

The life history and seasonal cycle of the ant, *Pheidole morrisi* Forel, as revealed by wax casting

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Received: 25 November 2014 / Revised: 23 March 2015 / Accepted: 25 March 2015 / Published online: 21 April 2015
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Abstract The dimorphic ant, *Pheidole morrisi* Forel, is the most common ant of the longleaf pine flatwoods ecosystem of northern Florida and occurs throughout the eastern USA. Although ecologically significant, its life history, and annual cycle have been little investigated. The effects of colony size and the annual cycle were described by collecting the full range of colony sizes during each of five seasonal phases ($n = 40$ total). Nests were cast in wax to capture and census all colony members, and to determine their vertical distribution within the nest. Colony sizes ranged from 800 to 49,000 ants, larger than previous literature reports. Colonies invested in worker production during the spring and fall and reduced worker production as they produced alates in the summer. Colonies over 3000 workers were able to produce alates, but the number was not related to colony size. Vertical distribution of nest volume was strongly top heavy, with the greatest proportion of the nest volume near the surface. The mean weight of majors was about four times that of minors. The mean percentage of total workers that were major workers (11 % by number, 30 % by weight) did not change significantly as colonies grew, but varied with season. Production of major pupae was highest in spring, so that the percentage of colony biomass represented by majors increased from about 27 % in spring to 38 % in late summer. The overall density of the ants was highest in medium-sized colonies, lowest in small colonies, and moderate in large colonies. Broadly, the colony moved lower in the nest from

summer to winter and higher from winter to summer. The seasonal vertical distribution of ants suggested that the ants responded to temperature, but not crowding. Temperatures in the nest varied from 12 to 17 °C in the winter to 23–33 °C in the late summer. Broods were nearly always found in the warmest regions. The distribution of callow workers was strongly correlated with that of the brood. The results are discussed in light of the utility of the wax-casting method and the nature of the data needed to describe and understand the superorganism.

Keywords Formicidae · Colony size · Seasonality · Worker size · Nest architecture · Brood production · Annual cycle

Introduction

An organism's environment selects for life span, growth rate, reproductive investment patterns and rates, and many other aspects of life history. For social insects, these life history characteristics play out not only at the individual insect level, but are critical features of the colony as a whole. Because colonies consist largely of sterile workers, most of the life history features of individual members of social insect colonies are shaped by selection at the level of the colony as a whole, the so-called superorganism level. It follows that revealing the properties and life histories at the superorganism level is needed to understand social insect biology and evolution. Tschinkel (1991, 2011) offered a simple method for sampling and assessment of social insect colonies that simultaneously reveals features of the colony's composition, its ontogeny, and its annual cycle. Such sociometric and sociogenic studies reveal colony-level attributes and their consequences at the individual and

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colony scale and permit reasoning about the forces of natural selection acting at the superorganism level. Application of these methods to ants (Tschinkel 1987, 1993, 1998a, b, 1999, 2002; Laskis and Tschinkel 2009; Smith and Tschinkel 2006; Wilson 1985) has shown that ant colonies change in size, composition, subterranean distribution, nest architecture, production, and efficiency throughout the annual cycle and during growth. Here, we apply this method to an ecologically important ant, *Pheidole morrisi*.

The genus *Pheidole* has received much attention for several reasons: it is hyperdiverse (Wilson 2003; Moreau 2008), nearly all of its species are dimorphic (Moreau 2008), and it is often ecologically dominant both in species and biomass (Wilson 2003; King and Tschinkel 2008). One of the prominent and easily cultured American species, *P. dentata*, has become a model for studies of division of labor (Wilson 1975, 1976) as well as for neurological development and behavior (Seid and Traniello 2006; Muscedere et al. 2009). The less well-known, but ecologically similar *P. morrisi* is widespread in the southern and eastern USA (Patel 1990) and is the most common ant in the pine savanna ecosystems widespread across the southeastern coastal plain (King and Tschinkel 2008; Tschinkel et al. 2012). The longleaf pine (*Pinus palustris*) savanna ecosystems found in the Apalachicola National Forest, the focus of this study, are a reasonable proxy for the natural longleaf pine forests that once covered much of the eastern US coastal plains (Frost 1993). *Pheidole morrisi* is a generalist predator/scavenger with large colonies that likely plays an important ecological role, both currently and historically (Gregg 1942; King and Tschinkel 2008; Tschinkel et al. 2012). Despite this apparent ecological importance of this species across an entire region, it has been little studied. Yang (2004) described behavioral and demographic adaptations to regional environmental conditions through latitudinal shifts in the proportion of majors, the size of the major workers and their capacity to store fat (Yang 2006).

The purpose of this work was to use whole colony studies to reveal the seasonal and growth-related life history (sociometry/sociogenesis) of the ecologically important *P. morrisi*. These timing, size, number, frequency, and ratio patterns are the functional, evolved adaptations of the superorganism to the habitat in which it lives. But conclusions about adaptations and functions must begin with a quantitative description, as provided here.

Materials and methods

Study sites

Four study sites with high densities of *P. morrisi* colonies were located approximately 23 km southwest of Tallahassee, FL,

and within 6 km of one another. The sites contained mature second-growth longleaf pine (*P. palustris*), saw palmetto (*Serenoa repens*), wiregrass (*Aristida stricta*), dense woody ground cover, and shrubs, including gallberry (*Ilex coriacea* and *I. glabra*), rusty and shiny staggerbush (*Lyonia ferruginea* and *L. lucida*), runner oak (*Quercus minima* and *Q. pumila*), and herbaceous groundcover.

Sampling and wax-casting procedure

We sampled a full range of colony sizes with emphasis on the extremes, thus adding power to regressions based on colony size. Colony size was initially estimated from the mound size, number of entrance tunnels, and mound activity. Mound volumes were calculated as hemispheroids ($V = 4/3\pi \cdot a/2 \cdot b/2 \cdot h/2$). Data on the subterranean temperatures were collected hourly with “i-buttons” (Maxim Integrated, Inc. <http://www.maximintegrated.com/products/ibutton>) buried at four depths close to the nest (5, 15, 30, and 80 cm) 1 week before casting until 1 week after casting.

We used the wax-casting method described by Tschinkel (2010) to sample whole colonies. The area around nests was watered with 40–50 L of water the day before casting to reduce the penetration of the molten wax into the sand at the upper levels of the cast where soil was driest. Incorporation of sand severely slowed the processing of the casts. Watering was omitted if substantial rain occurred prior to casting.

On the day of casting, the mound was removed and all ants that emerged immediately, those in the mound, and any returning foragers were captured with a portable shop vacuum (DeWalt DC500) and an aspirator. The mound ants and foragers were classified together as “foragers”, but were probably not a single labor group. Removal of the mound exposed the openings of the vertical shafts into which molten wax at 80 °C was poured within 1–5 min. Once the cast hardened, a large pit was dug next to the cast (Fig. 1) and the cast’s position was determined by digging toward it. It was then retrieved in 10 cm sections as measured from the ground surface. Each section was packaged separately and labeled, allowing the vertical distribution of the ants to be determined later. Returning foragers were captured throughout the excavation (~2 h).

Laboratory census

The completed wax cast sections were processed in the laboratory. Each 10 cm section was weighed and photographed after arrangement in a rough simulation of the nest’s architecture (Fig. 2). Next, the sections were melted in an oven at 65 °C and the ants strained out of the molten wax with fine-meshed screen. The screen with the ants was transferred to a pad of paper towels on a warm hotplate to

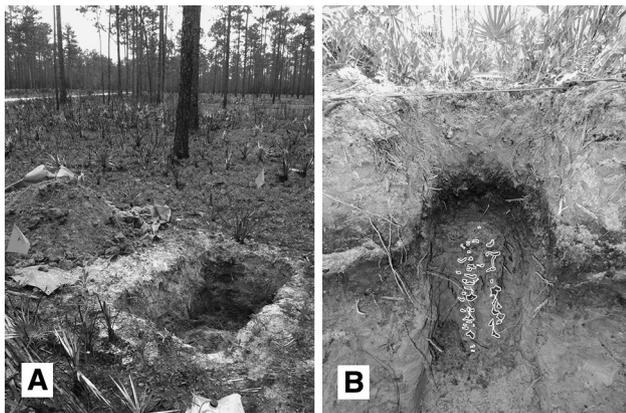


Fig. 1 A large pit (a) dug next to a nest that has been cast in wax (b). The wax was dyed blue so that it could be easily seen in the sand and is outlined in white in this image (b)

soak off more wax, and then to *n*-heptane, or Coleman[®] camp fuel (white gasoline) to dissolve away any remaining wax. Finally, the ants were removed from the solvent, allowed to dry, and moved to alcohol, in which they were stored until census.

The first 10 cm section of a cast often contained wax-soaked sand, in spite of the watering prior to casting (Fig. 2). In such cases, the volume of wax was determined either by laboriously scraping away the wax-soaked sand or by determining the volume of the entire composite, melting it, removing the ants from the sand, and then subtracting the volume of the sand from the volume of the two together. In the latter method, the ants were separated from the sand by floating them in chloroform (in which sand sinks) about ten times. Usually, no ants were found after six to seven iterations.

When samples contained very large numbers of ants, they were sub-sampled by spreading them evenly in a gridded Petri dish and the ants counted in a random selection of grid units. Common colony members were counted in 15 of the 65 grids, and the less common majors and major brood in 30 grids. These tallies were then used to estimate the total of each type in the entire sample. This method's estimates were consistently within 10–15 % of the exact counts. The significant reduction of processing time offset the associated uncertainty. Complete counts were always made for alates because of their low numbers and large size. Complete counts were also used for smaller samples.

Minor and major workers were counted separately and within each of these categories, callow (young) workers were distinguished from dark (older) workers. Other counts included major and minor worker pupae, alate pupae, and male and female adult alates. Worker larvae and sexual larvae were distinguished by the larger size of the latter, but earlier, smaller sexual larvae could not be separated from

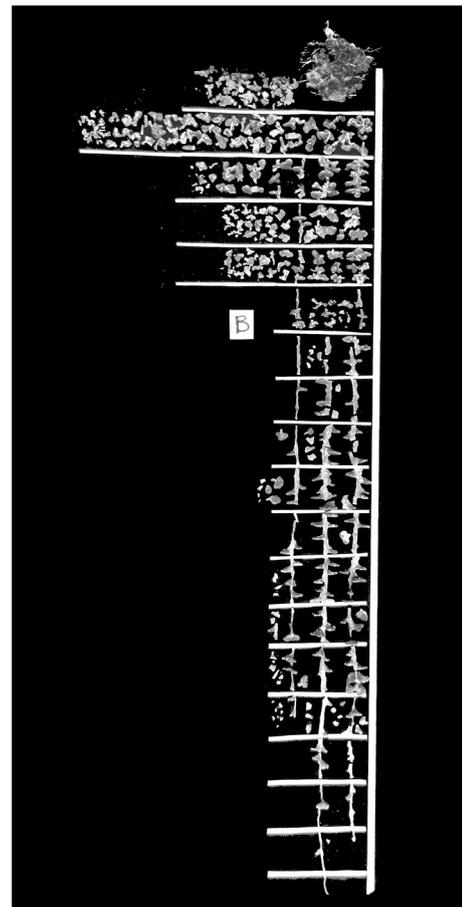


Fig. 2 A wax cast reconstructed to approximate the architecture of the nest, allowing a visualization of nest volume distribution

worker larvae. For some purposes, worker larvae and pupae were combined as worker brood.

Estimation of biomass

Total major, minor, and colony dry weights were estimated in three steps. (1) The head widths and dry weights of 20 minors and 20 majors from five colonies (not wax cast) were measured with a calibrated digital microscope and a microbalance, and regressed against one another (Table 2; regression 1). (2) The head widths of minors and majors sampled from the five smallest and five largest colonies of the 40 wax cast colonies were measured with a wedge micrometer (Porter 1983). These ten mean head widths were regressed against the total number of workers in the colony to yield how head widths of minors and majors were related to colony size. (3) The mean minor and major weights from step 2 were multiplied times the number of each type of worker to yield a total weight of each (Table 2; regressions 4, 5), and their sum produced the total worker dry weight (Table 2; regressions 6–8).

Data analysis

Data were analyzed by ANOVA, or with multiple regression with and without indicator variables. When necessary, data were log transformed to satisfy the assumptions of the statistical tests.

Nomenclatural conventions

For analyses focusing on biomass, the total number of individuals of all types and stages of ants is the most meaningful measure and is referred to as “colony size” throughout. For analyses focusing on the labor force, the number of workers is most meaningful and is referred to as “total workers” hereafter.

Results

Colony size in *P. morrisi* ranged from 800 to 48,900 (Table 1), and total workers from 500 to 37,400. Total weight of workers ranged from 0.0651 to 6.99 g, an approximately 100-fold size difference. Of this, the total weight of minor workers ranged from 0.049 to 4.69 g, and of majors from 0.0155 to 2.30 g. Majors thus comprised a mean of 30 % of colony dry weight, but only 11 % of worker number. The mean individual weight of minors was 0.12 mg and of majors 0.47 mg, making majors about four times as heavy and at least four times as costly to produce as minors.

Nests were between 0.5 and 2 m deep and ranged in volume from 33.6 to 2920 cm³. The increase in nest volume was primarily associated with increased number and size of chambers. Very small nests did not have an associated mound. When present, mounds ranged from 0.08 to 31 L. Polygyny was only found in two large colonies, each of which contained two queens.

Seasonal variation in colony composition

For most analyses, colony composition was calculated as mean percent of colony size. The seasonal changes in

composition can be seen in Figs. 3 and 4 and were similar for colonies of all sizes. Dark and callow minors and majors, larvae, and a single queen were present throughout the year. Worker brood (larvae and pupae) was produced in two distinct phases, one in the spring and one in the fall (Fig. 3a, b). Pupae were absent in the winter (Fig. 3b), and it is likely that the larvae produced in the fall were overwintered in larval diapause and contributed to the brood counts in the spring. Possibly as a result, the percentage of worker brood in the spring was higher than in the fall. This suggested a similar investment in worker brood during both phases with the fall phase contributing to the next spring phase, making it appear like a larger investment. It is also possible that some of the overwintered larvae were early instars of alates. Alates were recognizable as larvae, pupae, or adults only during the summer (May–August).

The peaks in larvae and pupae were followed by peaks in pupae and callows, respectively, as one stage developed into the next. The worker larvae composed the highest proportion of the colony in the winter and spring, decreased over the summer, and rose again in the fall (Fig. 3a). Eclosion of the overwintered larvae into pupae gave rise to the ca. 55/45 pupae to worker ratio during the spring peak in brood (Fig. 3b). During the remainder of the year, larval production maintained a ratio of ca. 30/70 pupae to workers. The pupae lagged behind the larvae by one seasonal phase (compare Fig. 3a, b). Because they developed from pupae, callow workers lagged pupal patterns, just as pupae lagged larval patterns (compare Fig. 3b, c). These lagged patterns suggest large (but diffuse) cohorts of developing ants in which the predominant stage shifts from larvae to pupae to callows.

Because callows are young workers, it is clear that the age structure of the whole colony shifted from predominantly old workers in the winter to an increasing proportion of young workers that peaked in the early summer as the overwintered larvae completed their development. The peak in callows preceded and overlapped the reproductive phase in summer (Figs. 3b, 4), a period that experienced a sizeable decrease in larvae and pupae. As all these patterns are based on proportions, it should be noted

Table 1 The sampled seasonal phases

Seasonal phase	Abbreviation	Months of year	<i>n</i>	Colony size range (total ants)
Winter	W	December and January	6 (2012)	1770–48,913
Spring	Sp	March and April	8 (2012)	779–47,378
Early summer	ES	May and June	9 (2012)	1125–44,513
Late summer	LS	August and September	11 (4 in 2011 and 7 in 2012)	3209–25,684
Fall	F	October and November	6 (2012)	3991–15,988

The summer was divided into two phases to determine precisely when the reproductive phase of *P. morrisi* occurs. Abbreviations for the seasons are used in some graphs. The sampling regime captured five major phases of the annual cycle. (But late summer was sampled in 2 years)

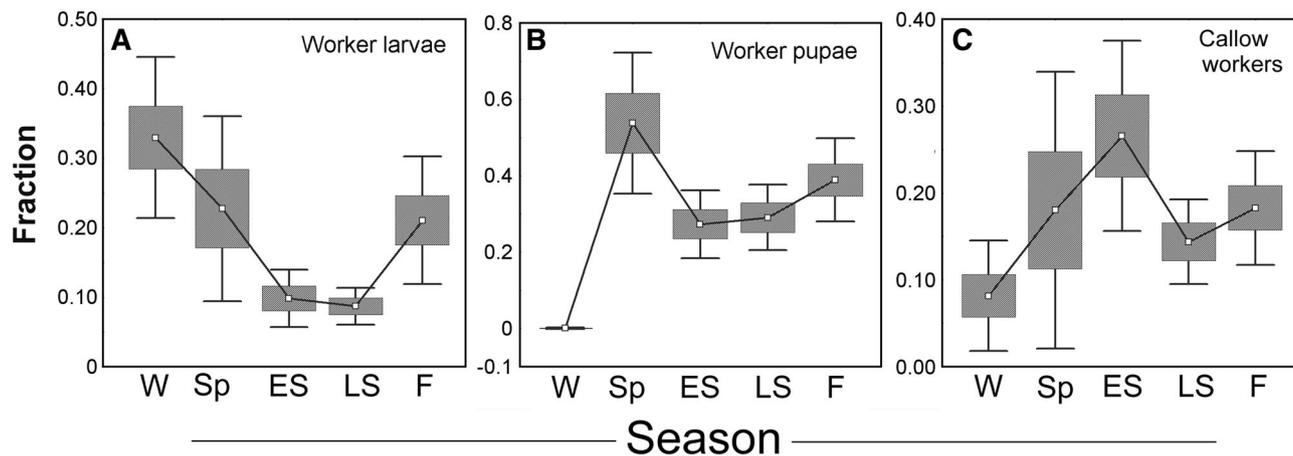


Fig. 3 a Seasonal phases of worker brood production as fraction of the total colony size. **a** Fraction worker larvae; **b** fraction worker pupae; **c** fraction callow workers. The larvae are the only brood present in the winter. The number of pupae rises rapidly in the spring and remains high throughout the remainder of the year. The appearance of pupae in

the spring is soon followed by a rise in callows, peaking one seasonal phase after the pupae. Callow production over the year results in colony growth. Center point is the mean, boxes show 1 SE, and whiskers 95 % CI

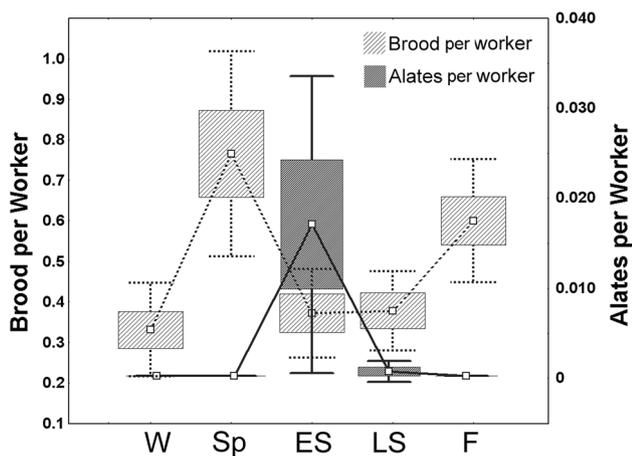


Fig. 4 The seasonal phases of colony investment in worker and sexual brood. Worker production was significantly higher in spring and fall than in winter, early summer, and late summer. Alates were few in number (note the different scale), and their production divides the (ergonomic) investment in worker brood into two phases. Boxes show 1 SE and whiskers 95 % CI. One-way ANOVA of brood/worker ratio by season $F_{1,35} = 11.7$; $p < 0.000005$

that these would be affected by seasonal worker mortality as well as the production of brood. We did not estimate worker mortality, but judging from the seasonally shifting age structure, worker mortality also varied seasonally.

Young alate larvae were not distinguishable from worker larvae, so it is possible that some sexual brood was present in the spring. By early summer, the sexual brood had reached the larger size that distinguishes them from worker brood. The most alates of any stage, larval, pupal, or adult, were found in the early and late summer, suggesting that investment in reproduction separates the two phases of worker larvae production (Fig. 4). As is the case in many ants, the high resource demands

of rearing sexuals probably reduced worker production during this phase (Kipyatkov 2006; Tschinkel 1993).

The workers collected in the mound and as returning foragers were classified together as mound workers, because the foraging behavioral caste cannot be reliably discerned by our study design. Mound worker percentage of the colony varied between ~5 and 20 %, but was not significantly related to colony size (ANOVA: $F_{1,26} = 0.96$; n.s.) or season ($F_{3,26} = 0.66$, n.s.). This suggested that either there is little seasonal pattern to allocation of labor to foraging or, more likely, mound workers and foragers cannot be assumed to be in the same labor group. More reliable estimation of the proportion foraging must await studies aimed specifically at this goal.

Major pupae as a percent of colony size varied significantly with season ($F_{4,35} = 5.01$, $p < 0.003$) and peaked in the spring as overwintered larvae pupated. The continued production of major pupae, even though at declining rates, built up the adult major worker proportions, both in number and percent of weight, to a late summer peak (Fig. 5). This accumulation occurred because the pupal stage is brief, but the life span of adult workers is far longer. As a consequence, major workers as a proportion of total workers fluctuated seasonally, showing a distinct peak in late summer followed by a drop in the fall as the late summer slump in larval production caught up (Fig. 5).

Variation by colony size

The effect of colony size on worker characteristics was estimated in several steps [see “Materials and methods”]. Analysis of size by ordinary linear regression (OLR) and reduced major axis regression (RMA) yielded almost

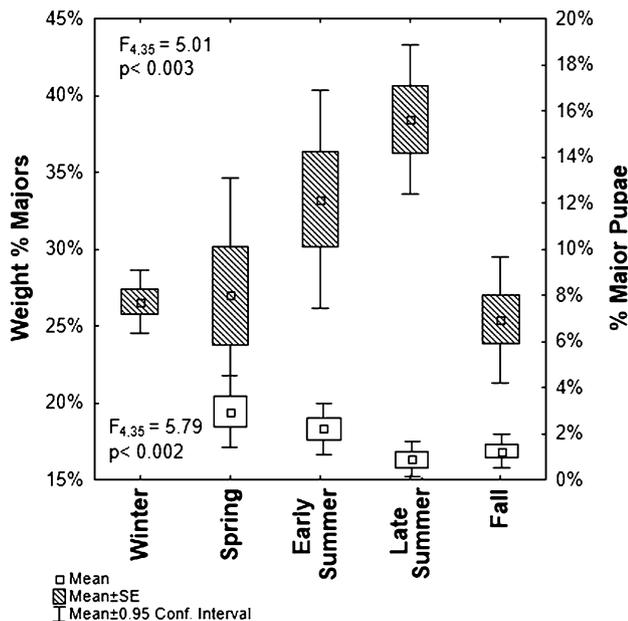


Fig. 5 The ratio of total major workers to total dark workers (*hatched boxes*) changes significantly with season, peaking in late summer and then decreasing to low values by the fall (**b**). The ratio of major pupae to total dark workers (*open boxes*) rises rapidly after the ratio of adult majors drops (**a**). Note differences in scale. *Boxes* show 1 SE and whiskers 95 % CI

identical parameters]. Head width and dry weight of workers were tightly related (Table 2; regression 1). The heads of majors were disproportionately larger than those of minors, so that dry weight increased more slowly than head width [slope of log–log regression = 2.24 instead of 3 if isometric (RMA slope = 2.20)]. The head widths measured on the five smallest and five largest wax cast colonies showed that across this approximately 60-fold colony size increase, minor worker head widths increased from a mean of 0.65–0.72 mm (11 %) and those of majors from 1.16 to 1.26 mm (9 %) (Table 2; regressions 2, 3). From the regression of dry weight against head width (Table 2; regression 1), this corresponded to an increase in mean minor weight from 0.12 to 0.14 mg (17 %) and of majors from 0.46 to 0.52 mg (13 %) (Table 2; regressions 4, 5).

The estimated dry weight (biomass) of the total workers of the smallest colonies was around 0.065 g, while that of the largest was 6.9 g, an approximately 100-fold increase. Although the mean weight of both minors and majors increased with total workers, only the total weight of minors was slightly, but significantly ($p < 0.05$) positively allometric (log–log slope = 1.04; Table 2; regression 6). This corresponded to an 11-fold increase in total weight of minors for every 10-fold increase in the number of workers. However, the relationship of number of workers to total weight of workers was isometric, i.e., a simple multiple of the number of workers (Table 2; regression 8).

These more or less isometric weight–number relationships suggest that total major weight as a proportion of total worker weight does not change with colony size, even though it varied by season (Fig. 6; Table 2; regression 9). Indeed, the seasonal patterns seen in Fig. 5 are similarly expressed in colonies of all sizes (Fig. 6, note inset of Fig. 5). This seasonal similarity for colonies of all sizes also applied to worker brood production. The ratio of worker brood to total ants varied significantly by season (Fig. 4), but within season this ratio did not change significantly with colony size (regression slope = 0.10; $t_{30} = 0.92$; $p = 0.37$).

Alate production was low, with a maximum of 540 alates. Moreover, although colonies with fewer than 3000 workers did not produce alates during the two summer samples, there was no relationship between colony size and alate production (regression vs. colony size for early and late summer, $p > 0.5$; n.s.). Indeed, many colonies with more than 3000 workers did not contain alates during the summer.

Colonies grow until birth and death rates come into equilibrium, and either or both can change as colonies grow, making estimates of these two rates critical to understanding colony growth and maximum size. We have no independent estimate of death rate, but the number of “new” ants produced per worker serves as a reasonable proxy for birth rate (Tschinkel 1993). By designating the sum of the number of pupae and callows as “new” ants, “births” over an extended time period produce a measure that is proportional to the birth rate (actual birth rates would require knowledge of the developmental periods for pupae and callows under the temperatures to which they were exposed in the nest). As the workers who produced these new ants were the older, dark workers, the relevant measure of colony size was the number of dark workers. New workers per dark worker were regressed against the birth rate proxy for all seasons except winter, during which production of adult workers ceased (Fig. 7).

“Birth rate” (new workers per dark worker) decreased significantly as colony size increased (Fig. 7; Table 2; regression 10). Season and the log of dark worker number explained 40 % of the variance in the birth rate. A tenfold increase in the number of dark workers was associated with 0.31 fewer new workers per dark worker, so that small colonies averaged one or more new workers per dark worker, whereas large colonies averaged about 0.4 (Fig. 7). This relationship was similar in all seasons except late summer when the rate of new worker production was lower (Table 2; regression 10; semi-partial correlation = -0.31). When adjusted for season, the number of dark workers still explained 27 % of new workers per dark worker (semi-partial correlation = -0.55). Late summer, in spite of having a significant effect on the dependent variable ($t_{28} = -2.27$; $p < 0.05$), contributed little explanatory

Table 2 Regression variables and parameters

Regr. #	x-Variable	y-Variable	Indicator variable	Parameter	Parameter value	s.e.	t	d.f.	p<	R ² (%)
1	Log HW	Log Wt.		Intercept	-0.51	0.0028	-183	97	0.00	99
				Slope	2.24	0.022	104	97	0.00	
2	Total workers	HW _{minors}		Intercept	0.65	0.0031	208	8	0.000000	93
				Slope	1.54 (10 ⁻⁶)	0.000000	10.9	8	0.000005	
3	Total workers	HW _{majors}		Intercept	1.19	0.00818	145	8	0.000000	76
				Slope	1.87 (10 ⁻⁶)	0.000000	5.06	8	0.001	
4	Total workers	Mean Wt. minors		Intercept	0.117	0.000023	5061	38	0.00	96
				Slope	6.56 (10 ⁻⁷)	0.000000	405	38	0.00	
5	Total workers	Mean Wt. majors		Intercept	0.454	0.000039	11507	38	0.00	99
				Slope	1.66 (10 ⁻⁶)	0.000000	601	38	0.00	
6	Log total workers	Log total weight minors		Intercept	-1.12	0.032	-35.1	38	0.000000	99
				Slope	1.04	0.0083	125	38	0.000000	
7	Log total workers	Log total weight majors		Intercept	-1.27	0.191	-6.65	38	0.000000	91
				Slope	0.990	0.0497	19.9	38	0.000000	
8	Log total workers	Log total weight workers		Intercept	-0.88	0.046	-19.1	38	0.000000	99
				Slope	1.02	0.012	85.4	38	0.000000	
9	Log total worker weight	Log total weight majors	Fall to early summer	Intercept	-0.481	0.099	-4.85	37	0.000022	96
			Late summer	Intercept	-0.344	0.036	3.81	37	0.000516	
				Slope	0.974	0.032	30.1	37	0.000000	
10	Log dark workers	New workers per dark worker		Intercept	1.80	0.27	6.57	28	0.000001	53
				Slope	-0.30	0.073	-4.08	28	0.0004	
			Late summer	Slope	0.48	0.24	2.01	28	0.054	
			early summer	Intercept	-0.125	0.097	-1.29	28	0.21	
			Late summer	Intercept	-2.04	0.90	-2.26	28	0.031	
			fall	Intercept	-0.126	0.11	-1.18	28	0.25	
11	Lot total weight dark workers, mg	Mg new workers per mg dark workers		Intercept	1.26	0.21	5.93	31	0.000001	13
				Slope	-0.215	0.072	-3.00	31	0.006	
12	Log colony size	Log nest volume	Late summer	Intercept	-0.21	0.076	-2.77	31	0.01	
				Intercept	-0.675	0.246	-2.75	38	0.001	81
				Slope	0.81	0.062	13.1	38	0.000001	
13	Log colony size	Log mound volume		Intercept	-4.25	1.33	-3.21	38	0.005	
				Slope	1.90	0.34	5.66	38	0.00001	
14	Log colony size	Maximum depth		Intercept	-79.1	38.2	-2.07	38	0.05	37
				Slope	45.3	9.57	4.74	38	0.00005	
15	Log colony size	Number of shafts		Intercept	-3.50	1.01	-3.47	38	0.002	29
				Slope	1.35	0.253	5.35	38	0.000005	

Fig. 6 Although the proportional investment in major workers changed seasonally, this pattern was not related to colony size. All colony sizes showed the same seasonal patterns. This can be seen in the significantly higher intercept in late summer while retaining a common slope. The inset shows the seasonal pattern of percent majors irrespective of colony size. The inset is taken from Fig. 5

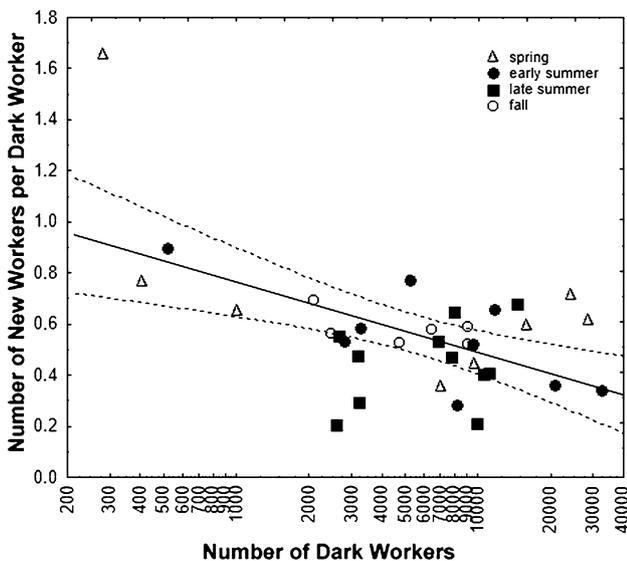
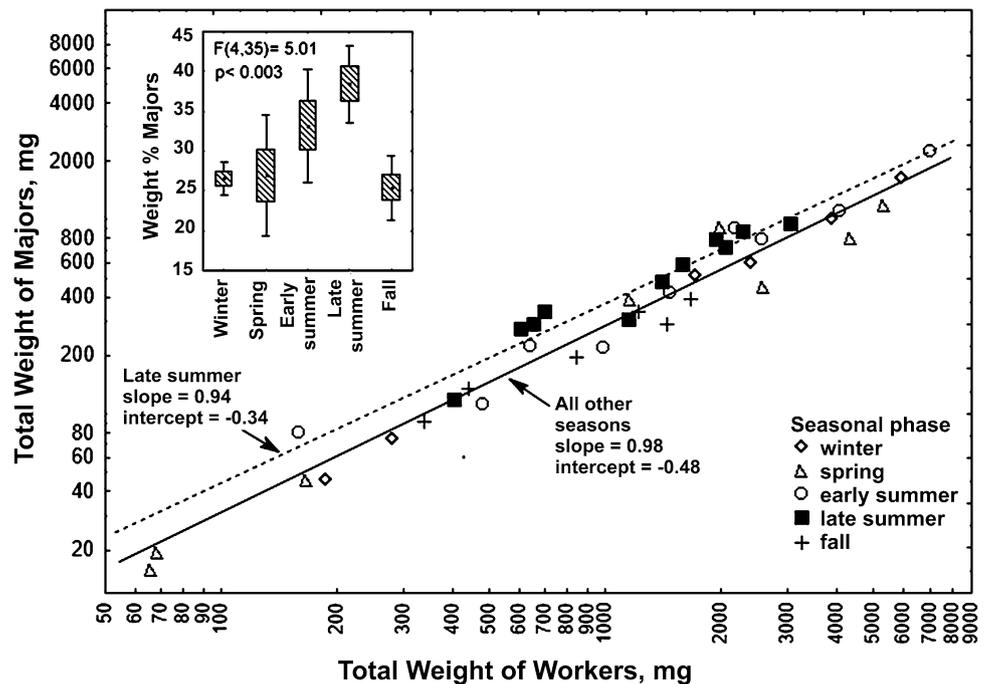


Fig. 7 The standing ratio of new workers to dark (old) workers serves as a proxy for birth rate, and shows that the “birth rate” of new workers declines by 0.31 for every tenfold increase in the number of dark workers. New workers included both pupae and callows, thus integrating the rate over a longer period. Estimation of the true birth rate in terms of workers per day requires knowledge of development rate at the experienced temperature, and was not available. Line and confidence intervals are fit to the equation $y = b + a \log x$. Winter was excluded from this analysis. Dashed lines 95 % CI

power (tolerance 0.006), whereas log number of dark workers contributed most ($t_{28} = -4.1$; $p < 0.005$; tolerance 0.88). The narrow range of colony sizes sampled in late

summer made the estimates of slope and intercept rather unstable and difficult to interpret.

The numerical birth rate decreased in part, because the size of the new workers increased with colony size. The production of new biomass through the production of new workers therefore decreased more slowly in relation to dark worker number than did the numerical birth rate. Whereas the numerical birth rate decreased by 0.31 for every tenfold increase in the number of dark workers, the milligrams of new workers per milligram dark workers decreased by only 0.21 (Table 2; regression 11) because minors increased 13 % and majors 17 % in size (see above, Table 2; regressions 4, 5).

Nest architecture

Nest architecture conformed to the basic patterns revealed by Tschinkel (2003 and unpublished) using molten aluminum casting. The underground nest consists of between one and five vertical shafts connecting lobed chambers, with far fewer chambers at greater depth and, below a few centimetres, almost no horizontal connections between shafts. Nests ranged from 0.5 to 2 m deep and often ended at the water table. All but the smallest colonies built mounds, but only the largest mounds contained chambers.

Total nest volume was strongly associated with colony size (Fig. 8). Over the entire range of colony size, nest volume increased more slowly than colony size, with a sixfold increase for every tenfold increase in colony size (Fig. 8). However, analysis of residuals showed that the

log–log relationship was significantly non-linear, with an increase in instantaneous slope suggesting that as colonies grew, they excavated more nest volume per ant (Figs. 8, 10). Season had no effect on this relationship. The increased nest volume resulted from more chambers and deeper nests (Fig. 9b) with more vertical shafts (Fig. 9c). Colonies with fewer than about 3000 ants did not build mounds, but above

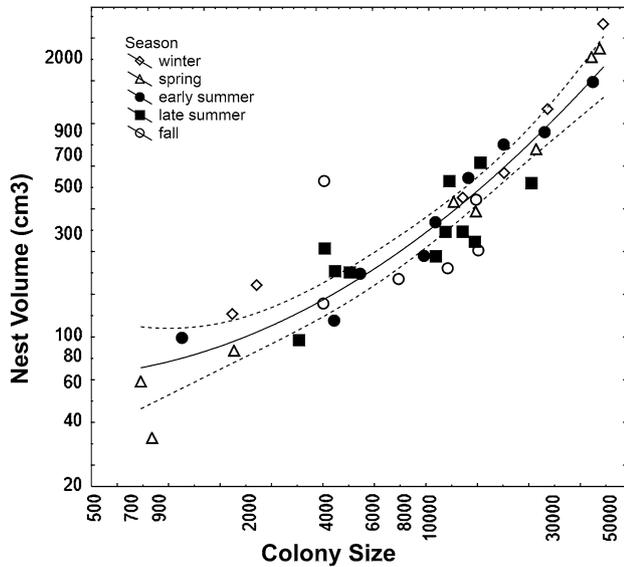


Fig. 8 The log of the nest volume versus the log of colony size indicated that volume increased more slowly than colony size. Averaged over the entire range, for every tenfold increase in colony size there was a sixfold increase in nest volume. However, a quadratic polynomial provided a better fit to the data, showing that the rate of volume addition per ant increased with colony size. Log total nest volume (cm^3) = $3.13 - 1.22x + 0.275x^2$. Dotted lines 95 % CI for the mean regression

this size, mound volume was not strongly related to log colony size (Fig. 9a; $R^2 = 22\%$). Regression parameters for nest attributes can be found in Table 2 (regressions 12–15).

Because nest volume generally increased more slowly than colony size, the volume per ant changed in a complex manner (Fig. 10), with a minimum in medium-sized colonies and generally higher values in very small and very large colonies (Fig. 10). The inverse of volume per ant described the changes in crowding, with greatest crowding in middle-sized colonies. Season had no effect on these crowding patterns.

Nest space was not evenly distributed vertically, but decreased with depth, so that over 60 % of total nest volume was in the top 20 % of the nest (Fig. 11a). The underground distribution of colony members showed broad trends when averaged across seasons. The dark (mature) major workers were located at the bottom of the nest and the dark minor workers toward the top (Fig. 11b, c). The larvae, pupae, and callow workers were all found together (Fig. 11d), validating the combination of larvae and pupae as “brood” and providing evidence that callow workers are involved in brood care, or have recently eclosed into their adult forms and have remained where they emerged. When crowding estimates take available space into account, it can be seen that the proportion of the colony found at depths does not parallel the space available for any type of ant (Fig. 11b–d).

Seasonal differences in subterranean distribution and temperature

Workers move brood up and down in response to daily changes in soil temperature. Near the surface, daily

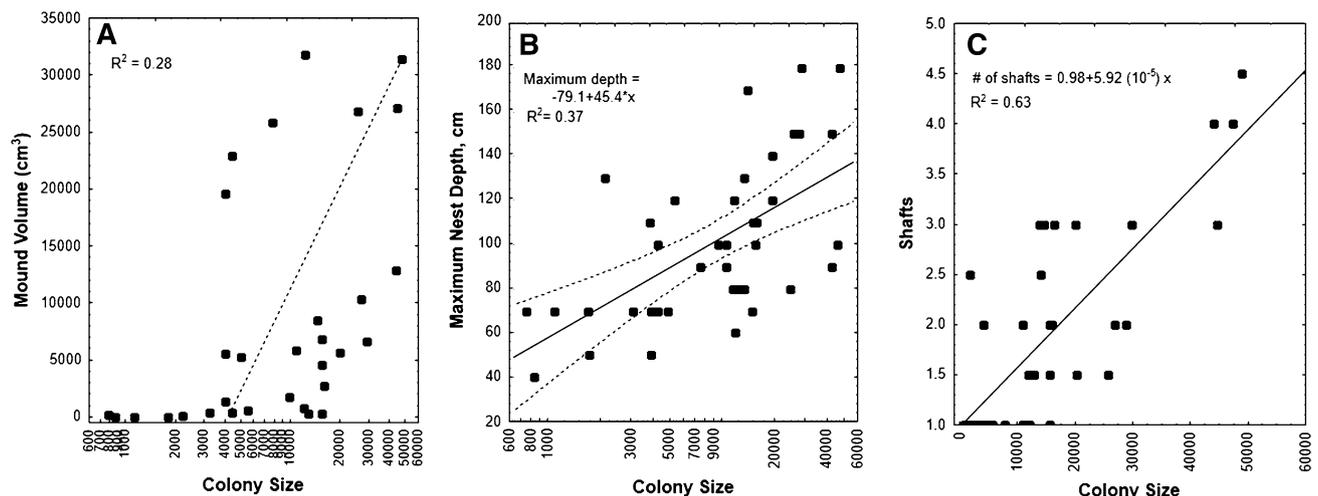


Fig. 9 Correlations between colony size and other nest attributes. As colonies grew, they excavated nests with **a** larger mounds ($R^2 = 0.23$), **b** greater nest depth ($R^2 = 0.34$), and **c** more shafts ($R^2 = 0.63$)

temperature variation may be 10–20 °C, whereas at 80 cm it is 2 °C or less. Averaged over the daily cycle, shallow soils are cooler during the winter than deeper soils, while in summer the opposite is true. In the course of a day, the shallowest depths switch from being the coolest in early morning to the warmest by early afternoon (Table 3). The

wax casts were always made in the morning just preceding this transition. In early summer, this resulted in very little variation in ant density at any depth (Table 3).

The vertical distribution of brood, callows, major and minor workers varied seasonally (Fig. 12a–j). Workers (minors and majors), and to a lesser degree brood and callows were located in the deeper regions of nest in the winter, moved dramatically upward in the spring and early summer to predominate in the top two deciles, and began shifting downward again in late summer. Their vertical distribution in the fall was bimodal, perhaps as some portions of the colony moved downward, while others did not. These shifts in vertical location were associated with changes in vertical soil temperature profile. In the winter and fall, the highest temperatures were found deepest underground (Figs. 12, 13; Table 3). The shift to the upper regions of the nests in spring and early summer (Fig. 12b, c, g, h) was associated with favorable temperatures at these levels, as was the subsequent downward movement in late summer and fall (Fig. 12d, i). Figures 11 and 12 understate the vertical changes in ant density, because the mass of the colony moved downward into smaller and fewer chambers in fall and winter, and upward into more and larger chambers in spring and summer.

The arrangement of colony members does not parallel the vertical distribution of nest volume in Fig. 11a, suggesting that the ants do not respond to available space but rather to environmental cues, possibly preferred temperatures, during these vertical seasonal rearrangements (Porter and Tschinkel 1993). The temperatures experienced by the brood are

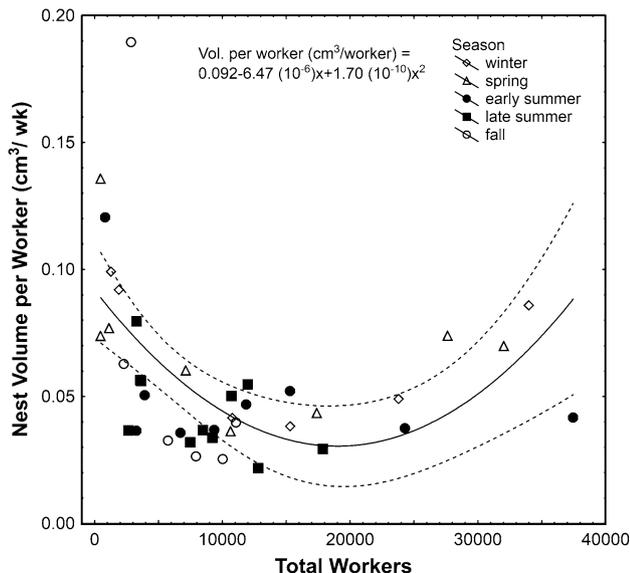


Fig. 10 The volume of nest per worker (the inverse of the density) changes with colony size. Medium-sized colonies had the smallest volume per worker and were the most crowded. Season is shown as symbols. The fitted line is a quadratic polynomial with 95 % confidence interval bands: Vol. per worker ($\text{cm}^3/\text{worker}$) = $0.092 - 6.47 (10^{-6})x + 1.70 (10^{-10})x^2$

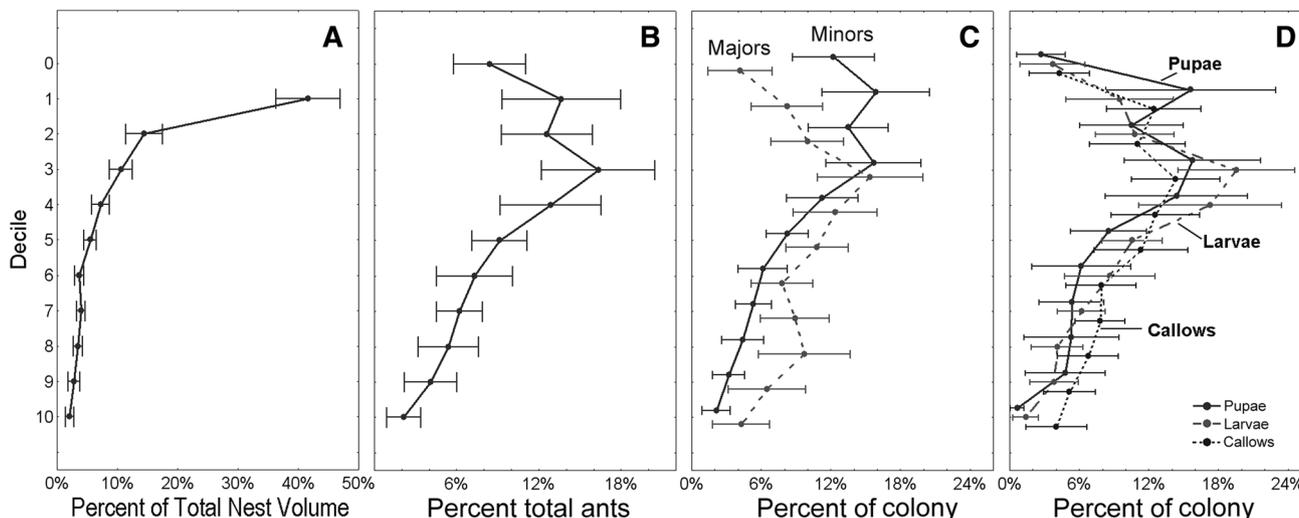


Fig. 11 Trends in subterranean vertical distribution based on averages of all seasons. Rather than actual depths, deciles were computed as 10 % increments of the maximum depth, to give a size-free representation of vertical distribution. An eleventh increment, “decile 0” represents the mound and ground surface. **a** Nest volume tapers

consistently with depth. **b** Total ants taper with depth. **c** Minors and majors had significantly different distributions ($p < 0.00001$), with minors being found more toward the surface and majors deeper in the nest. **d** Distributions of larvae, pupae, and callows are not significantly different. Whiskers indicate 95 % CI

Table 3 The regions of the nest and the temperatures where the broods were located along with the minimum and maximum temperatures available in the nest

Seasonal phase	Brood location in nest	Approximate temperature (°C) of brood location at casting time	Mean min and max temperatures available in nest
Winter	Middle	14	12.1–17.2
Spring	Upper middle	20	17.9–20.3
Early Summer	Upper	25	23.5–25.3
Late Summer	Middle	25–26	24.1–26.2
Fall	Bottom	20	14.6–20.7

The broods are generally kept at the warmest temperatures

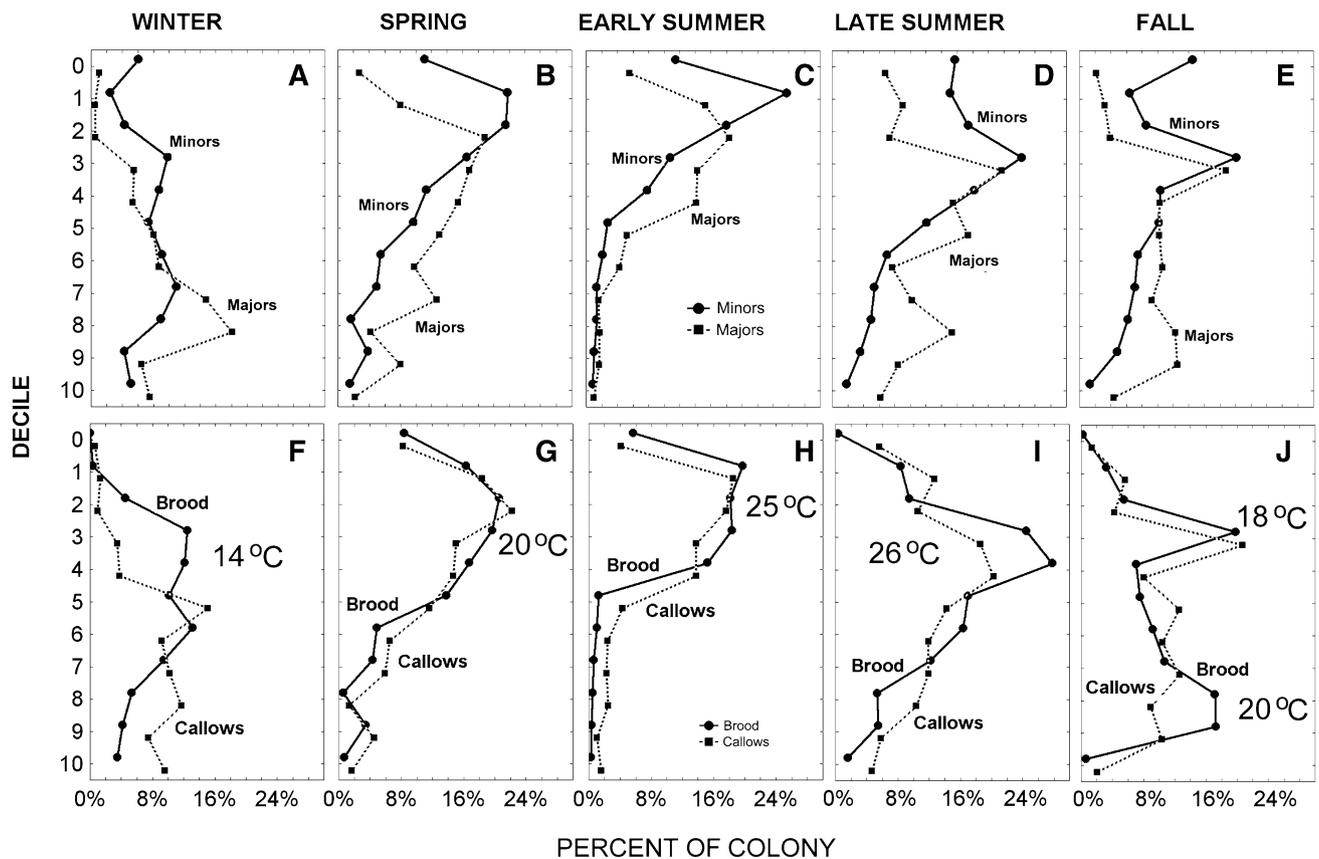


Fig. 12 The seasonal changes in the vertical subterranean distribution of the main categories of colony members (major and minor workers, a–e; brood and callows, f–j). In the spring and early summer the ants are found toward the top of the nest. By late summer they are found in

the middle of the nest. The ants are found deepest in the nest during the colder seasonal phases of fall and winter. The soil temperature associated with the peaks of brood and callow distribution are shown

most critical to colony fitness, because brood development rate is subject to both optimum and minimum temperatures. Based on the temperature of brood location for each season, their optimum temperature is ~ 25 °C (Table 3; Fig. 12).

The vertical distribution of brood during the annual cycle is shown in Fig. 12f–j, and the distribution of temperatures at the time of casting in Fig. 13. Table 3 presents the temperatures available to the ants during the sampling dates.

The broods were found in the middle of the nest during the winter at 14 °C, even though the bottom of the nest was the warmest region at 18 °C. However, this was still well under the optimum 25 °C. In the spring they were in the upper middle region of the nest at about 20 °C, which was just slightly cooler than the deeper regions. The brood showed the strongest preference for the upper region of the nest in the early summer, taking advantage of the highest

temperatures available in the nest. During the late summer, the brood was found in the middle of the nest, at approximately 25–26 °C. During the two summer samples, there was little vertical temperature gradient at the time of casting (Fig. 13), and the ants had access to temperatures in the mid-20s during much of the day. This raises the question of why broods were nevertheless found mostly in the middle region of the nest, rather than more evenly distributed.

Available space is not a likely explanation, as only a small proportion of the nest volume is located in this middle region. Factors other than temperature must be at play. In the fall the broods were located in part in the bottom of the nest, the warmest region, but in part higher up.

Density distribution by season

One of the obvious benefits of collecting ant colonies with wax casts is the preservation of their vertical subterranean distribution, as well as preserving the volume in which they resided. This makes it possible to determine the distribution of density of ants. Because nests vary in maximum depth, direct comparison of vertical distribution can obscure common within-nest patterns. For comparison among nests of different sizes, size-free vertical distributions were rendered as the percent of the ants residing in deciles (i.e., ten percent increments of the maximum depth). An eleventh increment, “decile 0” represented the mound and ground surface. Such plots are size independent on both axes, allowing comparisons across sizes. In Fig. 14, the shaded shapes show the 95 % confidence bands for size-free nest volume distribution by decile. The proportion of the total ant density (sum of the density over all deciles) found in each decile is shown for each season as points and lines (Fig. 14). Ant density moves up and down seasonally, from low and middle depths during cooler seasons to shallower depths during warm seasons. A comparison of the ant

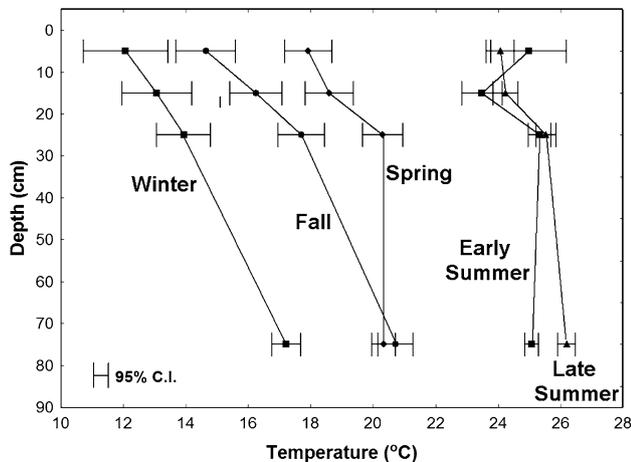


Fig. 13 Mean subterranean temperatures at 10 a.m. (casting time) for each season. The warmest temperatures were nearly always found at the greatest depths, but were sometimes available at both the top and bottom of the nests

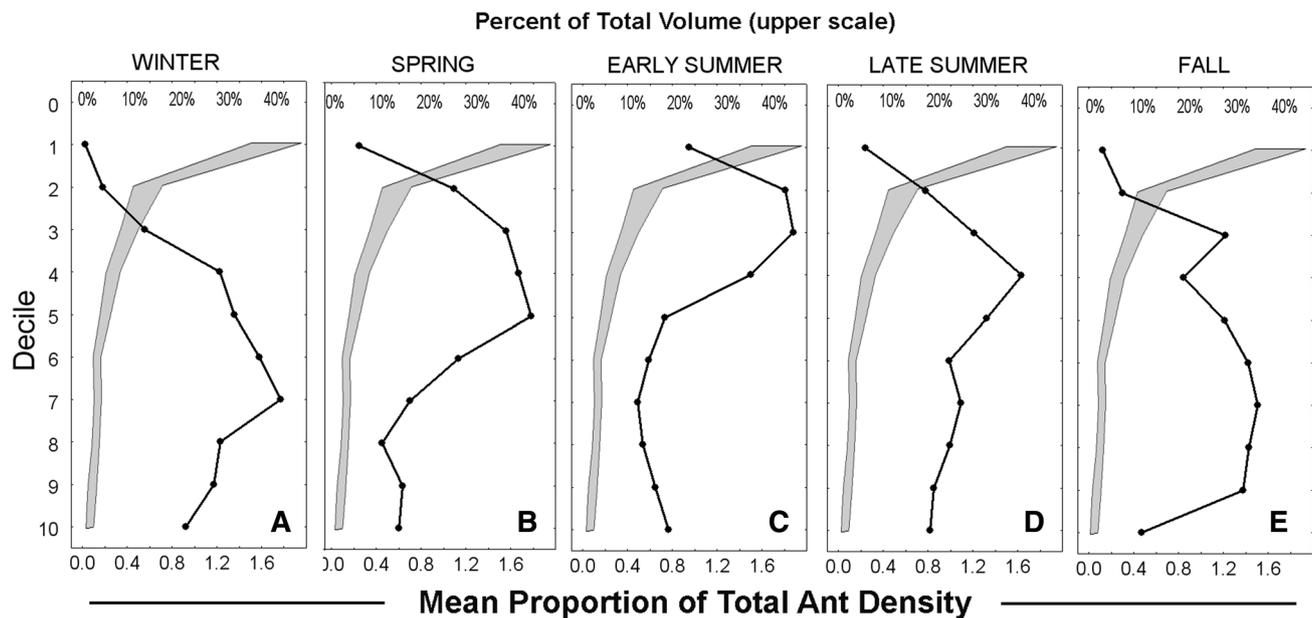


Fig. 14 The seasonal cycle of subterranean density changes. The different shapes of the density distributions reveal how drastic the rearrangement of the ants is underground. It is important to note that the tapering of the nest volume with depth was fixed (gray shaded

plots), while the density varied vertically with season. Shaded figure mean ± 95 % CI of proportion of nest volume. Mean standard error of density of all deciles for each season: winter = 0.24, spring = 0.24, early summer = 0.16, late summer = 0.18, fall = 0.29

density with the available space emphasizes that available space seems not to be a primary factor regulating vertical distribution of the ants within the nest. During some seasons, density is highest in zones with the lowest proportion of space, whereas in others it is more aligned with space availability, but in no case is ant density distributed parallel to available space (Fig. 14).

Discussion

This study focuses on organization at the colony level which, in parallel to that of a unitary organism, has its own consistent and measurable qualities, and its own seasonal and lifetime rhythms. Each colony of *Pheidole morrissi* consists of many components, including major and minor workers, old and young workers, brood in various stages, male and female alates, and a subterranean nest, but each colony in our population divided its resources among those pieces in the same way during each season. Each colony has predictable architecture, periods of growth, general size at first reproduction, and a synchronized reproductive period, just like a traditional unitary organism. These colony features are organized by temperature, birth rate, and demography. Colony attributes change during growth—the nest volume generally does not parallel the number of ants, so that average crowding changes in complex ways. The colony components each move up and down in the nest in a still more complex, three-dimensional dance in relation to seasons and time of day. Both minor and major workers become larger, but the colony's relative investment in them remains similar for colonies of all sizes. The ratio of old (dark) workers to new workers, a proxy for birth rate, declines with colony size, possibly playing a role in limiting the maximum size of colonies. Taken together, our findings provide compelling evidence that the social insect colony is a superorganism analogous to the unitary organism, with natural selection acting on the whole entity to shape the colony size, number, size, and abundance of the workers, their seasonal birth, life span and death, the investment in sexuals, and many other aspects of colony composition and function. These are the attributes that adapt the superorganism to its environment.

This study demonstrates the utility of the wax-casting method for capturing and analyzing entire colonies of ground-nesting ants (Tschinkel 2010). All nest contents, including brood, workers, alates, prey items, myrmecophiles, and anything else that was in the nest are frozen in their exact location at the time the wax is poured. Vertical arrangement of these items is a functional part of the superorganism, as is their vertical movement in relation to life stage, age, and environmental conditions (Tschinkel 2004). However, a measure that is not currently available from the

wax-casting method is body fat, whose variation with season, colony size, and worker size is an integral part of the life cycle and reproductive strategy of many ants (Tschinkel 1993, 1999).

Ph. morrissi colonies ranged in size from a few hundred workers to 38,000, with nest volumes up to almost 3 L, composed of up to five shafts often penetrating the water table (1.5–2 m). The large size of mature colonies as well as their abundance confirms the ecological dominance of *Ph. morrissi* in the native piney flatwoods, as previously reported by King and Tschinkel (2008) through pitfall trapping. Our maximum colony sizes were more than threefold larger than those reported by Yang (2004). This is rather puzzling, as Yang worked in the same habitat we did, but used a different method of capture and estimation.

Ant colonies must typically reach a minimum size for alate production, switching from the ergonomic to the reproductive phase of the life cycle (Oster and Wilson 1978). This switch point is clearly an important life history characteristic, no doubt linked to other characteristics such as growth rate, life expectancy, founding success, and more (Tschinkel 1991, 2011). For *Ph. morrissi*, this transition occurred in colonies with more than 3000 workers, less than 10 % of the maximum colony size. This transition also occurred at about 10 % of maximum size in the fire ant, *S. invicta* (25,000 workers; Vargo 1988; Markin et al. 1973; Tschinkel 1993), and in the harvester ant, *P. badius* (700 workers; Smith and Tschinkel 2006). Whether other species also make this transition at a similar proportion of maximum size is not known, but in any case colonies continue growing well beyond the minimum size for alate production. In *Ph. morrissi*, the unexpected finding that alate production is not related to colony size suggests that the reproductive strategy of *Ph. morrissi* is not simply to maximize alate output. It is possible that colonies “mast”, producing highly variable numbers of sexuals in different years, perhaps depending on certain narrowly defined conditions. Other possibilities are that the alates reside in the nest only briefly after adult enclosure and were missed by the sampling or that they may be produced and fly in a long trickle, rather than as coordinated batches and mating flights.

Seasonality

Many temperate zone ants undergo a seasonal cycle with distinct growth, reproductive, and dormant phases (Hölldobler and Wilson 1990, for examples, see: Tschinkel 1987, 1991, 1998a, 1999, Laskis and Tschinkel 2009, Kipyatkov 1993, 1995, 2006; Kipyatkov et al. 2001). In *Ph. morrissi*, the general, size-related changes are overwhelmed by seasonal patterns of production and vertical distribution within the nest. Worker brood is overwintered as larvae, possibly contributing to the spring peak in worker pupae, followed by

the early summer peak in callow workers. The proportion of major workers increased until late summer as a result of the greater production of majors in the spring and early summer, and possibly because majors live longer than minors and therefore accumulate. In contrast to our study, Yang (2006) found no seasonal variation in the proportion of major workers, perhaps because he sampled only in June–July and October.

Worker brood peaks at 40 % of colony size in the spring, slackens in summer, while alates mature and rises again to 35 % in the fall. Kipyatkov (2006) described similar two-phase worker brood rearing separated by the reproductive phase in north temperate ants. However, in *Ph. morrisi* the low number of sexuals produced seems not to be in accord with this dip in worker production. Worker and sexual production are largely complementary in other ant species as they switch productive capacity from workers to alates, e.g., *S. invicta* (Tschinkel 1993), *P. badius* (Tschinkel 1999; Smith and Tschinkel 2006), and *Crematogaster ashmeadi* (Tschinkel 2002), but in all of these, sexual production is a larger share of total production. It is possible that low alate production is a consistent and adaptive characteristic of *Ph. morrisi* resulting from a high degree of K-selection in this stable, long-lived longleaf pine ecosystem.

All ants have age-based division of labor (Hölldobler and Wilson 1990; Oster and Wilson 1978). The seasonal differences in brood production ensure that the worker age structure changes during the annual cycle. In early summer, over one-quarter of the workers are young (callow), and by winter this has shrunk to about 8 %. In *P. badius*, allocation of labor to foraging is driven by changes in the age structure of the worker population during the annual cycle (Kwapich and Tschinkel 2013). If this is also true of *Ph. morrisi*, the “labor sectors” swell and shrink greatly through the seasons, with brood-rearing labor predominating in the first half of the year, and foraging in the second. Seasonal patterns of forager availability could not be determined in our study. Seasonal changes in colony size, such as that occur in the fire ant, *S. invicta* (Tschinkel 1993), were not obvious in *Ph. morrisi*.

In *S. invicta*, the ratio of major to minor workers changes drastically during colony growth (Tschinkel 1988), suggesting large shifts in the type of labor available, but the lack of such change in *Ph. morrisi* suggests that the need for whatever work majors perform does not change with colony size. The degree of size difference between majors and minors is correlated to the degree of labor specialization by majors (Wilson 1984). Wheeler (1986) provided evidence that the proportion of majors was regulated by feedback, but in *S. invicta* this proportion was simply correlated with colony size. Another factor affecting relative labor availability could be worker life span, as majors probably live longer than minors. In *S. invicta*, larger worker body size is associated with much longer life span (Calabi and Porter 1989).

Vertical organization

In *Ph. morrisi*, the colony as a whole moved upward from winter to summer and downward from summer to fall and winter, suggesting that this movement is driven primarily by soil temperature. All ants have a preferred temperature (Hölldobler and Wilson 1990) and rearrange themselves and their nest-mates to take advantage of these temperatures (Porter 1990; Porter and Tschinkel 1993; Penick and Tschinkel 2008). If optimal temperatures are not available, the ants do the best that they can, but may make different choices depending on whether food is in short supply or not (Porter and Tschinkel 1993). Based on the observed distributions in *Ph. morrisi*, the preferred temperature for the brood is about 25 °C.

Averaged across seasons, minor workers were found higher in the nest and majors lower, suggesting differences in the distribution of tasks handled by these two types. It is possible that the flow of the wax carried more majors deeper into the nest than minors, but the differences in seasonal ant distribution patterns mitigates against this cause. Callow distribution was often not quite congruent with other worker distribution, but this stratification by age was weaker than it was in *P. badius* in which workers were strongly vertically segregated by age, with young workers deep in the nest and old near the surface (Tschinkel 1998a) and only the oldest acting as foragers (Kwapich and Tschinkel 2013). This vertical organization is an essential element in division of labor and is detectable in most ants as a centripetal movement of workers away from brood as they age (Hölldobler and Wilson 1990; Conway 2003; Sendova-Franks and Franks 1995; Tschinkel 1993, 1998a; Jandt and Dornhaus 2009). However, the location of the majority of brood clearly varies among species. The seasonal vertical movement of brood in *Ph. morrisi* and *S. invicta* (Tschinkel 2006) contrasts with the unvaried location of brood in the deeper regions of the nest in *P. badius*. In *S. invicta*, this movement is clearly stimulated by temperature (Penick and Tschinkel 2008; Porter 1990) and this seems to be the case in *Ph. morrisi* as well, but not in *P. badius*.

Vertical movement within the nest creates extreme variation of ant density, variation not driven by available space as density does not parallel the vertical availability of chamber area. Little is known about the function of crowding or its preferred levels in social interactions, but ants aggregate even in spacious laboratory nests, especially around brood.

The correlation between larger colonies and deeper nests, while intuitive, cannot be rationalized by the relationship between temperature and depth, because the temperature gradient reverses with the seasons and does not change appreciably below 80 cm, a depth far exceeded by many colonies. Nest volume could also be added laterally in the

upper regions of the nest, so the tendency to increase nest depth with colony population indicates a difference in the dynamics of large and small colonies.

Birth rate

The standing number or weight of new workers per weight of dark workers is an imperfect estimate of the true birth rate or production rate (respectively), as it does not include the element of developmental time. To be expressed as rates per day, the standing ratio of pupae and callows to dark workers must be divided by how long it takes a pupa to become a callow, and a callow to become a dark worker, and both of these depend on the temperature. Figure 12 shows that the temperatures to which brood and callows are exposed changes drastically throughout the year, from about 14 to 26 °C. Thus, with lower available nest temperatures, development would take longer and the ratio of new to dark workers would overestimate birth rates relative to the warmer temperatures of summer. In the current absence of the stage duration of *Ph. morrisi* in relation to temperature, these corrections cannot be made. In addition, the temperatures in Fig. 12 are those at the time of wax casting in the morning and do not reflect daily up and down movement of brood as the surface warms and cools. If *Ph. morrisi* move brood as actively as do *S. invicta* (Penick and Tschinkel 2008), brood development rate may be drastically manipulated by workers.

Significance

This study of *Ph. morrisi* provides more evidence that superorganisms in their subterranean nests exist in complex dimensions of time and space, distributing their parts in relation to season, life stage and time of day, as well as changing attributes during growth and reproduction. In contrast to field studies such as ours, laboratory studies in single-chambered, two-dimensional, constant-temperature laboratory nests lacking an annual cycle cannot reveal many of the superorganism's critical functional adaptations.

Acknowledgments Ryan Reynolds, Christine Stevens, Caleb Vaughn, and Alice Mallory provided help in the field. The Florida State University Antarctic Research Facility allowed us to store wax casts in their freezer. Christina Kwapich provided helpful comments and discussion. This work was partially supported by National Science Foundation IOS 1021632.

References

Calabi PS, Porter SD (1989) Worker longevity in the fire ant *Solenopsis invicta*: ergonomic considerations of correlations between temperature, size and metabolic rates. *J Insect Physiol* 35:643–649

- Conway JR (2003) Architecture, population size, myrmecophiles, and mites in an excavated nest of the honey pot ant, *Myrmecocystus mendax* Wheeler in Arizona. *Southw Nat* 48(3):449–450
- Frost CC (1993) Four centuries of changing landscape patterns in the longleaf pine ecosystem. In: Proceedings of the Tall Timbers Fire Ecology Conference no. 18, pp. 17–43
- Gregg RE (1942) The origin of castes in ants with special reference to *Pheidole morrisi* Forel. *Ecology* 23:295–308
- Hölldobler B, Wilson EO (1990) The ants. The Belknap Press of Harvard University Press Cambridge, Massachusetts
- Jandt JM, Dornhaus A (2009) Spatial organization and division of labour in the bumblebee *Bombus impatiens*. *Anim Behav* 77:641–651
- King JR, Tschinkel WR (2008) Experimental evidence that human impacts drive fire ant invasions and ecological change. *PNAS* 105:20339–20343
- Kipyatkov VE (1993) Annual cycles of development in ants: diversity, evolution, regulation. In: Kipyatkov VE (ed) Proceedings of the colloquia on social insects, vol 2. Russian-speaking section of the IUSSI, Socium, St. Petersburg, pp 25–48
- Kipyatkov VE (1995) Role of endogenous rhythms in the regulation of annual cycles of development in ants (Hymenoptera, Formicidae). *Entomol Rev* 74(7):1–15
- Kipyatkov VE (2006) The evolution of seasonal cycles in cold-temperate and boreal ants: patterns and constraints. Life cycles in social insects: behavior, ecology and evolution. St. Petersburg University Press, St. Petersburg, pp 63–84
- Kipyatkov VE, Elmes G et al (2001) Physiological and ethological adaptation by *Myrmica* ants to short summers. In: Proceedings of the 2001 Berlin Meeting of the European Sections of IUSSI, Berlin, p 111, 25–29, September 2001
- Kwapich CM, Tschinkel WR (2013) Demography, demand, death and the seasonal allocation of labor in the Florida harvester ant (*Pogonomyrmex badius*). *Behav Ecol Sociobiol* 67:2011–2027. doi:10.1007/s00265-013-1611-9
- Laskis KO, Tschinkel WR (2009) The seasonal natural history of the ant, *Dolichoderus mariae* (Hymenoptera: Formicidae) in northern Florida. *J Insect Sci* 9:2
- Markin GP, Dillier JH, Collins HL (1973) Growth and development of colonies of the red imported fire ant, *Solenopsis invicta*. *Ann Entomol Soc Amer* 66:805–808
- Moreau CS (2008) Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). *Molec Phylog Evol* 48:224–239
- Muscudere ML, Willey TA, Traniello JFA (2009) Age and task efficiency in the ant *Pheidole dentata*: young minor workers are not specialist nurses. *Anim Behav* 77(4):911–918
- Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton
- Patel AD (1990) An unusually broad behavioral repertory for a major worker in a dimorphic ant species: *Pheidole morrisi* (Hymenoptera: Formicidae). *Psyche* 97:181–191
- Penick CA, Tschinkel WR (2008) Thermoregulatory brood transport in the fire ant, *Solenopsis invicta*. *Insect Soc* 55:175–172
- Porter SD (1983) Fast, accurate method of measuring ant head widths. *Ann Entomol Soc Amer* 76:866–867
- Porter SD (1990) Thermoregulation in the fire ant *Solenopsis invicta*. In: Veeresh GK, Mallik B, Viraktamath CA (eds) Social insects and the environment, vol 660. Oxford & IBH Publ. Co. Pvt. Ltd, New Delhi
- Porter SD, Tschinkel WR (1993) Fire ant thermal preferences: behavioral control of growth and metabolism. *Behav Ecol Sociobiol* 32:321–329
- Seid MA, Traniello JFA (2006) Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): a new perspective on temporal polytheism and behavioral plasticity in ants. *Behav Ecol Sociobiol* 60:631–644

- Sendova-Franks AB, Franks NR (1995) Spatial relationships within nests of the ant *Leptothorax unifasciatus* (Latr.) and their implications for the division of labour. *Anim Behav* 50:121–136
- Smith CR, Tschinkel WR (2006) The sociometry and sociogenesis of reproduction in the Florida harvester ant (*Pogonomyrmex badius*). *J Insect Sci* 6:32
- Tschinkel WR (1987) Seasonal life history and nest architecture of a winter-active ant, *Prenolepis imparis*. *Insect Soc* 34:143–164
- Tschinkel WR (1988) Colony growth and ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. *Behav Ecol Sociobiol* 22(2):103–115
- Tschinkel WR (1991) Insect sociometry, a field in search of data. *Insect Soc* 38:77–82
- Tschinkel WR (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecol Monog* 63(4):425–457
- Tschinkel WR (1998a) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insect Soc* 45:385–410
- Tschinkel WR (1998b) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*, in relation to colony size and season: distribution of workers and seeds in the nest. *Ecol Entomol* 24:222–237
- Tschinkel WR (1999) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex radius*: distribution of workers, brood and seeds within the nest in relation to colony size and season. *Ecol Entomol* 24:222–237
- Tschinkel WR (2002) The natural history of the Arboreal ant, *Crematogaster ashmeadi* (Hymenoptera: Formicidae). *J Insect Sci* 2:12
- Tschinkel WR (2003) Subterranean ant nests: trace fossils past and future? *Palaeogeogr Palaeoclimatol Palaeoecol* 192:321–333
- Tschinkel WR (2004) The nest architecture of the Florida harvester ant, *Pogonomyrmex badius*. *J Insect Sci* 4(21):19
- Tschinkel WR (2006) *The fire ants*, vol 747. Harvard University Press, Cambridge
- Tschinkel WR (2010) Methods for casting subterranean ant nests. *J Insect Sci* 10:88
- Tschinkel WR (2011) Back to basics: sociometry and sociogenesis of ant societies (Hymenoptera: Formicidae). *Myrmecol News* 14:49–54
- Tschinkel WR, Murdock T, King JR, Kwapich C (2012) Ants and groundwater in the north Florida flatwoods. *J Insect Sci* 12:114
- Vargo EL (1988) Effects of pleometrosis and colony size on the production of sexuals in monogyne colonies of the fire ant *Solenopsis invicta*. In: Trager JC (ed) *Advances in myrmecology*. Brill EJ, Leiden, pp 217–225
- Wheeler DE (1986) Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am Nat* 128:13–34
- Wilson EO (1975) Enemy specification in the alarm-recruitment system of an ant. *Science* 190:798–800
- Wilson EO (1976) The organization of colony defense in the ant *Pheidole dentata* Mayr (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 1(1):63–81
- Wilson EO (1984) The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 16:89–98
- Wilson EO (1985) The sociogenesis of insect colonies. *Science* 228:1489–1495
- Wilson EO (2003) *Pheidole* in the new world: a dominant and hyperdiverse ant genus. Harvard University Press Cambridge, Massachusetts
- Yang AS (2004) Geographic variation of caste structure among ant populations. *Curr Biol* 14:514–519
- Yang AS (2006) Seasonality, division of labor, and dynamics of colony level nutrient storage in the ant *Pheidole morrisi*. *Insect Soc* 53:456–462