

Two Closely Related Species of Desert Carpenter Ant Differ in Individual-Level Allocation to Fat Storage

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ABSTRACT

Comparison of closely related species that differ in their life histories is a powerful method for studying the underlying physiological mechanisms contributing to life-history variation. I investigated whether two closely related members of the *Camponotus festinatus* species complex of desert carpenter ants, *C. nr. festinatus* Desert Light and *C. nr. festinatus* Desert Dark, differed in their life-history tactics with respect to fat storage. Newly mated queens were collected in the field, and colonies were reared under common conditions in the laboratory for 2 yr before sampling. I show that the two species differ in fat storage at the individual level. While the basic scaling relationship between lean mass and fat content did not differ between the two species, Dark workers and soldiers stored significantly more fat per unit lean mass than Light workers or soldiers. There were no significant demographic differences in the proportions of workers or soldiers involved in fat storage between the two species, although there was a trend toward Light colonies having a greater proportion of soldiers storing large amounts of fat. There was also no significant difference in the total amount of fat stored by the two species at the colony level. The detection of strong individual-level effects but no colony-level effects was likely due to the low statistical power of colony-level analyses. Showing that these two closely related species differ in fat storage at the individual level in a common environment demonstrates their utility as a model for understanding the physiological and behavioral mechanisms regulating life-history variation in fat storage in ants.

Introduction

A fundamental problem in physiological ecology is identifying mechanisms that control variation in patterns of resource al-

location to phenotypic and life-history traits (Roff 1992; Stearns 1992; West-Eberhard 2003). One approach to identifying mechanisms begins with comparing allocation patterns between closely related species with differing life histories. Social insects provide an interesting perspective on resource allocation because selection occurs at a number of levels in insect societies, with the colony as an important unit of selection (Bourke and Franks 1995). However, most colony-level traits are products of integrating multiple individual-level traits (Bourke and Franks 1995). Therefore, understanding resource allocation in social insects necessitates studying traits at both individual and colony levels.

Allocation of nutritional resources to storage is an important physiological life-history trait in most organisms. Storage is functionally important because it allows organisms to decouple times of resource need from times of availability, which allows organisms to optimally time their investments into various life-history traits and survive periods of nutrient limitation. Among social insect species, nutrients can be stored either internally as fats, carbohydrates, and proteins or externally as honey, pollen, or seeds (Hölldobler and Wilson 1990; Hunt and Nalepa 1994; Martinez and Wheeler 1994). In the ants, the most common type of nutrient reserve is internal fat storage, whereby workers accumulate triglycerides in the fat body during times of excess nutrient availability and mobilize these stores from the fat body during times of nutrient limitation, passing them to colony members through either lipid-rich oral secretions from the postpharyngeal gland or unfertilized trophic eggs from queens and workers containing functional ovaries (Voss 1981; Hölldobler and Wilson 1990).

In social insects, nutrient storage is a central characteristic of seasonal life cycles (Ricks and Vinson 1972; Jensen 1978; Tschinkel 1987, 1993, 1998; Hunt and Nalepa 1994; Moritz 1994; Bourke and Franks 1995; Børgesen 2000). For example, in many ant species, the timing of sexual production and maximal nutrient need occurs during the spring, preceding the peak of nutritional resource availability, which usually occurs in the summer (Ricks and Vinson 1972; Jensen 1978; Tschinkel 1987, 1993, 1998; Børgesen 2000). Several species of ant have been shown to accumulate fat reserves during times of high resource availability that are depleted coincident with sexual production several months later (Ricks and Vinson 1972; Jensen 1978; Tschinkel 1987, 1993, 1998; Børgesen 2000). In these cases, nutrient reserves provide the resources necessary for colonies to produce sexuals at the most advantageous time, even if resource availability is low at that time. In addition, fat reserves

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are important for the survival of colonies in climates where nutritional resources can be unavailable for months at a time, such as north-temperate ants that experience long overwintering periods (Lachaud et al. 1992; Cushman et al. 1993; Kaspari and Vargo 1995).

Nutrient storage also affects individual-level behavior in social insects and has been correlated with colony-founding strategies. Social insect workers performing risky off-nest tasks, such as foraging or defense, tend to be older and leaner (Porter and Jorgensen 1981; O'Donnell and Jeanne 1995; Blanchard et al. 2000; Toth and Robinson 2005). The adaptive value of exposing low-cost "disposable" workers to risky tasks has long been recognized, but it was unclear whether declining fat stores motivated individuals to forage or were a product of the high energetic demands of foraging. Recent pharmacological manipulations of fat storage in honeybee workers has shown that, among same-age individuals, leaner workers were more likely to initiate foraging, demonstrating a causative effect of fat stores on behavior (Toth et al. 2005). This rationale has been extended by Hunt and Amdam (2005), who have developed a model predicting that nutritional reserves affect foraging behavior and dominance interactions during the founding phase of the colony life cycle, thereby promoting division of labor in groups of *Polistes* wasps, a model system for the evolution of social behavior. Furthermore, nutrient reserves are generally correlated with colony-founding strategies among ant species. Queens of independent claustral-founding species, which seal themselves in underground chambers and rear their first brood without foraging, contain greater reserves than either species in which queens forage during the founding period or species that found colonies dependent either on workers from the natal nest or as social parasites (Keller and Passera 1989; Passera and Keller 1990; Johnson 2002; Hahn et al. 2004). Therefore, understanding the regulation of nutrient reserves, particularly fat storage, at the individual and colony levels is critical to understanding both the division of labor characteristic of social insect colonies and the evolution of important colony life-history traits, such as the timing of reproduction, founding mode, and overwintering behavior.

Like all social insects, ant colonies can increase their fat stores through both individual- and colony-level tactics (Tschinkel 1993). First, individual workers can increase the amount of fat they store per unit lean mass. Second, colony demography can shift so that a greater proportion of individuals within the colony can participate in storing significant amounts of fat. Most ant species employ a combination of these two tactics (Ricks and Vinson 1972; Jensen 1978; Tschinkel 1987, 1993, 1998; Hasegawa 1993). In some ant species with polymorphic workers, fat storage scales allometrically with body size so that larger individuals contain proportionally greater fat stores; as a result, fat storage becomes a function of the larger soldier caste in these species, with individuals storing vast quantities of nutrients termed repletes (Wilson 1974; Porter and Tschinkel

1985; Tsuji 1990; Lachaud et al. 1992; Hasegawa 1993; Tschinkel 1993, 1998). Interestingly, internal storage of liquid foods in the crops of the honey-pot ant *Myrmecocystus mexicanus* is also size dependent, with the largest workers in the colony becoming repletes regardless of the body size distribution within the colony, suggesting that body size plays an important role in storage capacity in ants in general (Rissing 1984). Larger colonies generally contain greater total fat stores because they have both more individuals and a greater mean individual size resulting from a higher proportion of large individuals (Jensen 1978; Hasegawa 1993; Tschinkel 1993, 1998). Therefore, understanding the regulation of fat storage in social insects, such as ants, requires understanding both the physiological mechanisms underlying liporegulation in individual colony members and the demography of storage among individuals within colonies through time.

To investigate the mechanisms underlying the regulation of fat storage in ants, I quantified patterns of allocation to fat storage at the individual, caste, and colony levels in two closely related desert carpenter ants that appeared to differ in fat storage. Members of the *Camponotus festinatus* (Buckley) species complex are distributed throughout the southwestern United States and northern Mexico, and the complex contains a wide range of morphological, ecological, and molecular genetic variation over its range (Creighton 1950; Goodisman and Hahn 2005; A. N. Lazarus, S. P. Cover, D. A. Hahn, and J. J. Wernegreen, unpublished data). In southern Arizona, where the complex has been best studied, four distinct species have been identified on the basis of consistent morphological and molecular variation (Goodisman and Hahn 2005; A. N. Lazarus, S. P. Cover, D. A. Hahn, and J. J. Wernegreen, unpublished data). Two of these species, which are genetically and morphologically distinguishable—*C. nr. festinatus* Desert Light (hereafter "Light") and *C. nr. festinatus* Desert Dark (hereafter "Dark"), so termed for differences in cuticle coloration—occur sympatrically in low-elevation desert areas in the Tucson Basin. Observation of these two species in the field and laboratory suggested that individuals differed in the amount of fat body development, which is indicative of fat storage (Rosell and Wheeler 1995).

To determine whether these two species differed in fat storage, I performed a common-garden experiment wherein queens were collected during mating flights and colonies were reared under controlled conditions in the laboratory for 2 yr, after which colonies were sampled to determine whether the two species differed in their fat storage tactics. Specifically, I asked the following questions. First, did individual workers or soldiers differ in the amount of fat they stored between the two species? Second, did colonies of the two species differ in the demography of fat storage among individuals in the colony? Third, was there caste-level specialization in nutrient storage in colonies of either species, as has been observed in other ants? Finally, was there

any difference between the two species in the total amount of fat stored by colonies?

Material and Methods

Insect Collection and Rearing

Queens of both species were collected at an ultraviolet light trap located at ca. 1,000 m elevation on the University of Arizona Santa Rita Experimental Range in Pima County, Arizona, on July 14 and July 19, 1999. Queens were confined in 30-mL glass test tubes filled with ca. 10 mL of deionized water and plugged with cotton to provide moisture. Queens and resulting colonies were maintained in an environmental chamber at 30°C for the duration of the experiment. Tubes were checked for brood every 3–5 d. After 8 wk, queens of both species had produced two to five pupae. Tubes containing pupae were checked every second day for eclosion of the first worker. Once the first worker had eclosed, tubes were unplugged and placed in a 200-mL plastic petri dish containing fresh cotton-plugged test tubes, which served as water sources and nesting substrates. Colonies resided in petri dish nests for approximately 9 mo while they grew in size. After 12 mo, colonies were moved to 33 × 18 × 10-cm plastic boxes containing more cotton-plugged test tubes as water sources and nesting substrates. Colonies were fed ad lib. with a combination of frozen immatures of the cockroach *Nauphoeta cinerea* and the moth *Manduca sexta* and a 1 : 1 v/v honey-water mixture supplemented with 0.5% Vanderzant's vitamin mix and 0.5% Wesson's salts mix from the time of first worker eclosion until sampling.

Sampling

After 2 yr in the laboratory, five colonies of each species were sampled during July 2001. Sampling consisted of separating all workers and soldiers from the colony. Workers and soldiers of both species are capable of accumulating significant stored fat and protein reserves in their fat bodies, causing the gaster to become distended (Martinez and Wheeler 1994; Rosell and Wheeler 1995). In this study, gaster distension was used as a visual proxy of fat body development and consequently fat storage. To investigate the demography of fat storage in the two species, workers and soldiers were each sorted into one of two categories based on observable gaster distension: low or high fat. Because the abdominal cuticle of these ants is very light and the intersegmental membranes are completely translucent when the gaster is distended, it is possible to distinguish between distension due to fat body development and distension of the crop with liquid. Individuals whose gasters were clearly distended because of full crops and had little fat body development were placed into the low-fat group. The number of workers and soldiers in each of the fat body groups was counted for each colony. Twenty individuals from each fat body group in each colony were haphazardly selected for further analyses.

Each individual was placed into a 1.5-mL plastic microcentrifuge tube and frozen at –20°C until further analysis. Colonies of each species were sampled alternately so that bias in sampling time throughout was minimized (i.e., Light colony 1 was sampled, then Dark colony 1 was sampled, etc.).

Fat Extraction and Quantification

Six to 12 individuals from each fat body development group within each colony were selected for fat content analysis, yielding 312 individuals in total. Samples were freeze-dried until constant weight, weighed, and stored at –20°C until fat analysis. Fat content was analyzed using a modification of Van Handel's (1985) procedure. In brief, samples were homogenized for 60–90 s at 250 rpm in 1 : 1 (v/v) chloroform-methanol solvent in 1.5-mL microcentrifuge tubes with a plastic pestle and centrifuged at 12,000 g for 20 min at 4°C. The supernatant was removed, and the above procedure was repeated once with the 1 : 1 chloroform-methanol mixed solvent and once with pure chloroform. To remove polar lipids, pooled supernatants were subsequently run through a column containing 0.2 g of 100-mesh silicic acid that had been dried at 100°C overnight. Columns were washed eight times with 1 mL pure chloroform to elute nonpolar lipids, of which triglycerides represented more than 95% of the total. The resulting solution was dried under nitrogen gas and resuspended in a known amount of pure chloroform. A subsample of this solution was used to spectrophotometrically estimate fat mass against a range of known triolein standards using the sulfophosphovanillin method.

Lean mass for each individual was calculated as total dry mass minus fat mass. Total fat storage for each fat body group within each caste and colony was estimated by taking the mean fat storage value for the individuals analyzed from that fat body group and multiplying it by the total number of individuals in the group. Colony-level fat storage was calculated by taking the sum of the total fat storage values for each fat body group within a colony.

Results

Individual-Level Allocation to Fat Storage between Species

Because there is often significant between-colony variation in social insects (Bourke and Franks 1995), nested ANOVAs with colony as a random factor nested within species were used for individual-level analyses. There was no significant difference in lean mass in either workers or soldiers between the two species (workers, whole model: $F = 1.60$, $df = 9$, $P = 0.117$; workers, species: $F = 2.80$, $df = 1$, $P = 0.096$; soldiers, whole model: $F = 2.19$, $df = 9$, $P = 0.028$; soldiers, species: $F = 0.15$, $df = 1$, $P = 0.701$; Fig. 1a). There were also no significant colony-level effects on lean mass in workers, but there was a significant effect of colony on lean mass in soldiers (workers, colony [species]: $F = 1.48$, $df = 8$, $P = 0.167$; soldiers, colony

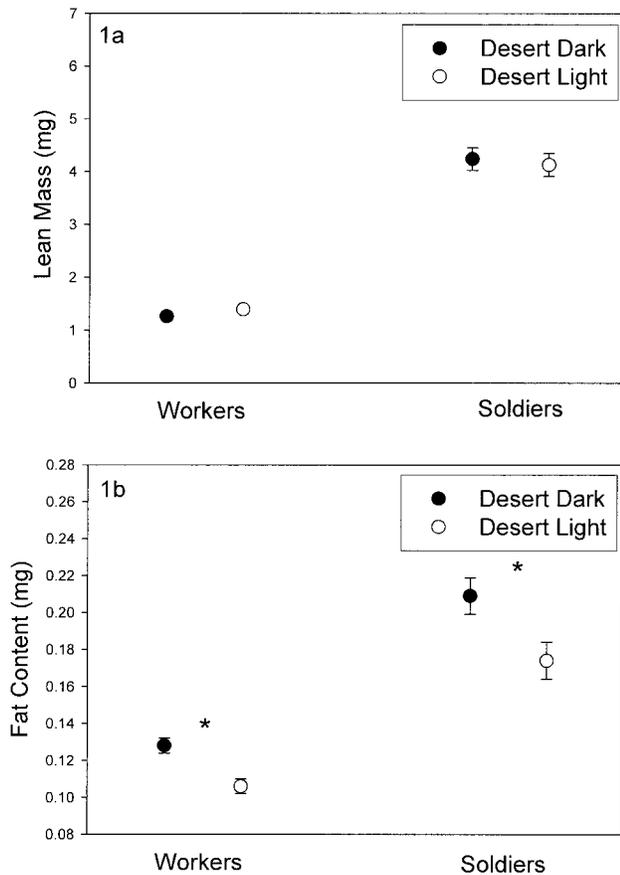


Figure 1. *a*, Individual-level comparisons of lean mass in workers and soldiers of the two species, using means adjusted for colony-level effects. *b*, Individual-level comparisons of fat content in workers and soldiers of the two species, using means adjusted for effects of colony and lean mass. Error bars represent 1 SE; bars are subsumed within some points. An asterisk denotes a statistically significant difference.

[species]: $F = 2.46$, $df = 8$, $P = 0.017$). Certain colonies in both species had larger soldiers than others.

Fat content was positively correlated with lean mass in both workers and soldiers of both species (Pearson’s correlations; Dark workers: $r = 0.54$, $n = 93$, $P < 0.001$; Light workers: $r = 0.60$, $n = 102$, $P < 0.001$; Dark soldiers: $r = 0.63$, $n = 55$, $P < 0.001$; Light soldiers: $r = 0.58$, $n = 62$, $P < 0.001$). Therefore, lean mass was used as a covariate, along with colony nested within species, in analyses of fat content. Dark workers and soldiers stored significantly more fat than Light workers or soldiers when lean mass and colony were accounted for (Table 1; Fig. 1*b*). There were also significant colony-level effects on fat content in both workers and soldiers when lean mass was held constant (Table 1). Individuals from certain colonies in both castes within both species stored more fat per unit lean mass than those in other colonies. There was no clear relationship between colony size and fat content in either species.

Therefore, the observed colony-level effects were not mediated by colony size.

Significantly greater fat storage per unit lean mass by Dark workers and soldiers becomes apparent when the scaling relationship between lean mass and fat mass is compared in each caste of the two species. To hold colony-level effects constant, the residuals of lean mass and fat mass from ANOVAs with colony nested within species were plotted against each other to determine whether the relationship between lean mass and fat storage differed between the two species in either caste. There was a significant positive linear relationship between lean mass and fat mass in both castes of both species (Fig. 2). Regression slopes did not differ between the two species in either caste (workers: $t = 0.32$, $df = 191$, $P > 0.05$; soldiers: $t = 1.03$, $df = 113$, $P > 0.05$). However, the elevations of the regression lines were significantly greater for both Dark workers and Dark soldiers (workers: $t = 3.30$, $df = 192$, $P < 0.005$; soldiers: $t = 2.37$, $df = 114$, $P < 0.01$). Therefore, the basic scaling relationship between lean mass and fat storage did not differ between the two species in either caste, but Dark workers and soldiers contained more fat per unit lean mass throughout the range of lean masses.

Demography of Fat Storage between Species

Individuals visually assigned to the high-fat group contained significantly greater fat reserves than individuals in the low-fat group for both castes in each species when colony-level effects were held constant (Table 2). Therefore, visual estimation of fat body development was a good proxy for individual fat content to assess fat storage demography within colonies. There was no difference between the two species in the proportion

Table 1: Nested ANCOVA for the effects of species, colony nested within species, and lean mass on lipid content

Caste, Source	df	F	P
Worker fat content:			
Whole model	10	25.54	<.001
Species	1	14.42	<.001**
Colony (species)	8	13.69	<.001
Lean mass (mg)	1	149.72	<.001
Error	184		
Total	194		
Soldier fat content:			
Whole model	10	13.30	<.001
Species	1	5.99	.016**
Colony (species)	8	4.60	<.001
Lean mass (mg)	1	62.69	<.001
Error	106		
Total	116		

** Significant species-level effect.

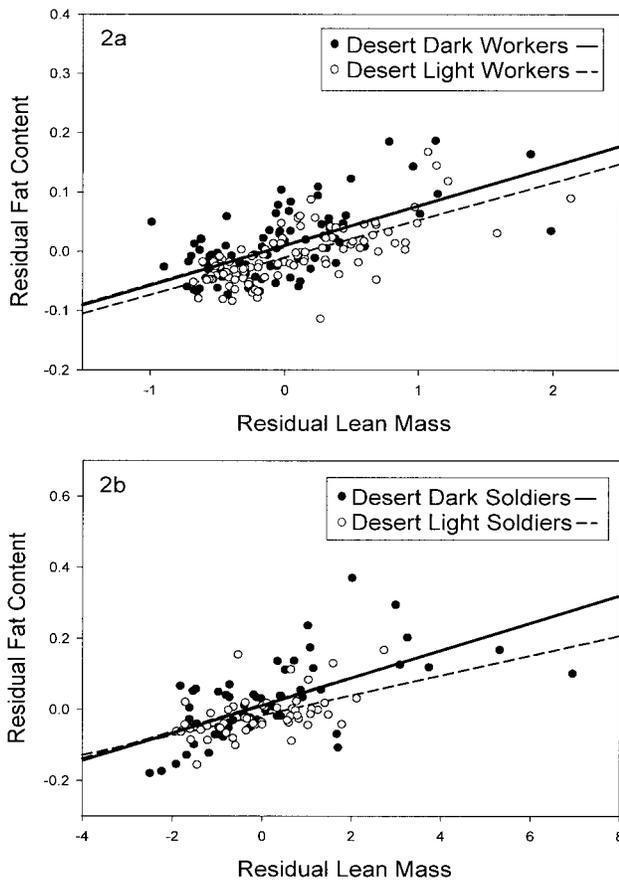


Figure 2. Plots of the relationships between colony-adjusted lean mass and fat mass in workers (a) and soldiers (b) of the Desert Dark (solid lines) and Light species (dashed lines). Dark workers fat mass = $0.011 + 0.067(\text{lean mass})$, $R^2 = 0.39$, $df = 92$, $F = 59$, $P < 0.001$. Light workers fat mass = $-0.010 + 0.063(\text{lean mass})$, $R^2 = 0.52$, $df = 101$, $F = 109$, $P < 0.001$. Dark soldiers fat mass = $0.014 + 0.038(\text{lean mass})$, $R^2 = 0.40$, $df = 61$, $F = 40$, $P < 0.001$. Light soldiers fat mass = $-0.017 + 0.028(\text{lean mass})$, $R^2 = 0.28$, $df = 54$, $F = 21$, $P < 0.001$.

of workers or soldiers in the low- and high-fat categories within colonies, although there was a moderately significant trend toward Light colonies containing a greater proportion of high-fat soldiers (low-fat workers: $t = 0.29$, $df = 8$, $P = 0.604$; high-fat workers: $t = 0.36$, $df = 8$, $P = 0.726$; low-fat soldiers: $t = 0.95$, $df = 8$, $P = 0.386$; high-fat soldiers: $t = 2.10$, $df = 8$, $P = 0.069$; Fig. 3). This trend suggests that although Dark workers and soldiers store more fat per unit lean mass than Light workers and soldiers, Light colonies may involve a slightly greater proportion of their soldiers in storing large quantities of fat.

Storage Attributes between Castes within Each Species

In both species, soldiers contained significantly greater total fat mass than workers (Table 3; Fig. 4a). However, for both species,

workers contained a significantly greater proportion of fat per unit lean mass than soldiers (Table 4; Fig. 4b). There were significant colony-level effects on both total fat mass and the fat-to-lean-mass ratio in both species (Tables 3, 4).

Colony- and Caste-Level Attributes between Species

Although individual colonies ranged between 540 and 1,221 individuals among the five colonies sampled from each species, there was no difference in total number of individuals, total number of workers, or total number of soldiers between the

Table 2: Two-way ANOVA for the effects of visual fat storage state and colony on fat content for each caste within each species and the resulting adjusted means for fat content for high- and low-fat-content individuals within each caste and species

Species, Caste, Source	df	F	P	Adjusted Mean Fat \pm SE (mg)
Dark workers:				
Whole model	5	18.55	<.0001	
Fat storage state	1	62.50	<.0001	
Colony	4	8.02	<.0001	
Error	87			
Total	92			
High-fat storage state				.177 \pm .008
Low-fat storage state				.098 \pm .006
Light workers:				
Whole model	5	21.92	<.0001	
Fat storage state	1	47.80	<.0001	
Colony	4	16.52	<.0001	
Error	96			
Total	101			
High-fat storage state				.149 \pm .007
Low-fat storage state				.092 \pm .005
Dark soldiers:				
Whole model	5	17.65	<.0001	
Fat storage state	1	52.43	<.0001	
Colony	4	8.90	<.0001	
Error	56			
Total	61			
High-fat storage state				.323 \pm .020
Low-fat storage state				.133 \pm .015
Light soldiers:				
Whole model	5	14.42	<.0001	
Fat storage state	1	3.53	<.0001	
Colony	4	16.26	<.0001	
Error	49			
Total	54			
High-fat storage state				.193 \pm .008
Low-fat storage state				.107 \pm .013

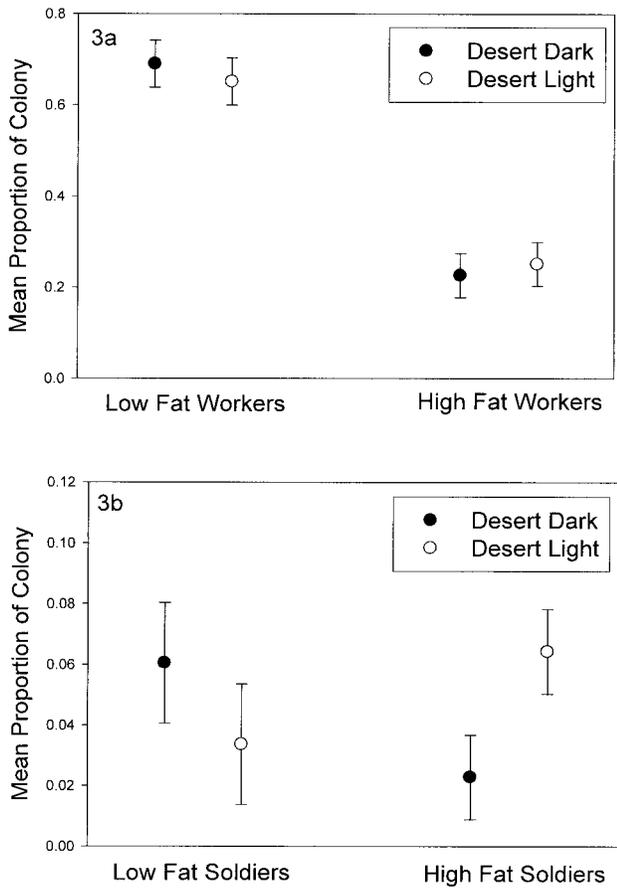


Figure 3. *a*, Comparisons of the proportion of the total number of individuals in the colony accounted for by high- and low-fat workers of the two species. *b*, Comparisons of the proportion of the total number of individuals in the colony accounted for by high- and low-fat soldiers of the two species. Error bars represent 1 SE.

two species (total individuals: $t = 0.71$, $df = 8$, $P = 0.498$; total workers: $t = 0.73$, $df = 8$, $P = 0.485$; total soldiers: $t = 0.12$, $df = 8$, $P = 0.907$; Fig. 5*a*). In addition to being the same size, on average, colonies of the two species did not differ significantly in either overall total fat storage or the total amount of fat stored by workers or soldiers, although there was a slight trend toward Dark colonies storing more fat overall (total fat content: $t = 0.74$, $df = 8$, $P = 0.483$; worker fat content: $t = 0.76$, $df = 8$, $P = 0.471$; soldier fat content: $t = 0.06$, $df = 8$, $P = 0.957$, respectively; Fig. 5*b*).

Discussion

I have shown that two species within the *Camponotus festinatus* species complex differ in individual-level fat storage tactics in a common environment. Dark workers and soldiers stored significantly more fat per unit lean mass than Light workers and soldiers. There was no detectable significant demographic dif-

ference in the proportions of workers or soldiers with different levels of fat body development between the two species, although Light colonies contained a noticeably greater proportion of high-fat body distension soldiers. Despite the differences in individual-level fat storage tactics between the two species, the total amount of fat stored by colonies of the two species did not differ.

Soldiers of both *C. festinatus* species stored significantly greater total amounts of fat than workers, but soldiers stored proportionally less fat than workers per unit lean mass in both species, which contrasts with species containing repletes (Wilson 1974; Porter and Tschinkel 1985; Tsuji 1990; Lachaud et al. 1992; Hasegawa 1993; Tschinkel 1993, 1998). Greater proportional fat storage in workers rather than soldiers, combined with much greater numbers of small workers in the colonies of both species, led to workers containing 87% and 84% of the total colony fat load in Dark and Light colonies, respectively, highlighting the importance of small workers relative to soldiers in colony-level fat storage in these two species. Many ant species with worker size variation contain a greater proportion of workers than soldiers, and the role of small workers in internal nutrient storage may be more important than is currently recognized across the ants.

Storing more fat per unit lean mass has been well documented as a tactic for increasing fat storage during ontogeny among colonies of a number of ant species (Jensen 1978; Hasegawa 1993; Tschinkel 1993, 1998) and now has been shown to contribute to between-species differences as well. Interestingly, neither mean individual size nor the basic scaling relationship between lean mass and fat storage differed in either caste of the two species (i.e., a positive linear relationship, with slopes that did not differ significantly). However, the elevation of the relationship was significantly greater in both Dark workers and Dark soldiers, reinforcing that the Dark species stores

Table 3: ANCOVA for caste differences in the natural log of total fat mass in the two species

Form, Source	df	F	P
Desert Dark:			
Whole model	9	9.50	<.001
Colony	4	7.77	<.001
Caste (colony)	5	10.64	<.001**
Error	145		
Total	154		
Desert Light:			
Whole model	9	15.02	<.001
Colony	4	16.57	<.001
Caste (colony)	5	12.26	<.001**
Error	147		
Total	156		

** Significant caste-level effect.

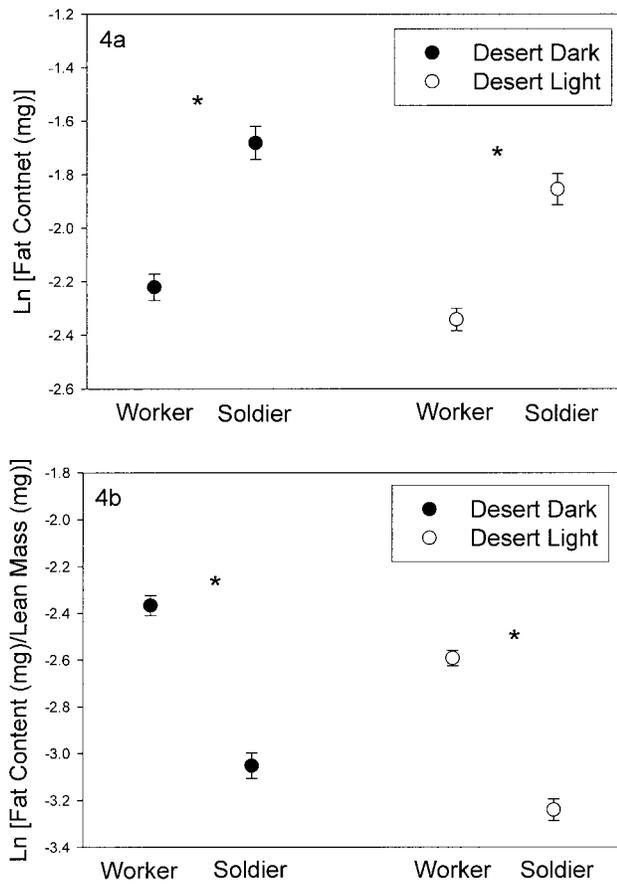


Figure 4. *a*, Comparisons of the natural log of total fat storage between workers and soldiers in each species. *b*, Comparisons of the natural log of the fat mass to lean mass ratio between workers and soldiers in each species. Symbols represent adjusted means from the nested ANOVAs in Table 3. Error bars represent one standard error. An asterisk denotes a statistically significant difference.

more fat per unit lean mass throughout its body size range. If fat storage in individual ants is maintained around an internally determined liporegulatory set point, as it is in mammals (Mercer 1998; Woods and Seeley 2000), these results suggest that Dark individuals have a higher liporegulatory set point than Light individuals over their entire range of body sizes and that liporegulatory set points are more labile than the basic scaling relationship between body size and fat storage in these ants. Showing that Dark individuals store significantly more fat in a common environment validates the use of this pair of species as a comparative model for understanding the physiological and behavioral mechanisms regulating fat storage in ants. Future work should focus on comparing behavioral mechanisms of fattening (e.g., the distribution of food among colony members) and physiological mechanisms of fattening (e.g., lipid synthesis and mobilization responses to nutrition and activity) in individuals of both species in a common environment.

It may seem contradictory that I found strong support for a difference in allocation tactics at the individual level between the two species and weak to no support for species differences in colony-level traits, such as total fat storage or proportion of the colony accounted for by various fat body development groups. If Dark workers and soldiers store more fat per unit lean mass, either Dark colonies should have contained significantly greater fat stores or the two species could have achieved the same colony-level fat stores through Light colonies containing a greater proportion of individuals storing significant amounts of fat (e.g., a greater proportion of high-fat individuals). I found strong support for neither. However, this was likely due to a lack of statistical power to detect colony-level differences, compared to my ability to detect individual-level differences between the two species, rather than to a true lack of colony-level differences. Post hoc power analyses of all the colony-level comparisons in this study revealed that statistical power was low for all comparisons, with no comparison having greater than 50% power, which is much lower than the 80%–95% power levels suggested as benchmarks by some authors (Lougheed et al. 1999; Walsh et al. 1999; Di Stefano 2003). Such low power estimates make it impractical to detect differences at the colony level with laboratory-reared colonies such as these. For example, one would have to sample 58 colonies between the two species to approximate 80% power and 71 colonies to approximate 95% power for the observed levels of variance in the colony-wide fat comparison, unrealistic goals considering the workload and space necessary to rear laboratory colonies. Although this study does not provide conclusive evidence for differences in colony-level traits between the two species, the strong evidence for individual-level differences in fat storage suggests that they must exist, and the trend toward Light colonies containing a greater proportion of high-fat soldiers merits further study.

Table 4: ANCOVA for caste differences in natural log of fat mass/lean mass ratio in the two species

Form, Source	df	F	P
Desert Dark:			
Whole model	9	19.28	<.001
Colony	4	9.98	<.001
Caste (colony)	5	20.64	<.001**
Error	145		
Total	154		
Desert Light:			
Whole model	9	29.66	<.001
Colony	4	18.51	<.001
Caste (colony)	5	31.42	<.001**
Error	147		
Total	156		

** Significant caste-level effect.

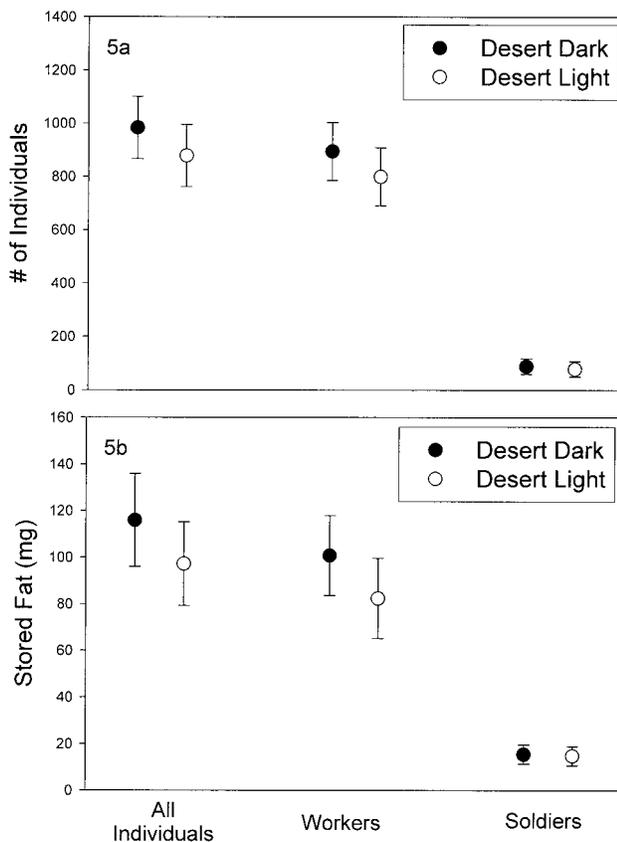


Figure 5. *a*, Colony-level comparisons between species of the total number of individuals, total number of workers, and total number of soldiers between species. *b*, Colony-level comparisons between species in total amount of fat stored by all individuals in the colony, by workers, and by soldiers. Error bars represent 1 SE.

Another explanation for why no significant differences in colony-level traits were found between the two species could be the young age of the colonies. Because colony-level fat storage is known to increase with colony age and size in several species of ants, the young age of the laboratory colonies used in this study may have contributed to the lack of detectable colony-level differences (Jensen 1978; Tschinkel 1993, 1998). These 2-yr-old colonies were smaller than their peak age and size in the lab and were clearly still in the ergonomic growth phase of their life cycle. Differences in fat storage at the colony level between the two species could become more pronounced with time and more easily detected as the colonies move from the ergonomic growth stage into the reproductive stage of their life cycle, where they will reach peak size, raising the question of whether the ontogeny of fat storage differs between the two species (Oster and Wilson 1978). Future efforts to assess colony-level fat storage should focus on mature colonies sampled from both the laboratory and the field.

There was significant variation among colonies within each

species. Individuals from certain colonies within each species had consistently higher lean masses or fat stores, although individuals from colonies with the greatest average lean masses did not always contain the greatest fat stores and fat storage was not related to colony size. This suggests that intraspecific variation in fat storage tactics may exist. Significant variation between colonies is a well-known feature of social insects that can be caused by numerous factors, including colony genotype, maternal, environmental, size, and age-related effects (Hölldobler and Wilson 1990; Bourke and Franks 1995). Because environment, age, and size were kept the same among colonies in this study, it seems likely that the strong observed colony-level effects were due to genetic effects, maternal effects, or perhaps demographic stochasticity.

Strong differences in fat storage tactics at the individual level prompt the question of why these two closely related species have evolved different fat storage tactics. Variation in resource availability has been implicated as a cause of increased nutrient storage in a number of animals, including insects, birds, and mammals (Boggs 1981; Houston and McNamara 1993; Perrin and Sibly 1993; Rogers et al. 1993; Bednekoff and Houston 1994; Diamond 2003). Deserts are notoriously variable places in terms of environmental conditions, particularly rainfall, which is directly related to productivity and resource availability in these moisture-limited systems (Inouye 1991; Pake and Venable 1996). For perennial organisms, such as ant colonies, investing heavily in nutrient stores when nutrient availability is high is a potential bet-hedging strategy for dealing with times of reduced resource availability (Rogers 1987; Philippi and Seger 1989; Rogers et al. 1993; Hopper 1999). Observations of colonies of these two species in areas of sympatry in the Tucson Basin suggest no significant differences in reproductive timing, foraging patterns, or habitat/microhabitat selection, making the observed differences in fat storage all the more interesting (D. A. Hahn, unpublished data). Further study of the two species in areas of allopatry and sympatry over a wider geographic range would be useful in determining whether ecological factors such as microhabitat selection differ between the two species in a way that could influence the costs and benefits of different fat storage patterns.

Differences in individual-level fat storage tactics may also have consequences for behavior in the two species. Studies in ants, bees, and wasps have shown that individuals performing risky off-nest behaviors, such as foraging or defense, contain less fat stores than those performing less risky in-nest behaviors, such as nursing larvae (Kondoh 1968; Porter and Jorgenson 1981; MacKay 1983; Lachaud et al. 1992; O'Donnell and Jeanne 1995; Blanchard et al. 2000; Toth and Robinson 2005). This correlation has led several authors to suggest that fat stores may influence the repertoire of behaviors an individual will perform and that this correlation has evolved because of the adaptive value of individuals with less energetic content, which would be less costly for the colony to lose, performing risky tasks

(Porter and Jorgenson 1981; MacKay 1983; O'Donnell and Jeanne 1995; Blanchard et al. 2000). Furthermore, recent work in honeybees has shown that manipulating fat reserves has a causative effect on foraging behavior, with leaner individuals more readily engaging in risky tasks such as foraging. If fat storage influences ant behavior in a similar way, differences in individual-level storage tactics between the two *C. nr. festinatus* desert species could lead to significant behavioral differences, perhaps in the rate that individuals progress through behavioral development during their lifetimes, or differences in their motivation to forage or defend their nests. In addition, Dark workers and soldiers containing more fat per unit dry mass throughout their body size range than Light workers and soldiers suggests that losing a worker or soldier may be more costly to Dark colonies, which could have implications for the evolution of colony-level traits, such as collective foraging and nest defense, colony growth rates, and sexual production. Clearly, further studies of colonies over a greater portion of the colony life cycle are needed in the two species to determine whether there are individual- or colony-level behavioral or life-history differences associated with fat storage. By demonstrating significant individual-level differences in fat storage among workers and soldiers of these two closely related desert carpenter ant species from an area of sympatry reared in a common environment, this study has laid the foundation for using the *C. festinatus* species complex as a model for understanding both the proximate physiological and behavioral mechanisms and the ultimate evolutionary mechanisms regulating nutrient storage at the individual and colony levels in social insects.

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