Storage Protein Content as a Functional Marker for Colony-Founding Strategies: A Comparative Study within the Harvester Ant Genus *Pogonomyrmex*

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ABSTRACT

Claustral colony founding, in which new queens rear their first clutch of workers solely from internal reserves, is common in the higher ant subfamilies and is believed to represent a major innovation in ant life histories. The ability to store large amounts of amino acids contained in storage proteins is an essential physiological trait for claustral colony founding by ant queens. To determine whether there is an association between storage protein content and colony-founding strategy, we identified and quantified two major storage proteins in queens of five harvester ant species in the genus *Pogonomyrmex* that differ in colony-founding strategy. Queens of the fully claustral nonforaging species *Pogonomyrmex rugosus* and *Pogonomyrmex maricopa* contained the greatest amount of these proteins. Facultatively foraging semiclaustral *Pogonomyrmex occidentalis* queens contained an intermediate amount. Obligately foraging semiclaustral *Pogonomyrmex californicus* queens from two different populations contained significantly less storage protein than the other independent-founding species. Queens of the dependent-founding social parasite *Pogonomyrmex anergismus* also contained little storage protein. Our results suggest that storage protein content has evolved in concert with colony-founding strategies in the genus *Pogonomyrme* and provides a good functional marker for colony-founding strategy.

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Introduction

Colony founding is the most vulnerable stage in the ant colony life cycle (Hölldobler and Wilson 1990; Bourke and Franks 1995). In response, many strategies to enhance founding success have evolved (Hölldobler and Wilson 1990; Bourke and Franks 1995; Rüppell and Heinze 1999; Brown and Bonhoeffer 2003). Ants display two major types of colony founding. First, in dependent founding, queens start a new nest with a group of workers. Social parasites, in which queens invade mature host colonies and species that adopt queens directly into mature nests, are also considered dependent founders. Second, in independent founding, queens start new colonies without the aid of workers.

Independent founding strategies also fall into two types. First, in semiclaustral founding, queens leave the nest and forage to meet the nutritional needs of themselves and their brood. Second, in fully claustral founding, queens seal themselves in the nest after mating and raise the first clutch of workers entirely from body reserves. Fully claustral colony founding is thought to be a major innovation in higher ants because it eliminates the need for queens to leave the nest to forage, reducing their exposure to predators, desiccation, and other sources of mortality (Hölldobler and Wilson 1990; Bourke and Franks 1995; Brown and Bonhoeffer 2003). The potential costs of queen foraging make it notable that several species in the higher ant subfamilies *Myrmicinae* and *Formicinae* have returned to semiclaustral founding when most of their congeners remain fully claustral (Hölldobler and Wilson 1990; Brown and Bonhoeffer 2003).

The diversity of founding strategies in ants is well documented, but the physiological correlates of these strategies are poorly known. Stille (1996) showed an association between the relative size of queens compared with their workers and founding. Independent single claustral foundresses were proportionally the largest, followed by independent foundresses that start colonies in multiple queen groups, followed by dependent social parasites. No semiclaustral species were considered. Keller and

Table 1: Collection locales (state, county, locale) and sample size for species of *Pogonomyrmex* that were analyzed in this study

Species	Latitude	Longitude	Elevation (m)	Sample Size[a]	Year
Pogonomyrmex californicus complex:					
P. californicus (Buckley); Arizona, Maricopa, Salt River Recreation Area, Blue Point Bridge	33°33′N	111°34′W	425	14 (9)	1999
				12 (6)	2001
P. californicus (Buckley); California, San Diego, Cameron Guard Station	32°43′N	116°28′W	990	22 (11)	2001
Pogonomyrmex maricopa Wheeler; Arizona, Pinal, 2.0 km north of Superior	33°18′N	111°08′W	825	14 (5)	1999
				6 (2)	2000
Pogonomyrmex occidentalis complex:					
P. occidentalis (Cresson); Arizona, Yavapai, Ash Fork	35°13′N	112°30′W	1,555	9 (3)	1999
				6 (3)	2001
P. occidentalis (Cresson); Arizona, Yavapai, Chino Valley	34°47′N	112°27′W	1,410	10 (4)	2001
Pogonomyrmex barbatus complex:					
Pogonomyrmex rugosus Emery; Arizona, Maricopa, 1.8 km southwest of McCartney Road and I-10	32°56′N	111°42′W	430	14 (8)	1999
				11 (4)	2001
Pogonomyrmex anergismus Cole; New Mexico, Grant, 6 km east of Separ	32°10′N	108°22′W	1,375	5 (1)	1998

Note. Taxonomy follows Bolton (1995). Voucher specimens are deposited in the R. A. Johnson collection, Tempe, Arizona.

[a] Number of alate queens (number of colonies) used to measure storage proteins.

Passera (1989) and Johnson et al. (1996) both showed a strong association between founding strategy and fat reserves. Independent fully claustral foundresses had the greatest relative fat stores, followed by independent semiclaustral foundresses, followed by dependent foundresses. However, dependent-founding queens still contained significant fat reserves. Dependent and semiclaustral queens do not require fat for brood provisioning, but they do require significant energy reserves to support mating, dispersal, and either digging nests or entering host colonies.

Fat reserves support multiple metabolic functions in queens besides provisioning brood, and body size is related to numerous functions, such as increased survival or dispersal ability (Nylin and Gotthard 1998; Brown and Bonhoeffer 2003). Therefore, protein reserves may be more closely linked to the colony-founding method and may provide a better predictor than either of these two traits. Ant queens require significant amounts of amino acids to rear their first brood (Wheeler and Buck 1995, 1996). Semiclaustral foundresses can obtain amino acids through foraging, and dependent foundresses can obtain them from their hosts, but fully claustral foundresses are limited to internal amino acid reserves accumulated during maturation in their natal colony. These amino acids come from two sources, histolysis of the flight muscles in the thorax and storage proteins in the hemolymph and fat body. Histolysis of the flight muscles begins immediately after queens locate a nest site, and the amino acids released are used to produce brood (Janet 1907; Wheeler and Buck 1996).

Storage proteins are large molecular-weight proteins associated with metamorphosis and reproduction in numerous insect species (Telfer and Kunkel 1991; Wheeler and Martinez 1995; Seo et al. 1998; Pan and Telfer 1999, 2001). In ants, these proteins occur in larvae, where they are used during metamorphosis; in queens, these proteins are used for egg production and brood provisioning during colony founding and occasionally in workers (Wheeler and Buck 1995; Wheeler and Martinez 1995). Wheeler and Buck (1996) demonstrated that storage protein reserves equal or surpass the flight muscles as an amino acid source during founding. Therefore, storage proteins are essential for a fully claustral queen to rear her first brood.

We tested the hypothesis that storage protein content is associated with colony-founding strategy by quantifying storage proteins in alate queens of five harvester ant species differing in founding strategies (Table 1). We predicted that storage protein content would increase as the method of colony founding changes from dependent to obligately semiclaustral to facultatively semiclaustral to fully claustral. Queens of *Pogonomyrmex rugosus* Emery and *Pogonomyrmex maricopa* Wheeler are fully claustral, producing their first brood of workers solely from internal reserves (Johnson 2002). *Pogonomyrmex occidentalis* (Cresson) queens are facultatively semiclaustral; queens

Table 2: Summary of ecological and physiological data related to nest founding for the species of *Pogonomyrmex* used in this study

Species	Method of Nest Founding	Queen Foraging	Queen Dry Mass (mg)	Queen : Worker Mass Ratio	Fat Content (%)
Pogonomyrmex rugosus	Independent	No	24.9 ± .3	4.6 ± .2	40.5 ± .8
Pogonomyrmex maricopa	Independent	No	16.4 ± .4	5.1 ± .2	41.8 ± .7
Pogonomyrmex occidentalis	Independent	Yes, facultative	13.6 ± .3	5.2 ± .3	45.4 ± .6
Pogonomyrmex californicus:					
Arizona population	Independent	Yes, obligate	5.7 ± .3	2.8 ± .1	31.8 ± .8
California population	Independent	Yes, obligate	6.2 ± .4	3.8 ± .2	39.0 ± .9
Pogonomyrmex anergismus	Dependent	No	4.3 ± .8	NA	27.2 ± 1.9

Note. Data are presented as means ± 1 SE. From Johnson et al. (1996), Johnson (2002), and R. A. Johnson, unpublished data. NA = not applicable because *P. anergismus* is workerless.

forage in the field but are capable of rearing the first brood solely from stored reserves (Billick et al. 2001; R. A. Johnson, unpublished data). *Pogonomyrmex californicus* (Buckley) queens are obligately semiclaustral. In Arizona populations, *P. californicus* queens found colonies singly and must forage to produce brood. Conversely, in a Southern California population, queens found colonies in groups where one or several individuals forage (Rissing et al. 2000; R. A. Johnson, unpublished data). We examined queens from both populations to examine potential intraspecific variation associated with differences in founding. As an example of a dependent founding species, we examined queens of the workerless social parasite *Pogonomyrmex anergismus* Cole, which found colonies by entering a mature colony of *P. rugosus* or *Pogonomyrmex barbatus* and duping the workers into rearing their brood (Cole 1968; Johnson 1994).

Material and Methods

Specimen Collection

Mature alate queens of five species of *Pogonomyrmex* were collected from their natal nests during the mating flight season in 1999 and 2001 (Table 2). Because of rarity, our analyses included queens from only one colony of *Pogonomyrmex anergismus*; all other species were collected from multiple colonies at multiple sites. Individuals from each nest were placed in 50-mL centrifuge tubes with a piece of moistened paper towel to prevent desiccation, taken to the laboratory, and frozen individually at −70°C within 36 h.

Sample Preparation and Electrophoresis

Queens were removed from the freezer, freeze-dried to constant mass, weighed to the nearest microgram, and returned to −70°C until analysis. For electrophoresis, each queen was homogenized in 0.3 mL of Tris-buffered saline (20 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 7.5) containing the following protease inhibitors: leupeptin, antipain, chymostatin, aprotinin (all at 17 μg/mL), 1.7 μg/mL pepstatin A, and 1 mM 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF, an irreversible serine protease inhibitor). Samples were homogenized in 1.5-mL plastic microcentrifuge tubes with a plastic pestle attached to a rotating shaft driven by a variable-speed motor. Each sample was ground for 60–90 s at 250 rpm and then centrifuged at 12,000 *g* for 20 min at 4°C. This procedure isolated soluble proteins in the supernatant.

Storage protein content differed greatly among queens of the five species. Consequently, we diluted the supernatant for each species to bring it into a quantifiable range. Samples were diluted using the Tris-buffered saline protease inhibitor solution as follows: 1 : 10 for *Pogonomyrmex rugosus* and *Pogonomyrmex maricopa,* and 1 : 5 for *Pogonomyrmex occidentalis.* No dilution was necessary for *P. anergismus* or *Pogonomyrmex californicus* queens from Arizona or California. From these dilutions, 10 μL aliquots were taken, mixed with 20 μL of sample loading buffer, and loaded onto gels.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to denature native hexamerins into their subunits for quantification. SDS-PAGE was performed following Laemmli (1970) and adapted to 6%–15% gradient slab gels. Gels were run at 20 mA constant current and stained with 0.1% Coomassie Brilliant Blue R 250 dissolved in a 5 : 4 : 1 solution of methanol, water, and acetic acid. Gels were destained in the same solution without Coomassie. To quantify storage proteins, gels were scanned at 633 nm using a laser densitometer (LKB Ultrascan XL). Standard curves were generated using known quantities of bovine serum albumin (BSA) that ranged from 0.2 to 4.0 μg. Each gel contained internal standards of 1.0 and 3.0 μg BSA to correct for gel-to-gel variation. Our focal proteins separated poorly on the 10 × 8-cm SDS gels (Hoefer Mighty Small II). Consequently, we estimated the amount of storage proteins in each species by summing the densities of the two storage protein bands.

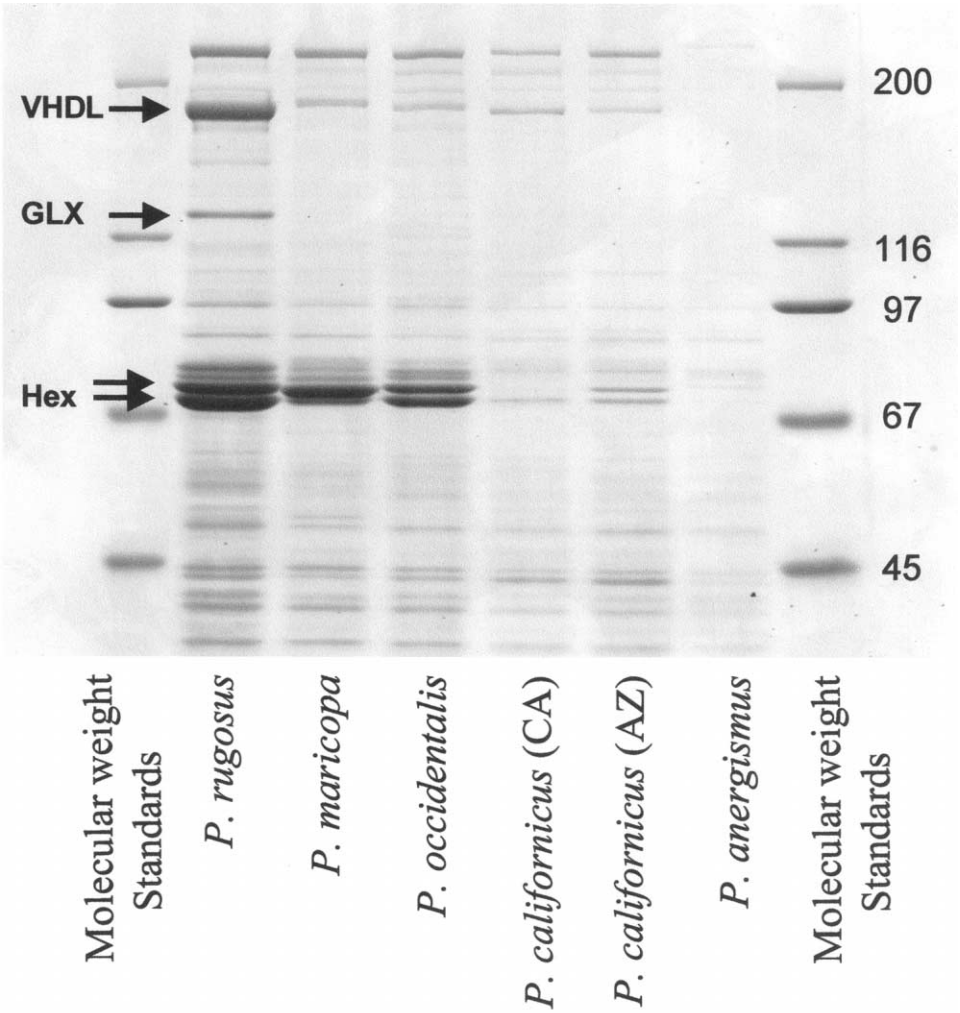

Figure 1. A 6%–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gradient gel, showing the two major storage hexamerins in *Pogonomyrmex* queens. Both populations of *Pogonomyrmex californicus* and *Pogonomyrmex anergismus* contain significantly less storage hexamerins than the fully claustral or facultatively foraging semiclaustral species. Each sample was extracted in 300-μL buffer, and 10 μg of extract was loaded. *Hex* = storage hexamerins; *GLX* = high glutamine/glutamic acid storage protein; *VHDL* = very high-density lipoprotein; *CA* = California; *AZ* = Arizona.

Statistical Analyses of Among-Species Differences

We analyzed the data using two separate nested ANOVAs, one for total storage protein content and one for percent storage protein. Independent variables were nested in this order: species, year, locale, and colony. An a posteriori Tukey's honestly significant difference (HSD) test was used to assess species differences. No transformations of the data were necessary to meet the assumption of homogeneity of variances. Populations of *P. californicus* from Arizona and California were considered separately in all cases.

Molecular Weights

Samples were run on large-format 20 × 16-cm SDS-PAGE 6%–15% gradient gels to obtain adequate separation of the subunits of the proteins to estimate their molecular weights. We estimated molecular weights of proteins in SDS-PAGE using standards in the high molecular weight calibration kit (Bio-Rad) containing the following: myosin (200,000 kDa), galactosidase (116,250 kDa), phosphorylase B (97,400 kDa), BSA (66,200 kDa), and ovalbumin (45,000 kDa).

Large format 20 × 16-cm 4%–12% native-PAGE gradient gels were used to assess the molecular weights of the proteins in their native (undenatured) state. For native-PAGE we used the buffer system of Laemmli (1970), with SDS and beta-mercaptoethanol omitted. We estimated molecular weights of native proteins using the following standards obtained from Pharmacia: thyroglobulin (669,000 kDa), ferritin (440,000), catalase (232,000 kDa), lactate dehydrogenase (140,000 kDa), and albumin (67,000 kDa).

Table 3: Amino acid composition of *Pogonomyrmex* storage hexamerins compared with values for the average ant storage hexamerin and the average protein in the SWISS-PROT database

	Pogonomyrmex rugosus		*Pogonomyrmex maricopa*		*Pogonomyrmex occidentalis*		Average Ant	Average Protein SWISS-
Amino Acid (mol %)	Hex 1	Hex 2	Hex 1	Hex 2	Hex 1	Hex 2	Hexamerin[a]	PROT
Asparagine/aspartic acid	11.44	10.71	11.14	10.61	9.76	10.28	13.70	9.61
Glutamine/glutamic acid	8.84	9.23	8.88	9.24	9.19	10.45	9.20	10.40
Serine	5.64	6.42	6.36	5.09	5.21	5.57	6.10	7.08
Glycine	6.19	9.74	8.36	9.28	9.36	7.26	8.00	6.85
Histidine	5.27	2.15	4.83	1.50	2.51	5.22	2.60	2.24
Arginine	4.13	4.03	3.84	3.86	3.98	4.31	4.10	5.19
Threonine	5.22	4.54	4.99	4.6	4.53	5.22	4.50	5.58
Alanine	5.41	6.33	5.58	6.59	6.49	5.15	5.50	7.61
Proline	6.48	6.39	6.77	5.27	5.11	6.23	5.60	4.80
Lysine	6.03	5.98	5.39	5.91	5.79	5.31	6.00	5.97
Valine	8.49	5.42	7.90	6.78	7.54	7.98	6.20	6.61
Isoleucine	5.12	4.88	4.91	6.17	5.92	5.19	5.20	5.85
Leucine	9.23	9.73	9.47	10.73	11.15	10.22	9.20	9.53
Phenylalanine	5.70	6.81	4.88	7.54	6.90	5.29	6.60	4.10
Tyrosine	6.32	6.68	6.13	6.26	5.92	5.91	6.30	3.16

[a] Values were taken from Wheeler and Buck (1995).

Amino Acid Composition

Purification of storage protein subunits was performed using 20 × 16-cm large-format 6%–15% SDS-PAGE gradient gels, which facilitated the separation of the bands representing the subunits of our two storage proteins. Proteins were electrophoretically transferred to a PVDF (polyvinylidene difluoride) membrane (Problott). After staining with 0.1% Coomassie brilliant blue R 250, bands of interest were cut out. Amino acid analysis was performed at the University of Arizona Biotechnology Core Facility using a dedicated Applied Biosystems Model 420A Amino Acid Analyzer with automatic hydrolysis (vapor phase at 160°C for 100 min using 6M HCL) and precolumn phenylthiocarbamyl-derivatization.

Statistical Analyses of Amino Acid Composition

Insect storage hexamerins generally contain higher than average amounts of the aromatic amino acids phenylalanine and tyrosine (Telfer and Kunkel 1991). We assessed whether the putative *Pogonomyrmex* storage hexamerins contained higher than average amounts of these two amino acids by plotting phenylanine content against tyrosine content for each *Pogonomyrmex* protein. These values were contrasted with those of the average ant hexamerin derived from Wheeler and Buck (1995) and the average composition of all proteins entered in the SWISS-PROT database (http://us.expasy.org/tools/pscale/A.A.SWISS-PROT.html). The amino acid composition of most proteins is astonishingly similar across numerous prokaryotic and eukaryotic taxa (King and Jukes 1969). Therefore, we assumed that the average amino acid composition across all taxa in the SWISS-PROT database was representative of the average amino acid composition of nonstorage hexamerin proteins in harvester ants. Similarity was assessed graphically by generating a 99% confidence interval ellipse around the *Pogonomyrmex* data. Values falling outside of the 99% confidence interval ellipse were considered significantly different from the putative *Pogonomyrmex* storage proteins. If the *Pogonomyrmex* proteins were ant storage hexamerins, we would predict that the average ant hexamerin would fall within the ellipse, whereas the average protein from the SWISS-PROT database would fall outside the ellipse. All statistical analyses were performed using the JMP IN statistical package (SAS Institute 1996).

Results

Protein Identification

Known ant storage proteins fall into three classes: (1) hexamerins, which contain moderately high amounts of aromatic amino acids (range = 7.3%–9.4%), (2) proteins that contain a high amount of glutamine/glutamic acid (high GLX, range = 20.3%–22.8%), and (3) very high density lipoproteins (VHDL; Wheeler and Buck 1995; Wheeler and Martinez 1995). The two major storage proteins found in *Pogonomyrmex* queens were hexamerins. Queens of the fully claustral species *Pogonomyrmex rugosus* and *Pogonomyrmex maricopa* and the facultatively semiclaustral species *Pogonomyrmex occidentalis* had the highest amounts of these two storage proteins (Fig. 1). In con-

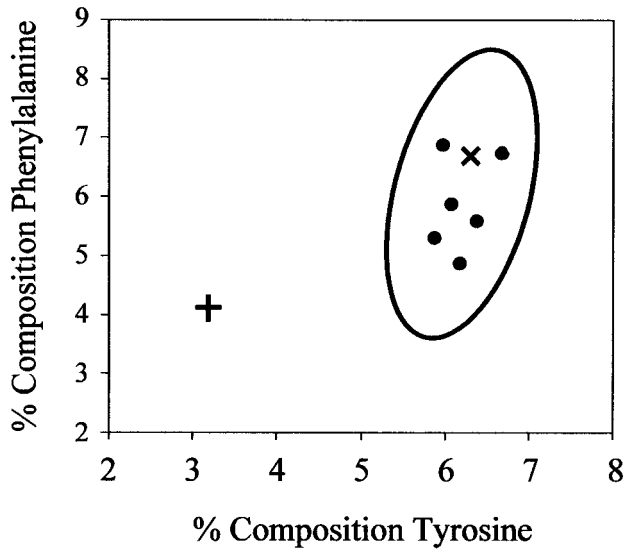

Figure 2. Composition of the aromatic amino acids, phenylalanine and tyrosine, for the storage hexamerins from *Pogonomyrmex rugosus*, *Pogonomyrmex maricopa*, and *Pogonomyrmex occidentalis* (*filled circles*); the average ant hexamerin (*x*) from Wheeler and Buck (1995); and the average protein in the SWISS-PROT database (*plus sign*). The black ellipse is the 99% confidence interval for the *Pogonomyrmex* data; points represent each of the two bands from the three species.

trast, both storage hexamerins were much less abundant in both populations of the obligately semiclaustral *Pogonomyrmex californicus*, and the dependent-founding social-parasite *Pogonomyrmex anergismus*. High GLX and VHDL storage proteins occurred very sporadically in queens of these five species, although apoproteins of the appropriate size for each of these classes of proteins can be observed in the *P. rugosus* queen in Figure 1. Therefore, we conclude that these classes of storage proteins are unlikely to be significant factors in the physiology of colony founding in these ants and are not considered further.

Molecular Weights

Mobility of the two focal proteins relative to known standards on denaturing SDS-PAGE gels yielded an estimated molecular weight for the apoproteins of 79 and 80 kDa, whereas the native proteins were both approximately 500 kDa, typical sizes for both subunits and native insect hexamerins, respectively (Telfer and Kunkel 1991; Wheeler and Buck 1995).

Amino Acid Composition

The mol percentage amino acid compositions for the putative storage hexamerins from three of our five species *P. rugosus*, *P. maricopa*, and *P. occidentalis* are listed in Table 3. *Pogonomyrmex californicus* and *P. anergismus* queens contained insufficient amounts of storage proteins for compositional analysis. The average protein fell well outside of the 99% confidence ellipse for our *Pogonomyrmex* storage hexamerins, with the latter containing a much higher tyrosine content and a slightly higher phenylalanine content (Fig. 2). In contrast, the aromatic amino acid compositions of our *Pogonomyrmex* storage hexamerins encompassed the average value for phenylalanine and tyrosine content of the known ant hexamerins (Fig. 2). Therefore, the aromatic amino acid compositions of our *Pogonomyrmex* proteins are consistent with the storage hexamerins of other ant species.

Protein Quantification across Species

The full-model nested ANOVAs were highly significant and explained >95% of the variance in both total and percent storage protein content (Table 4). Species was the largest contributor to both models, with species differences strongly associated with the colony-founding method. Storage protein content was lowest in the dependent-founding parasite *P. anergismus* and in both populations of the obligately foraging semiclaustral *P. californicus*, intermediate in the facultatively foraging semiclaustral *P. occidentalis*, and highest in the fully claustral *P. rugosus* and *P. maricopa* (Fig. 3). While *P. rugosus* and *P. maricopa* did not differ in percentage dry weight storage protein, the larger queens of *P. rugosus* contained significantly higher total storage hexamerin content than *P. maricopa* (Fig. 3). Colony was also a significant effect in both analyses, although it

Table 4: Results of nested ANOVAs for total storage protein and percent storage protein per queen

	Total Storage Protein per Queen				Queen Dry Mass Storage Protein (%)			
Source	df	*F*	*P*	Whole Model (%)	df	*F*	*P*	Whole Model (%)
Whole model	71	41.4	<.001	100	71	28.0	<.001	100
Species	5	240.5	<.001	77.8	5	186.4	<.001	75.0
Year (species)	4	7.2	<.001	1.8	4	.7	.599	.2
Locale (species, year)	3	.9	.406	.2	3	4.0	.014	1.0
Colony (species, year, locale)	59	5.3	<.001	20.2	59	5.0	<.001	23.8
Error	46				46			
Total	117			Whole model, $r^2 = .99$	117			Whole model, $r^2 = .98$

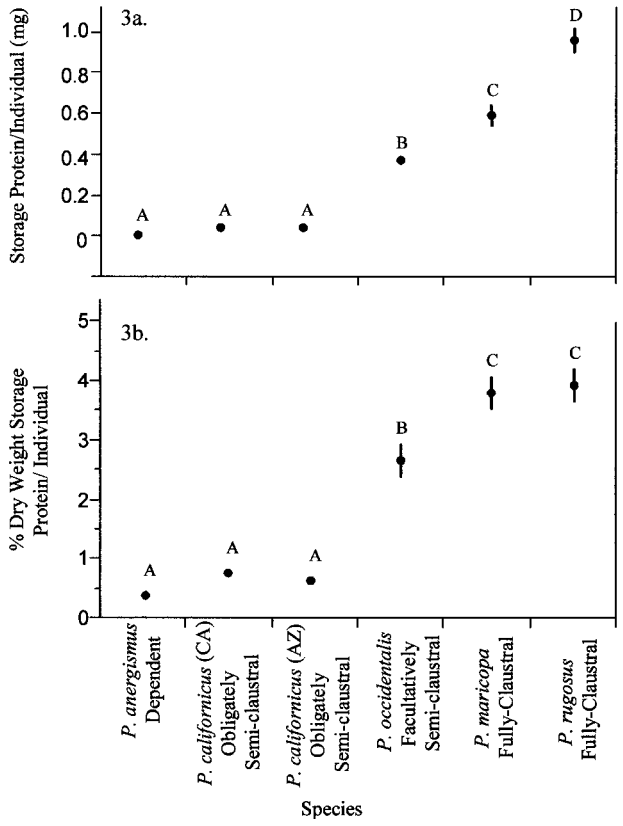

Figure 3. Total mass (*a*) and percentage dry mass (*b*) of storage hexamerins in *Pogonomyrmex* queens (adjusted means from the nested ANOVAs ± 1 SE). Error bars are subsumed within the points for some groups. Significant differences among groups are denoted by the letters A–D: D > C > B > A. Groupings are based on a nested ANOVA followed by Tukey's HSD test. See Table 1 for sample sizes. Mode of colony founding is given beside the name of each species. *CA* = California; *AZ* = Arizona.

explained far less of the full model variance than did species (Table 4). Year and collection locale contributed little to either whole model, and their effects differed between the two analyses (Table 4).

Discussion

Storage Protein and Mode of Colony Founding among Pogonomyrmex *Species*

Ours is the first study to quantify storage protein content in ant queens in relation to colony-founding strategy. Queens of the five harvester ant species in this study contained two major hexameric storage proteins. Species and colony of origin had large significant effects on storage protein content, accounting for roughly three-quarters and one-quarter of the whole model variance.

In agreement with our predictions, the fully claustral *Pogonomyrmex rugosus* and *Pogonomyrmex maricopa* queens contained the highest amounts of storage proteins. Facultatively foraging semiclaustral *Pogonomyrmex occidentalis* queens contained an intermediate amount of storage proteins, and obligately foraging *Pogonomyrmex californicus* queens from both the single foundress Arizona population and the multiple foundress California population and the dependent-founding social parasite *Pogonomyrmex anergismus* contained the lowest amounts. The low storage protein levels in *P. californicus* and *P. anergismus* queens may be indicative of the minimum protein reserves necessary for queens to produce eggs, when feeding larvae from internal reserves is not necessary.

Johnson (2002) found that *P. californicus* queens contained less fat reserves than claustral-founding *P. maricopa* and *P. rugosus* queens or facultative semiclaustral *P. occidentalis* queens (Table 1). Although both fat and storage protein content correlate with colony-founding mode, queens of all founding types store significant fat reserves, probably for use as fuel in flight and nest excavation. Therefore, storage protein content is a better predictor of colony-founding mode in *Pogonomyrmex* (Table 1; Fig. 3).

In addition to containing less stores, *P. californicus* and *P. anergismus* queens are small compared with their claustral-founding congeners, and *P. californicus* has a significantly smaller queen-to-worker mass ratio than the other *Pogonomyrmex* species in this study. *Pogonomyrmex californicus* and *P. anergismus* are in separate species groups within the genus, indicating that small body size and poor queen provisioning have evolved at least twice in association with nonclaustral founding. Interestingly, small queen size and poor provisioning are associated with divergent life histories in these two species; one is an obligate forager and the other a social parasite.

Potential Advantages of Nonclaustral Founding

Large queens rich in nutritional stores may enjoy increases in survival in the perilous founding period by avoiding foraging-associated mortality. However, trade-offs may exist between increased founding survival and the costs of producing large well-provisioned queens. Dependent or independent semiclaustral founding species can produce smaller sexuals provisioned with less fat and protein. Therefore, colonies could produce more sexuals for a given amount of resources. The colony-level reproductive advantage of producing more queens could have contributed to the evolution of small body size and minimal stores, followed by queen foraging in *P. californicus*.

Another potential selective advantage of queen foraging is the ability to break the size/number trade-off inherent to queens producing their first brood from limited internal reserves. In claustral-founding species, individuals in the first cohort are usually significantly smaller than later cohorts (Porter and Tschinkel 1986; Hölldobler and Wilson 1990). If some minimum number of workers is necessary for successful colony

founding, queens with fixed reserves will have to trade off worker number and size. While *P. californicus* queens are smaller and contain less fat and protein reserves than all the other species, when provided access to food, they produced more and proportionally heavier brood than their fully claustral congeners, breaking the size/number trade-off inherent in claustral founding (Johnson 2002). If this ability to break the size/number trade-off was selected for, foraging could release *P. californicus* queens from requiring large nutrient reserves and a large body to contain them, allowing the evolution of smaller body size and scant stores.

Alternatively, the evolution of small body size and scant reserves in *P. californicus* could have been the product of some other selective force on founding strategy, such as low mortality during the queen foraging period (Brown and Bonhoeffer 2003). In Arizona, *P. californicus* queens generally initiate mating flights and begin founding 6–8 wk earlier than their congeners in the same habitats (R. A. Johnson, unpublished data). This difference in timing may be associated with differences in foraging mortality that would contribute to the evolution of semiclaustral founding in *P. californicus* but not its later founding congeners (Brown and Bonhoeffer 2003).

Other Factors Affecting Storage Protein Content

The significant effect of colony on storage protein content showed there is notable colony-level variation in queen provisioning, which could have implications for the evolution of reproductive strategies both within and between populations. Alternatively, colony-level effects could be due to differences in the maturation of queens between colonies. Nutrient reserves are accumulated by queens after eclosion and before flying (Boomsma and Isaaks 1985; Nielsen et al. 1985). All the alates collected in this study appeared to be mature, and mating flights had begun in the populations at the time of collection. However, asynchrony in mating flight schedules between ant colonies within a population is common, and it is possible that some of the colonies in the study had not completed provisioning their sexuals (Hölldobler and Wilson 1990). In addition, year and collection locale had relatively small effects in our model, but because each factor had a small but significant effect on storage protein content, these factors also merit further study.

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