-image/>).

Data Analysis

Survival and mass loss by queens

I analysed queen survival both across treatments and within treatment cells. Survival across treatments was analysed by a logistic regression that used a binomial distribution and logit link function (PROC GENMOD in SAS 6.12; SAS Institute 1997). Queen status (live or dead) was the dependent variable and queen number and food level were the independent variables. The model uniquely coded each treatment cell (queen number × food level), which resulted in comparing survival rates across all treatment cells simultaneously. Significance levels were based on the Wald chi-square statistic. Within each treatment cell, I examined the effect of initial wet mass on queen status at the end of the experiment. This analysis used a *t* test within each treatment cell to compare initial wet mass of queens that survived with those that died; *P* values were adjusted within each year using the Bonferroni sequential technique.

Mass loss by queens was examined in two ways. First, percentage mass loss during nest founding may vary with group size (e.g. Tschinkel 1993). For the 1997 experiment, I tested for this effect using a one-way ANOVA. The 1998 experiment was a two-factor design (two queen levels ×

two food levels), but no queens survived in the one-queen unfed treatment. Consequently, I used a one-way ANOVA to compare percentage mass loss across the three remaining treatment cells. In the 1999 experiment, percentage mass loss was analysed using a two-way ANOVA; type IV sum of squares was used because of a missing treatment cell (SPSS 1990). Percentage mass loss was the dependent variable and queen number and food level were the independent variables. An a posteriori one-way ANOVA followed by a Duncan's multiple range test determined the nature of within-treatment differences. For each queen, the percentage mass loss was calculated as: [(initial wet mass – final wet mass)/initial wet mass] × 100. The percentage mass loss was averaged across all queens in each bottle, and this value was used in the analysis. The second method to examine mass loss related to observations that percentage mass loss may be positively associated with initial wet mass of queens (i.e. heavier queens lose a higher percentage of their mass; see Mintzer 1987). I tested for this effect by performing correlation analysis of percentage mass loss versus initial wet mass within each treatment cell using the product-moment Pearson coefficient.

Brood production

Number of bottles that contained workers or pupae was analysed by a logistic regression that used a binomial distribution and logit link function (see above); presence or absence of workers/pupae was the dependent variable, and queen number and food level were the independent variables. The purpose of this analysis was to compare treatment cells for the ability of queens to rear brood to the point of nutritional self-sufficiency (i.e. to the pupal stage). I also examined treatment effects relative to the total number of brood per bottle and size of minim

workers. For total number of brood, I used the same ANOVA models as those described above for percentage mass loss by queens. I examined size of minim workers by using head width and dry mass as the dependent variable in separate two-way ANOVAs; queen number and food level were the independent variables.

I standardized analyses for mass loss and brood production by including only data for bottles in which all queens survived. For all statistical tests, variables were log or square-root transformed, as necessary, to meet ANOVA assumptions.

Primary Polygyny and Queen Adoption

The possibility that *P. californicus* exhibits primary polygyny was tested by determining survival of pleometrotic queens at several months beyond the time required for the first workers to emerge. Nests consisted of 16-ounce (473-ml) plastic bottles that had been filled with moistened, sieved soil (≤ 2 mm). Queens were collected at La Posta Road near Interstate 8 (32°43'N, 116°26'W; 1025 m), San Diego County, then placed in bottles without regard to their nest association in the field; 22 bottles contained three queens and one bottle contained seven queens. Bottles were covered with a plastic petri dish that contained several holes. I added water to the bottles, as needed, to maintain moisture, and I provided Kentucky bluegrass seeds ad libitum. Bottles were emptied and live queens were counted approximately 16 weeks after emergence of the first workers.

Brood raiding also occurs in several pleometrotic species (Bernasconi & Strassman 1999), and sometimes involves the queens defecting into the successful nest (Bartz & Hölldobler 1982; Rissing & Pollock 1987; Tschinkel 1992a). In species that display primary polygyny, such queen defection might increase queen number early in the colony cycle. I tested this prediction with a laboratory experiment that used pairs of bottle nests. One bottle contained two queens, and the other, five queens, with the queens in each bottle marked with a different colour of Tamiya paint. Following worker emergence, I placed the two bottles into holes cut at the bottom of a plastic nestbox that contained a common foraging area; the top of each bottle was flush with the bottom of the plastic box (see Rissing & Pollock 1987). I added water to the bottles, as needed, to maintain moisture, and provided Kentucky bluegrass seeds ad libitum. Six months later, I emptied the bottles and recorded the location of all live queens.

RESULTS

Queen Survival

Survival of queens was positively associated with queen number and food level in all 3 years. In 1997, survival of unfed queens was significantly higher in bottles that had three queens and five queens compared with bottles that had one queen (logistic regression: $\chi^2_1 \geq 32.7$, $N = 191$ queens, $P < 0.0001$; Fig. 1). Queen survival varied by food level and queen number in both 1998 ($\chi^2_1 \geq 9.7$, $N = 392$ queens, $P < 0.01$; Fig. 1) and 1999 ($\chi^2_1 \geq 4.1$, $N = 891$

queens, $P < 0.05$; Fig. 1); in the latter year, queen survival was also affected by an interaction between food level and queen number. That is, survival was higher in fed than in unfed treatments at low queen numbers, but survival was similar for both food levels at high queen numbers.

Initial wet mass affected queen survival in some treatment cells in the 1998 and 1999 experiments (Fig. 2). In unfed treatments, initial wet mass was significantly higher ($P < 0.05$) for queens that survived compared with those that died when queen associations were small (≤ 2), but not in larger queen associations (Fig. 2). For fed treatments, initial wet mass was significantly higher for queens that survived compared with those that died for the two-queen association in 1999 ($t_{99} = 2.1$, $P = 0.034$; Fig. 2). Differences in the two unfed treatment cells in the 1999 experiment remained significant after adjusting P values within each year using the Bonferroni sequential technique (Fig. 2).

Mass Loss by Queens

Wet mass of queens decreased significantly during nest founding. The only consistent pattern across treatments, however, was that unfed queens lost a higher percentage of their mass than did fed queens (Fig. 3). In 1997, percentage mass loss was similar for one, three and five unfed queens (one-way ANOVA: $F_{2,29} = 0.4$, NS; Fig. 3). Percentage mass loss varied by food level and queen number in both 1998 ($F_{2,55} = 34.7$, $P < 0.001$) and 1999 ($F_{6,175} = 56.8$, $P < 0.001$; Fig. 3); however, the interaction term in the 1999 experiment was not significant (Table 2). In 1999, percentage mass loss varied by queen number in unfed treatments ($F_{3,75} = 5.4$, $P = 0.002$), but not in the fed treatments ($F_{2,100} = 2.3$, NS; Fig. 3).

Exploratory analysis revealed a consistent positive correlation between initial wet mass of queens and their percentage mass loss by the end of the experiments in both 1998 and 1999 (Table 3). Six of the seven correlations remained significant after adjusting P values within each year using the sequential Bonferroni technique (Table 3).

Brood Production

The percentage of bottles with pupae/workers varied significantly between food levels and across queen numbers in all 3 years. In 1997, the percentage of queens that produced workers was significantly lower in bottles that had one unfed queen compared with those that had three and five unfed queens (logistic regression: $\chi^2_1 \geq 5.3$, $N = 32$ bottles, $P < 0.02$; Fig. 4). This percentage also varied across treatment cells in both 1998 ($\chi^2_1 \geq 11.0$, $N = 61$ bottles, $P < 0.05$; Fig. 4) and 1999 ($\chi^2_1 \geq 4.9$, $N = 184$ bottles, $P < 0.05$; Fig. 4); in both years this percentage was lowest for unfed treatments with low queen numbers.

Total number of brood produced also differed significantly across treatment cells. In 1997, the total number of workers increased from low to high queen numbers (one-way ANOVA: $F_{2,29} = 18.0$, $P < 0.001$; Duncan's multiple range test: $P < 0.05$; Fig. 5). In 1998, the total number of

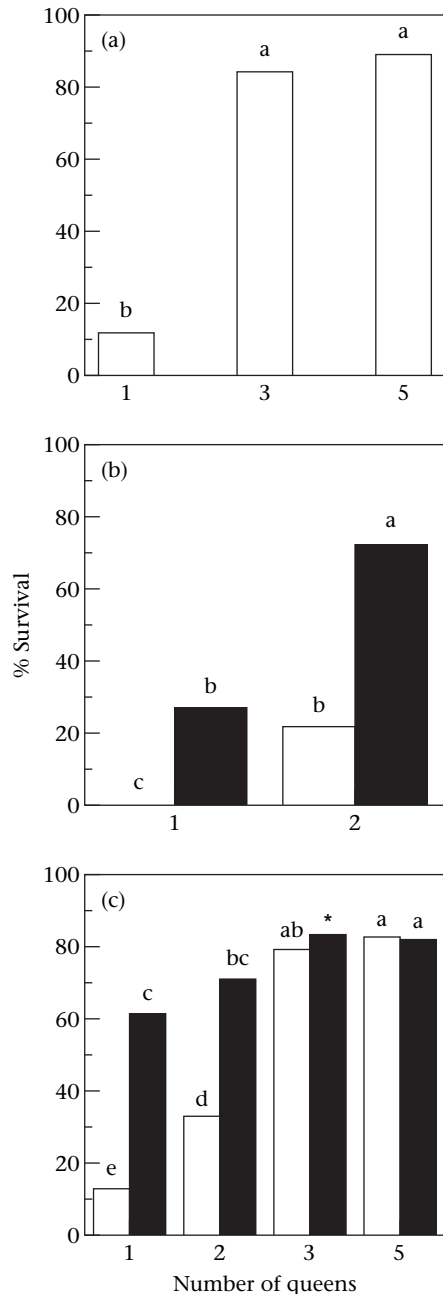


Figure 1. Percentage survival for unfed *Pogonomyrmex californicus* queens in a one-factor (queen number) bottle experiment in 1997 (a), and for fed (■) and unfed (□) *P. californicus* queens in two-factor (queen number*food level) bottle experiments in 1998 (b) and 1999 (c). Within each panel, significant differences ($P < 0.05$) across treatment cells are indicated by the letters a–e: $a > b > c > d > e$. Groupings are based on the Wald chi-square statistic. Sample size is given in Table 1. An asterisk indicates a treatment cell omitted from statistical analysis because of a low sample size.

brood was lowest in the two-queen unfed treatment, intermediate in the one-queen fed treatment, and highest in the two-queen fed treatment ($F_{2,58} = 16.0$, $P < 0.001$; Duncan's multiple range test: $P < 0.05$; Fig. 5). In 1999, the total number of brood varied by queen number and food level with food level being the largest contributor to

the model; the interaction term (queen number*food level) was also significant for number of brood per bottle (Table 4). Number of brood was higher at each queen number in fed compared with unfed treatments (Fig. 5), and was also positively associated with queen number in the fed ($F_{2,101} = 74.5$, $P < 0.001$) and unfed treatments ($F_{3,76} = 17.2$, $P < 0.001$; Fig. 5).

I also examined brood production on a per-queen basis. In 1997, the number of workers per queen differed across queen numbers (one-way ANOVA: $F_{2,29} = 10.7$, $P < 0.001$; Fig. 5), and was significantly higher ($P < 0.05$) for the three-queen and five-queen treatments than for the one-queen treatment. Number of brood per queen also varied across treatment cells in 1998 ($F_{2,58} = 17.8$, $P < 0.001$), but this difference was related to food level rather than queen number (Fig. 5). In 1999, per-queen brood production differed with food level and queen number ($P < 0.02$; Table 4), but the interaction term (queen number*food level) was not significant (Table 4). Number of brood per queen varied with queen number in unfed treatments ($F_{3,75} = 4.0$, $P = 0.01$), but not in the fed treatments ($F_{2,101} = 0.5$, NS; Fig. 5).

Size and Mass of Minim Workers

Dry mass of minim workers varied by food level, with those from fed treatments weighing significantly more than those from unfed treatments (Fig. 6). In contrast, head width of minims varied by queen number but not food level; the interaction factor of food level*queen number was not significant in either ANOVA (Fig. 6, Table 5). For both variables, I used minims from the fed treatments to examine patterns related to queen number. Head width of minims varied across queen numbers (one-way ANOVA: $F_{4,76} = 5.1$, $P < 0.001$; Fig. 6), whereas dry mass did not vary significantly ($F_{4,82} = 1.8$, NS; Fig. 6). However, neither measure varied in a consistent manner relative to queen number.

Primary Polygyny and Queen Adoption

Multiple queens were alive in 22 of the 23 bottles approximately 16 weeks after workers emerged. For the three-queen bottles, all queens survived in 17 bottles, two queens survived in four bottles, and no queens survived in one bottle. All seven queens survived in the other bottle. Overall, 66 of 73 (90%) queens were alive more than 4 months after the first workers emerged. Many of these colonies were then transferred to plastic nestboxes (18 × 13 × 5 cm) that contained a test-tube partially filled with water trapped by a cotton plug and ad libitum Kentucky bluegrass seeds. No aggressive interactions were observed between workers and queens or between queens in these colonies, and multiple queens persisted in laboratory colonies for more than 1 year.

Incipient nests of *P. californicus* can also become polygynous via queen adoption. Five of the plastic nestboxes contained bottles with live queens approximately 6 months after the first workers emerged; queens from

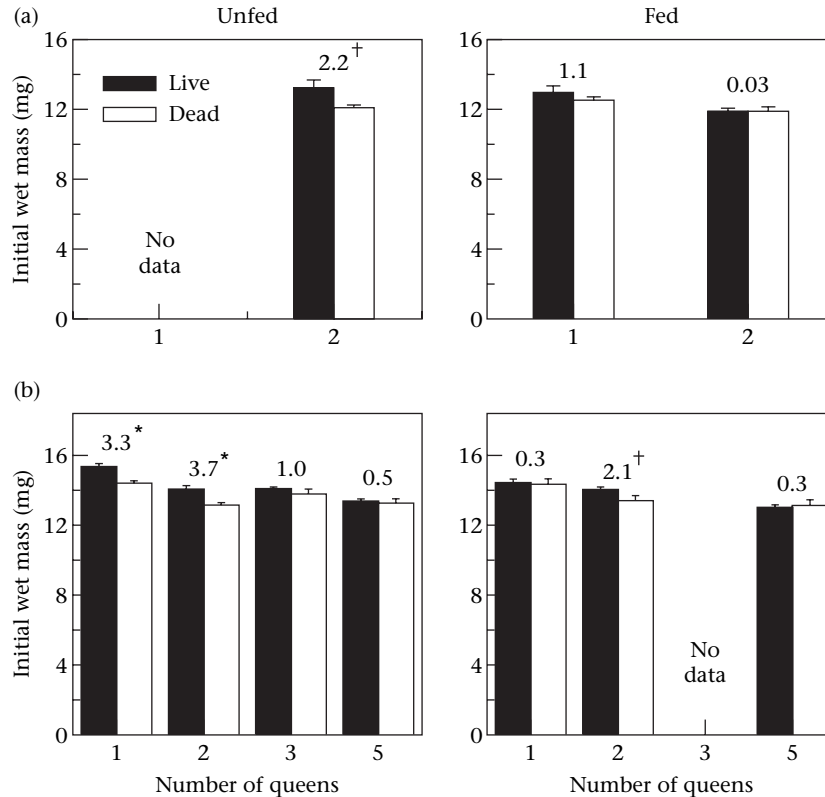


Figure 2. Effect of initial wet mass (mean \pm SE) on survival of *Pogonomyrmex californicus* queens in two-factor (queen number*food level) bottle experiments in (a) 1998 and (b) 1999. Within each treatment cell, a *t* test was used to compare the initial wet mass of queens that survived to those that died; *t* values are given above each comparison (*df* = 33–198). Asterisks denote significance level for each treatment cell after correcting *P* values within each year using the sequential Bonferroni technique. **P* < 0.01. †Treatment cells that became nonsignificant (*P* > 0.05) after adjusting *P* values using the Bonferroni sequential technique.

the two initial colonies occurred in the same bottle in three of the plastic boxes.

DISCUSSION

Pleometrosis and Primary Polygyny

The pleometrotic associations of most ant species undergo queen reduction until one queen remains. Thus, each queen faces a trade-off between investing energy to produce brood that enhances colony survival and withholding energy to enhance her own competitive ability in the fights that follow worker emergence. This trade-off typically results in pleometrotic queens producing fewer brood per capita than solitary queens, effecting a non-linear correlation between queen number and number of first brood workers (Waloff 1957; Taki 1976; Bartz & Hölldobler 1982; Tschinkel 1993; Sommer & Hölldobler 1995; Bernasconi & Keller 1998; but see Rissing & Pollock 1991). As a result, these pleometrotic queens lose less mass than solitary queens (Mintzer 1987; Tschinkel 1993; Bernasconi & Keller 1998; Bernasconi & Strassman 1999), giving them a potential competitive advantage during the process of queen reduction. In *Solenopsis invicta*, the decreased mass loss by pleometrotic versus solitary queens results from presence of additional queens rather than brood care (Bernasconi & Keller 1998). In sharp

contrast, queens of *P. californicus* do not undergo queen reduction, and thus lack the intense competitive pressure to survive mortal fights following worker emergence (see also Cahan & Fewell 2004). This correlates with their similar queen investment strategy in single- versus multiple-queen associations, as manifested by the linear correlation between queen number and brood number (at least up to five queens) and the similar percentage mass loss across queen numbers in both fed and unfed treatments.

Pleometrosis is undoubtedly the derived state in this population, given that nest founding occurs via haplopleometrosis in congeners and in other populations of *P. californicus* (Hölldobler 1976; Rissing et al. 2000). Current data indicate that pleometrosis occurs over a geographical area that is approximately 50–65 km in length; the localized geographical extent of pleometrosis also supports that this behaviour was derived recently. The apparently localized extent of pleometrosis is exemplified by occurrence of a haplopleometrotic population in similar valley grassland habitats at Warner Springs Ranch (unpublished data), which is about 50 km north of the pleometrotic population at Pine Valley; Warner Springs Ranch represents the closest locale north of Pine Valley in which *P. californicus* is known to occur (personal observation). Overall, the patchy distribution of habitat suitable for *P. californicus* suggests that haplopleometrosis and pleometrosis do not co-occur at a site. In contrast, the

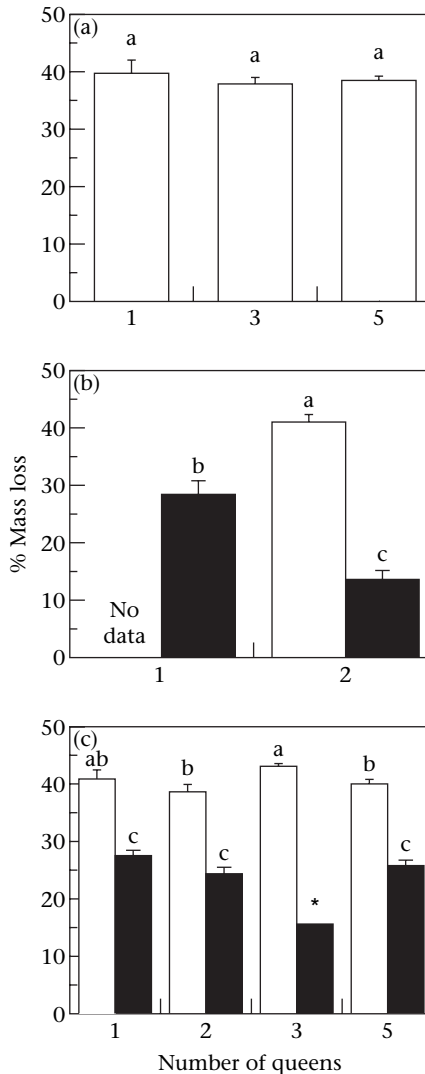


Figure 3. Percentage decrease of initial wet mass for unfed *Pogonomyrmex californicus* queens in a one-factor (queen number) bottle experiment in 1997, $N = 32$ bottles (a), and for fed (■) and unfed (□) *P. californicus* queens in two-factor (queen number*food level) bottle experiments in 1998, $N = 58$ bottles (b) and 1999, $N = 182$ bottles (c). Significant differences ($P < 0.05$) within each panel are denoted by the letters a–c: a > b > c. Groupings are based on an ANOVA followed by a Duncan’s multiple range test. An asterisk indicates a treatment cell that was excluded from statistical analyses because of a low sample size.

seed-harvester ant *Messor pergandei* displays a relatively smooth transition from haplometrosis to pleometrosis in nearby desert habitats; pleometrosis is associated with areas of decreased precipitation and lower plant biomass (Cahan et al. 1998; Cahan 2001b). It is doubtful that such abiotic factors have selected for pleometrosis in *P. californicus*, given that this population occurs in mild upland areas (>900 m elevation), where precipitation and plant biomass are much higher than desert sites inhabited by haplometrotic populations.

The factor(s) selecting for pleometrosis and primary polygyny is unknown at this time. The most likely factor

Table 2. Results of a two-way ANOVA (type IV sum of squares) for the effect of queen number and food level (independent variables) on the percentage loss of wet mass (dependent variable) by queens of *Pogonomyrmex californicus* in the 1999 bottle experiment

Source	% Mass loss by queens		
	df	F	P
Corrected model	6	56.8	<0.001
Food level	1	159.3	<0.001
Queen number	3	3.0	0.03
Food level*queen number	2	0.1	0.94
Error	175		
Total	182		
R^2		0.66	

The analysis included only bottles in which all queens survived.

seems to involve interactions between *P. californicus* and the seed-harvester ant *Messor andrei*. Observations indicate that large numbers of *P. californicus* workers, sometimes several hundred, occur in the external middens of *M. andrei* nests (personal observation). Mature colonies of *M. andrei* may thus exert intense interspecific competition upon incipient colonies of *P. californicus*, and colonies with more queens may better withstand attacks from these neighbours (see Jerome et al. 1998). Primary polygyny and queen adoption may likewise enhance colony survival under these harsh competitive conditions because both behaviours would speed colony growth and maintain colony size in the face of worker depletion.

Queen Survival

Survival of *P. californicus* queens increased in the presence of other queens (i.e. group augmentation of survival; Fig. 1). Interestingly, this effect was most

Table 3. Results of correlation analysis between initial wet mass and percentage mass loss for *Pogonomyrmex californicus* queens in the bottle experiments

Treatment	r	N	P
1998			
1-Fed	0.34	20	0.14
2-Unfed	0.73	14	0.003**
2-Fed	0.37	64	0.002**
1999			
1-Unfed	–0.34	13	0.25
1-Fed	0.28	61	0.029
2-Unfed	0.57	26	0.003*
2-Fed	0.38	58	0.003*
3-Unfed	0.47	117	0.001**
3-Fed	No data		
5-Unfed	0.22	70	0.07
5-Fed	0.41	65	0.001**

Treatment gives the number of queens placed in each bottle and whether or not seeds were provided. Analyses only included data for bottles in which all queens survived. * $P < 0.05$; ** $P < 0.01$ after adjusting P values in each year using the sequential Bonferroni technique.

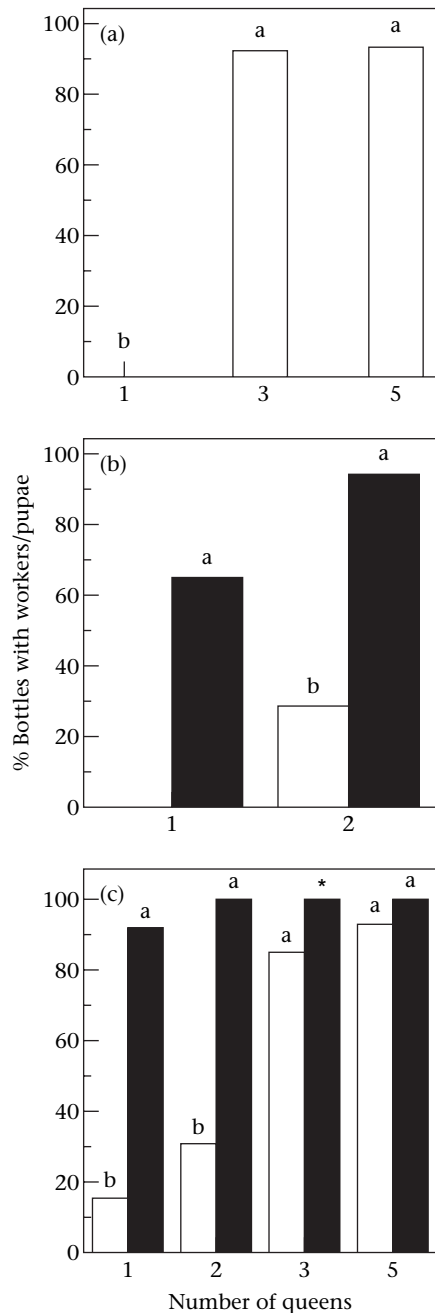


Figure 4. Percentage of bottles in which unfed *Pogonomyrmex californicus* queens produced workers/pupae in a one-factor (queen number) bottle experiment in 1997, $N = 32$ bottles (a), and in which fed (■) and unfed (□) *P. californicus* queens produced workers/pupae in two-factor (queen number*food level) bottle experiments in 1998, $N = 61$ bottles (b) and 1999, $N = 184$ bottles (c). Analyses include only bottles in which all queens survived. Significant differences between treatments are indicated within panels by the letters a–b: a > b. Groupings are based on the Wald chi-square statistic. An asterisk indicates a treatment cell that was excluded from analyses because of a low sample size.

pronounced in the unfed queen treatments, where survival for groups of three and five queens rose to levels similar to those of the respective fed queen treatments. The strength of this increase is compounded by the fact

that starvation (energy depletion) appeared to cause mortality in the one-queen and two-queen unfed treatments, but not in the three-queen and five-queen unfed treatments (Fig. 2), and by the fact that the five-queen unfed treatment had the lowest initial mass among these treatments (Table 1). Thus, survival increased with queen number in the face of lower per capita energy reserves, which supports the occurrence of strong mutual cooperation.

Group augmentation of survival occurs for queens of other ant species (Waloff 1957; Bartz & Hölldobler 1982; Mintzer 1987), and aphid, *Toxoptera citricida*, foundresses (Michaud & Belliure 2000), but the causal mechanism for this augmentation effect is unknown. In vertebrates and some invertebrates, fitness of all group members can increase with group size because of an increased ability to catch or defend food, to detect or repel predators, or to rear young successfully (Clutton-Brock 2003). This study did not explicitly test mechanisms related to group augmentation of queen survival, but data from the bottle experiments address aspects of this phenomenon.

Potential mechanisms that might cause group augmentation of survival among ant queens include enhanced resistance to desiccation (Sigal & Arlian 1982; Miramontes & DeSouza 1996; Yoder & Grojean 1997), reduced mortality from pathogens (Waloff 1957; Rosengaus et al. 1998; Rosengaus & Traniello 2001; Hughes et al. 2002), and a decreased mass loss in groups (Bernasconi & Keller 1998). With regard to desiccation resistance, groups of individuals sometimes survive longer than single individuals (Sigal & Arlian 1982; but see Lighton & Bartholomew 1988), probably because huddling reduces surface area and hence rate of desiccation. It is unlikely that this mechanism occurred in *P. californicus* in the present study because I placed moderate amounts of water in the bottles to minimize this confounding effect. The only other hypothesis that can be examined relates to a reduced mass loss in queens when they are placed in groups (Bernasconi & Keller 1998), thus conferring a lower risk of starvation by the end of the founding stage. This group effect does not occur in *P. californicus* because the percentage mass loss was not associated with number of queens in the fed or unfed treatments.

Enhanced survival of queens in larger groups may also, in part, be explained by differential patterns of mass loss within an association. Individuals could potentially enhance survival using behaviours such as unequal egg laying and/or differential oophagy (Tschinkel 1993; Bernasconi et al. 1997). The positive association between initial wet mass and percentage mass loss that occurred within associations of *P. californicus* queens (see also Mintzer 1987) suggests that larger queens lay more eggs and/or eat fewer eggs than smaller queens.

Benefits of Queen Foraging

Fully claustral queens have a fixed amount of energy that must be allocated in a trade-off between size and number of minim workers (Oster & Wilson 1978; Porter &

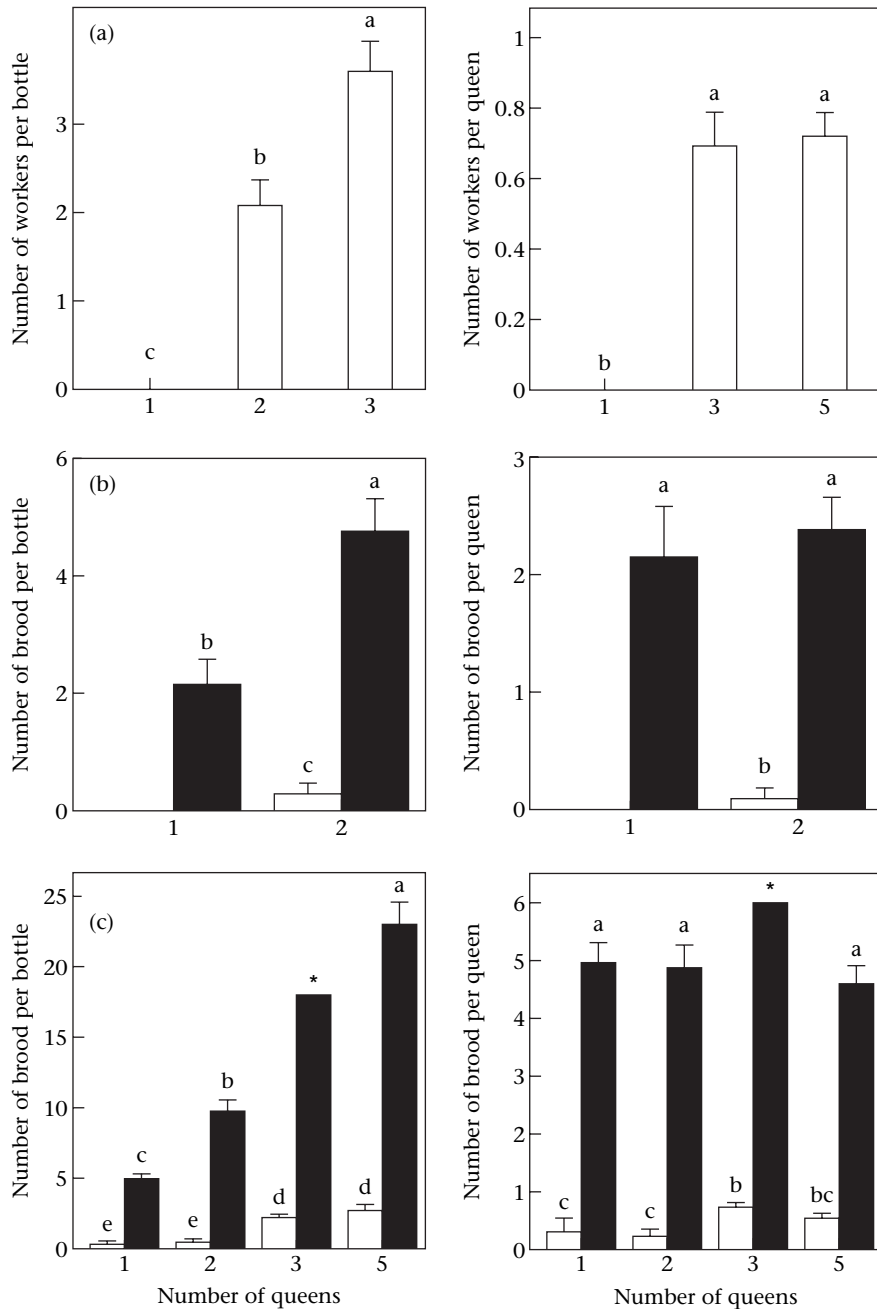


Figure 5. Brood production by unfed *Pogonomyrmex californicus* queens in a one-factor (queen number) bottle experiment in 1997, $N = 32$ bottles (a), and by fed (■) and unfed (□) *P. californicus* queens in two-factor (queen number*food level) bottle experiments in 1998, $N = 61$ bottles (b) and 1999, $N = 184$ bottles (c). Panels on the left show the mean \pm SE number of workers or brood per bottle; panels on the right show the mean \pm SE number of workers or brood per queen. Significant differences ($P < 0.05$) between treatments are indicated within panels by the letters a–e: $a > b > c > d > e$. Groupings are based on an ANOVA followed by a Duncan's multiple range test. See Table 4 for ANOVA results. An asterisk indicates a treatment cell that was excluded from analyses because of a low sample size.

Tschinkel 1986). The primary advantage of foraging appears to lie in providing queens of *P. californicus* with the energy to decouple this trade-off, allowing them to rear significantly more brood than possible by congeners that use only body reserves (Johnson 2002). This companion study used regression analysis to examine the trade-off, however, because single, unfed queens of *P. californicus* lacked the resources to survive and produce

minim workers (Johnson 2002; Hahn et al. 2004). In contrast, multiple, unfed queens of *P. californicus* survive and occasionally produce workers, thus providing an intraspecific test of the benefits of queen foraging. As predicted, queen foraging also decouples this trade-off at the intraspecific level because fed queens produced minim workers that were significantly heavier than those produced by unfed queens (but head width did not differ).

Table 4. Results of a two-way ANOVA (type IV sum of squares) for the effect of queen number and food level (independent variables) on total number of brood produced (dependent variable) by queens of *Pogonomyrmex californicus* in the 1999 bottle experiment (see Fig. 5c)

Source	df	Brood per bottle		Brood per queen	
		F	P	F	P
Corrected model	6	67.9	<0.001	57.6	<0.001
Food level	1	309.5	<0.001	254.4	<0.001
Queen number	3	42.5	<0.001	3.5	0.016
Food level*queen number	2	6.1	0.003	1.0	0.36
Error	176				
Total	183				
R ²		0.70		0.66	

The analysis included only bottles in which all queens survived.

The greater mass of minims probably resulted from their obtaining additional energy from the queen or from food harvested by the queen.

Queens typically lose more than 50% of their body mass to produce the first worker cohort (Tschinkel 1993, 2002;

Johnson 1998), leaving them nearly depleted of energy by the time the first workers begin to forage. Queen foraging decreased this cost by effecting an approximately 50% lower mass loss by fed queens compared with unfed queens (see also Tschinkel 1993). Note, however, that this study provided queens of *P. californicus* with ad libitum food. Consequently, these data probably reflect optimal conditions, such that benefits related to queen mass loss and the number and mass of minim workers probably decrease when food availability is low.

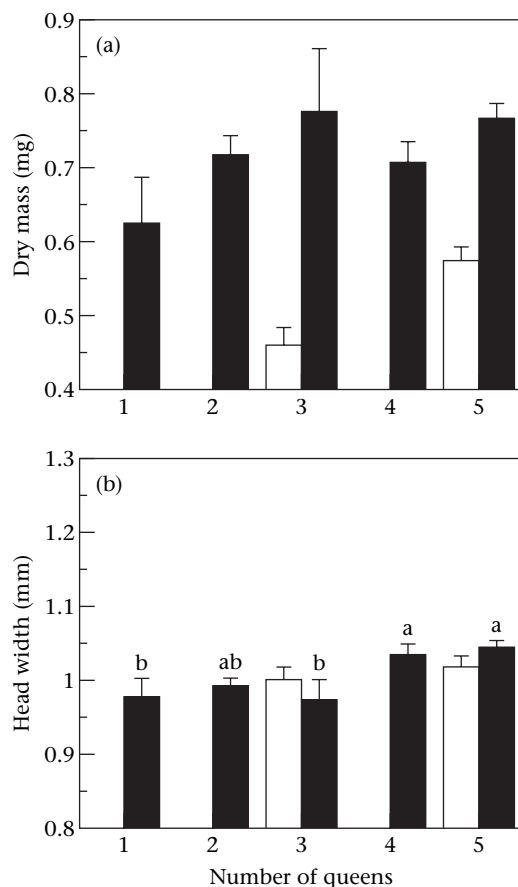


Figure 6. (a) Dry mass ($N = 112$) and (b) head width ($N = 103$) for minim workers of fed (■) and unfed (□) *Pogonomyrmex californicus* queens from two-factor (queen number*food level) bottle experiments. Values are means \pm SE. Dry mass and head width of minims from the fed treatment were compared across number of queens using a one-way ANOVA. Significant differences ($P < 0.05$) between queen numbers are indicated by the letters a–b: a > b. Groupings are based on a Duncan's multiple range test. See Table 5 for ANOVA results.

Brood Production

In most ants, pleometrosis provides a mechanism for fully claustral queens to pool their energy reserves, which results in the association producing more workers and/or producing workers in a shorter time compared with solitary queens (Rissing & Pollock 1988; Bernasconi & Strassman 1999). In contrast, multiple, unfed queens of *P. californicus* produced few, if any, workers because of their lack of energy reserves (Johnson 2002; Hahn et al. 2004). Fed queens, however, produced a large number of workers, with their number increasing linearly as a function of queen number. That these queens were not food limited suggests that the underlying basis of brood production (and consequently of queen energy reserves) relates to factors such as limitations on the number of eggs the queen can lay or the number of brood that she can care for and rear (see also Porter & Tschinkel 1985). As such, brood number can only increase via the resources provided by multiple queens.

Size of minim workers is affected by queen number and food level, but the pattern differs by species. In *Solenopsis invicta*, minim mass increases progressively with queen number (Tschinkel 1993), whereas minim mass does not vary with queen number in *Messor pergandei* (Rissing & Pollock 1991) or *P. californicus* (this study). Presence of food increases minim mass in both *S. invicta* and *P. californicus*. Regardless of the mechanism by which it is attained, an increased minim mass undoubtedly has positive effects on the energy balance of minims and may also increase their longevity and resistance to environmental stress, all of which probably facilitate survival of

Table 5. Results of a two-way ANOVA (type IV sum of squares) for the effect of queen number and food level (independent variables) on the dry mass and head width of minor workers (dependent variables) produced by queens of *Pogonomyrmex californicus* (see Fig. 6)

Source	Minor dry mass (mg)			Minor head width (mm)		
	df	F	P	df	F	P
Corrected model	6	12.0	0.001	6	3.5	0.003
Queen number	4	2.4	0.06	4	5.0	0.001
Food level	1	47.7	0.001	1	0.01	0.98
Queen number*food level	1	3.2	0.08	1	2.3	0.13
Error	105			96		
Total	112			103		
R ²		0.41			0.18	

the incipient colony (see also Tschinkel 1993). Additional studies that dissect patterns of queen mass allocation and the trade-offs between worker number and size are crucial to understanding the evolution of different methods of colony founding.

Overall, this study shows the positive and interactive effects of pleometrosis and queen foraging relative to survival and brood production of incipient ant colonies. Pleometrosis enhances queen survival in a situation in which single queens experience high risk, and foraging provides the resources necessary for queens to produce more and heavier brood. Understanding the evolution of pleometrosis in this system requires surveys to document the occurrence of haplometrotic and pleometrotic populations, along with gathering relevant ecological and physiological data and conducting tests on possible causal factors.

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References

- Adams, E. S. & Tschinkel, W. R. 1995. Effects of foundress number on brood raids and queen survival in the fire ant *Solenopsis invicta*. *Behavioral Ecology and Sociobiology*, **37**, 233–242.
- Bartz, S. H. & Hölldobler, B. 1982. Colony founding in *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae) and the evolution of foundress associations. *Behavioral Ecology and Sociobiology*, **10**, 137–147.
- Beauchamp, R. M. 1986. *A Flora of San Diego County, California*. National City, California: Sweetwater River Press.
- Bernasconi, G. & Keller, L. 1998. Phenotype and individual investment in cooperative foundress associations of the fire ant, *Solenopsis invicta*. *Behavioral Ecology*, **9**, 478–485.
- Bernasconi, G. & Strassman, J. E. 1999. Cooperation among unrelated individuals: the ant foundress case. *Trends in Ecology and Evolution*, **14**, 477–482.
- Bernasconi, G., Krieger, M. J. B. & Keller, L. 1997. Unequal partitioning of reproduction and investment between cooperating queens in the fire ant, *Solenopsis invicta*. *Proceedings of the Royal Society of London, Series B*, **264**, 1331–1336.
- Brockman, H. J. 1997. Cooperative breeding in wasps and vertebrates: the role of ecological constraints. In: *The Evolution of Social Behavior in Insects and Arachnids* (Ed. by J. C. Chao & B. J. Crespi), pp. 347–371. New York: Cambridge University Press.
- Brown, M. J. F. 2000. From the laboratory to the field: the advantage of pleometrotic colony founding. *Trends in Ecology and Evolution*, **15**, 116.
- Cahan, S. H. 2001a. Cooperation and conflict in ant foundress associations: insights from geographical variation. *Animal Behaviour*, **61**, 819–825.
- Cahan, S. H. 2001b. Ecological variation across a transition in colony-founding behavior in the ant *Messor pergandei*. *Oecologia*, **129**, 629–635.
- Cahan, S. H. & Fewell, J. H. 2004. Division of labor and the evolution of task sharing in queen associations of the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology and Sociobiology*, **56**, 9–17.
- Cahan, S., Helms, K. R. & Rissing, S. W. 1998. An abrupt transition in colony founding behaviour in the ant *Messor pergandei*. *Animal Behaviour*, **55**, 1583–1594.
- Cahan, S. H., Blumstein, D. T., Sundström, L., Liebig, J. & Griffin, A. 2002. Social trajectories and the evolution of social behavior. *Oikos*, **96**, 206–216.
- Clutton-Brock, T. 2003. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, **296**, 69–72.
- Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology and Systematics*, **29**, 141–177.
- Emlen, S. T. 1984. Cooperative breeding in birds and mammals. In: *Behavioural Ecology* (Ed. by J. R. Krebs & N. B. Davies), pp. 305–339. Oxford: Blackwell.
- Hahn, D. A., Johnson, R. A., Buck, N. A. & Wheeler, D. E. 2004. Storage protein content as a functional marker for colony-founding strategies: a comparative study within the harvester ant genus, *Pogonomyrmex*. *Physiological and Biochemical Zoology*, **77**, 100–108.
- Hatchwell, B. J. & Komdeur, J. 2000. Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour*, **59**, 1079–1086.
- Herbers, J. M. 1986. Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behavioral Ecology and Sociobiology*, **19**, 115–122.
- Hölldobler, B. 1976. Recruitment behavior, home range orientation and territoriality in harvester ants, *Pogonomyrmex*. *Behavioral Ecology and Sociobiology*, **1**, 3–44.

- Hughes, W. O. H., Eilenbert, J. & Boomsma, J. J. 2002. Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society London, Series B*, **269**, 1811–1819.
- Jerome, C. A., McInnes, D. A. & Adams, E. S. 1998. Group defense by colony-founding queens in the fire ant *Solenopsis invicta*. *Behavioral Ecology*, **9**, 301–308.
- Johnson, R. A. 1998. Foundress survival and brood production in the desert seed-harvester ants *Pogonomyrmex rugosus* and *P. barbatus* (Hymenoptera, Formicidae). *Insectes Sociaux*, **45**, 255–266.
- Johnson, R. A. 2002. Semi-claustral colony founding in the seed-harvester ant *Pogonomyrmex californicus*: a comparative analysis of founding strategies. *Oecologia*, **132**, 60–67.
- Keller, L. 1995. Social life: the paradox of multiple-queen colonies. *Trends in Ecology and Evolution*, **10**, 355–360.
- Lighton, J. R. B. & Bartholomew, G. A. 1988. Standard energy metabolism of a desert seed-harvester ant, *Pogonomyrmex rugosus*: effect of temperature, body mass, group size, and humidity. *Proceedings of the National Academy of Sciences, U.S.A.* **85**, 4765–4769.
- Michaud, J. P. & Belliure, B. 2000. Consequences of foundress aggregation in the brown citrus aphid *Toxoptera citricida*. *Ecological Entomology*, **25**, 307–314.
- Mintzer, A. C. 1987. Primary polygyny in the ant *Atta texana*: number and weight of females and colony foundation success in the laboratory. *Insectes Sociaux*, **34**, 108–117.
- Miramontes, O. & DeSouza, O. 1996. The nonlinear dynamics of survival and social facilitation in termites. *Journal of Theoretical Biology*, **181**, 373–380.
- Oster, G. F. & Wilson, E. O. 1978. *Caste and Ecology in the Social Insects*. Princeton, New Jersey: Princeton University Press.
- Pedersen, J. S. & Boomsma, J. J. 1999. Effect of habitat saturation on the number and turnover of queens in the polygynous ant, *Myrmica sulcinodis*. *Journal of Evolutionary Biology*, **12**, 903–917.
- Pfennig, D. W. 1995. Absence of joint nesting advantage in desert seed harvester ants: evidence from a field experiment. *Animal Behaviour*, **49**, 567–575.
- Porter, S. D. & Tschinkel, W. R. 1985. Fire ant polymorphism (Hymenoptera: Formicidae): factors affecting worker size. *Annals of the Entomological Society of America*, **78**, 381–386.
- Porter, S. D. & Tschinkel, W. R. 1986. Adaptive value of nanitic workers in newly founded red imported fire ant colonies (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, **79**, 723–726.
- Queller, D. C. & Strassman, J. 1998. Kin selection and social insects. *Bioscience*, **48**, 165–175.
- Rissing, S. W. & Pollock, G. B. 1987. Queen aggression, pleometrotic advantage and brood raiding in the ant *Veromessor pergandei* (Hymenoptera: Formicidae). *Animal Behaviour*, **35**, 975–981.
- Rissing, S. W. & Pollock, G. B. 1988. Pleometrosis and polygyny in ants. In: *Interindividual Behavioral Variability in Social Insects* (Ed. by R. L. Jeanne), pp. 179–221. Boulder, Colorado: Westview Press.
- Rissing, S. W. & Pollock, G. B. 1991. An experimental analysis of pleometrotic advantage in the desert seed-harvester ant *Messor pergandei* (Hymenoptera; Formicidae). *Insectes Sociaux*, **38**, 205–211.
- Rissing, S. W., Johnson, R. A. & Martin, J. W. 2000. Colony founding behavior of some desert ants: geographic variation in metrosis. *Psyche*, **103**, 95–101.
- Rosengaus, R. B. & Traniello, J. F. A. 2001. Disease susceptibility and the adaptive nature of colony demography in the dampwood termite *Zootermopsis angusticollis*. *Behavioral Ecology and Sociobiology*, **50**, 546–556.
- Rosengaus, R. B., Maxmen, A. B., Coates, L. E. & Traniello, J. F. A. 1998. Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). *Behavioral Ecology and Sociobiology*, **44**, 125–134.
- SAS Institute. 1997. *SAS/STAT Software: Changes and Enhancements Through Release 6.12*. Cary, North Carolina: SAS Institute.
- Sigal, M. D. & Arlian, L. G. 1982. Water balance of the social insect *Formica exsectoides* (Hymenoptera: Formicidae) and its ecological implications. *Physiological Zoology*, **55**, 355–366.
- Sommer, K. & Hölldobler, B. 1995. Colony founding by queen association and determinants of reduction in queen number in the ant *Lasius niger*. *Animal Behaviour*, **50**, 287–294.
- SPSS. 1990. *SPSS Reference Guide*. Chicago, Illinois: SPSS.
- Taki, A. 1976. Colony founding of *Messor aciculatum* (Fr. Smith) (Hymenoptera: Formicidae) by single and grouped queens. *Physiology and Ecology*, **17**, 503–512.
- Tschinkel, W. R. 1992a. Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. *Ecological Entomology*, **17**, 179–188.
- Tschinkel, W. R. 1992b. Brood raiding in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): laboratory and field observations. *Annals of the Entomological Society of America*, **85**, 638–646.
- Tschinkel, W. R. 1993. Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology*, **33**, 209–223.
- Tschinkel, W. R. 2002. The natural history of the arboreal ant, *Crematogaster ashmeadi*. *Journal of Insect Science*, **2**, 1–15.
- Tschinkel, W. R. & Howard, D. F. 1983. Colony founding by pleometrosis in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology*, **12**, 103–113.
- Vargo, E. L. 1988. Effect of pleometrosis and colony size on production of sexuals in monogyne colonies of the fire ant *Solenopsis invicta*. In: *Advances in Myrmecology* (Ed. by J. C. Trager), pp. 217–225. New York: Brill.
- Waloff, N. 1957. The effect of the number of queens of the ant *Lasius flavus* (Fab.) (Hym., Formicidae) on their survival and on the rate of development of the first brood. *Insectes Sociaux*, **4**, 391–408.
- Yoder, J. A. & Grojean, N. C. 1997. Group influence on water conservation in the giant Madagascar hissing-cockroach, *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). *Physiological Entomology*, **22**, 79–82.