RESEARCH ARTICLE

A seasonal natural history of the ant, *Odontomachus brunneus*

L. M. Hart · W. R. Tschinkel

Abstract A north Florida population of *Odontomachus brunneus*, a species of ponerine ants, was studied for a one-year period to determine the annual cycle of reproduction and colony growth, including the foraging biology and seasonal changes in nest architecture. The life cycle of *O. brunneus* is strongly seasonal. Colonies produce brood for 6 months and are broodless for 6 months. Alates are produced in mixed broods at the beginning of each season, consuming much of the colony's energy reserves. These reserves recover slowly through foraging during the summer's worker production, and rapidly after brood production ceases in October. The foraging population was estimated to average 77% (SD 22) of the workforce. This proportion was not related to colony size and female alates were also found to forage. Nest architecture was found to change seasonally, with winter nests being more than twice as deep as the average summer nest.

Keywords *Odontomachus brunneus* · Annual cycle · Seasonal nest architecture · Foraging

L. M. Hart · W. R. Tschinkel
Department of Biological Science, Florida State University,
Tallahassee, FL 32306-4370, USA
e-mail: tschinkel@bio.fsu.edu

Present Address:
L. M. Hart (✉)
Division of Plant Sciences, University of Missouri—Columbia,
1-31 Agriculture Building, Columbia, MO 65203, USA
e-mail: lmhd74@mizzou.edu

Introduction

Deciphering life history strategies is a key element in understanding a species and its interactions with the environment. For social insects such as ants, it is the life history of the superorganism that is of interest—the individual colony members are the parts that make up the superorganism. Natural history studies may include, but are not limited to the seasonal cycle, colony size, queen number, number of nests per colony, worker size, alate size and number, nest location, and nest architecture (Hölldobler and Wilson, 1990; Gadgil and Bossert, 1970; Tschinkel, 1991, 1993; Laskis and Tschinkel, 2009).

A large component of life history analysis is "understanding the diversity of reproductive allocation strategies" (Bourke and Franks, 1995, p. 301); that is, the patterns of how colonies allocate resources among the essential tasks of seasonal and size-related colony growth, sexual reproduction and colony maintenance (Gadgil and Bossert, 1970; Kipyatkov, 1993, 1995, 2001; Oster and Wilson, 1978; Tschinkel, 1993, 1998). The seasonal and life-cycle allocation patterns are best seen in the rates at which the different types of production (i.e., workers, sexuals) and maintenance proceed in relation to colony size and season (Tschinkel, 1993). Each species of ant has a characteristic annual cycle organized so as to capitalize on the warmest period of the year for larval development (Kipyatkov, 1993). In considering this, a temperate annual cycle should have brood production beginning as early in the spring as possible and continuing until the fall weather becomes too cold for the successful development, with only the stages capable of successful over-wintering present at the beginning of the winter season (Kipyatkov, 1993, 2001).

One important component of the annual cycle is the timing of sexual (alate) versus worker production. In many of

the temperate ant species that have been studied so far, the rearing of sexual brood generally occurs prior to worker production (Brian and Elmes, 1974; Elmes, 1987; Kipyatkov, 1996, 2001; Smith and Tschinkel, 2005; Tschinkel, 1993, 1998). By this account, most of the colony's initial investment in brood should be toward the creation of alates for the sexual reproduction of the colonies followed by a secondary investment in worker brood to increase the size of the colony as well as create a worker force that will survive the winter and begin the cycle all over again. This cyclical colony growth and reproduction has also been shown to create a seasonal age structure with young workers predominant in the fall and older workers in the spring (Rissing, 1987; Tschinkel, 1998). Because ants have an age-based division of labor, it is possible that such seasonal variation in worker age-distribution has consequences for the allocation of labor.

In addition to the reproductive cycle, cyclical variations in physical structure as a colony moves, grows or prepares for the environmental change can be an important element of its yearly phenotype. Nest architecture is species-specific and presumably serves particular biologically important functions (Cerquera and Tschinkel, 2009; Tschinkel, 2004). Descriptive studies of the general nest architecture are in their infancy, with little if any information on seasonal adaptations. *Odontomachus brunneus* nests have only recently been described as simple, consisting of a single vertical shaft connecting a varying number of chambers (Cerquera and Tschinkel, 2009). Despite having only 4 months of nest excavation data, Cerquera and Tschinkel's data show a trend toward increasing nest depth into the cooler winter months (unpublished data). Also previously described is the seasonal vertical movement of fungus gardens in the fungus gardening ant *Trachymyrmex septentrionalis* (Seal and Tschinkel, 2006). Such data suggest that ants may have a physiological response to temperature that induces the workers to alter the structure of their nest and its components to accommodate these climatic changes.

Along with the species-specific cycle of colony composition, the flow of energy within a colony provides insight into the seasonal functions of ant colonies. Annual cycles of energy allocation have been determined for several other ant species found in the Apalachicola National Forest including the Florida harvester ant, *Pogonomymex badius* (Tschinkel, 1998), and the fire ant *Solenopsis invicta* (Tschinkel, 1993), both of which can co-occur with *Odontomachus brunneus*. Energy allocation patterns are determined by measuring fat, the primary energy stores, as well as the weight of new lean and fat biomass in the various life stages throughout the annual cycle, revealing colony energy investment patterns. Additionally, in several ant species, the proportion of fat in a worker's body has been shown to decline with age and can be used to estimate relative age (Wilson, 1985; Tschinkel, 1993, 1998).

In addition to allocating energy, colonies must also allocate labor. At the most basic level, colonies show an age-based division of labor in which young workers perform mostly brood care, and old ones forage. Workers of intermediate age carry out a variety of other tasks. In species with subterranean nests, this results in upward movement of older workers (who are also becoming leaner), creating a vertical pattern of decreasing average fatness and increasing average age. Perhaps because they are a primitive species of ant, *O. brunneus* colonies generate a division of labor through competitive duels referred to as "interaction-based task allocation" by Powell and Tschinkel (1999). Dominant individuals locate themselves closer to the brood within a nest, participating in brood care while older, subordinate individuals are forced into the risky task of foraging. This dominance interaction, coupled with the well-documented phenomenon of age-based polyethism in most other ant species was used to decipher the internal social structure in the laboratory *O. brunneus* colonies. What is unclear is how faithful workers are to their designated roles; in laboratory observations, marked workers were capable of moving among the foraging arena, broodless and brood zones frequently (personal observation). However, it is unclear if this process also operates in natural nests to produce vertical age stratification of *O. brunneus* workers as it does in other ant species (Beshers and Fewell, 2001; Tschinkel, 1998). It is also unclear whether worker fatness is a useful proxy for age in *O. brunneus*.

Foraging biology, while a rather active area of myrmecological research, has largely neglected the determination of the proportion of a colony that is actively involved in foraging. Previous studies of forager populations using *Formica polyctena* (Kruk-De Bruin et al., 1977) and *Pogonomymex badius* (Porter and Jorgensen, 1981) suggest that (1) forager populations function almost as a separate entity from the rest of the nest, and (2) that the foraging population should be comprised of primarily older workers (Golley and Gentry, 1964; Kruk-De Bruin et al., 1977; Porter and Jorgensen, 1981). Despite such studies, it is unknown if the proportion of foragers in all ant colonies is related to the size of the colony as it is in *Solenopsis invicta* (Tschinkel, pers. comm.), if it is a species-specific trait or if this proportion is an evolutionarily derived trait such that primitive ants (i.e., *Odontomachus* species) and more derived ants (i.e., *Pogonomyrmex* species) would have differing proportions of their workforce participate in foraging.

Methods

Description of the site

This study was completed in management compartment 219 of the Apalachicola National Forest in Leon County, Florida

(30°22′ 11N, 84°19′ 32W). The sand hills ecotype is dominated by longleaf pine with an understory of turkey oak and a ground cover of wiregrass, saw palmetto, assorted shrubs and *Smilax* vines. Most of the study was carried out in a low-lying area near several depression ponds with a fluctuating water table, resulting in moist, poorly drained soils. The site was dominated by live oaks and other broadleaf trees along with occasional longleaf pines, and the ground was largely covered with a dense layer of decaying oak leaves (for seasonal temperature and rainfall information, see Appendix).

Excavation and collection

Six to eight nests were excavated monthly for a total of 76 nests. Excavations were done by digging a pit adjacent to a focal colony, with the edge of this pit not less than 15 cm from the nest entrance(s). This distance accommodates varying chamber sizes as well as the slight deviation of nest shafts from being entirely vertical. Chambers were exposed sequentially from the top and their contents accumulated in 20 cm increments down to 60 cm in the total depth (initial nest depths did not exceed this). Inhabitants residing deeper than 60 cm were placed in a separate container and the maximum nest depth recorded (procedure modified from Tschinkel, 1998).

Nest inhabitants were killed by rapid freezing, then dried at 50°, sorted and counted to determine the number and vertical locations of the workers, brood, alates and the queen. For analysis, nests were categorized by size class, with Class 1 nests having ≤50 works; Class 2, 51–100 workers; Class 3, 101–150 workers; Class 4, 151–200 workers.

Pupal cocoon dissections

To determine the type of brood early cocoons contained, dried cocoons from May to July were dissected under a dissection microscope, and the brood within identified as sexual, worker or unknown pupae. Sexual pupae were recognized by the presence of developing wings, and males by the lack of large mandibles. Unknown pupae were too early in the developmental process for their caste to be determined, or were still last instar larvae.

Determination of seasonal energy allocation throughout 1 year

Dried ants were weighed (mg), assigned an identification number and placed in a labeled, perforated gelatin capsule. Capsules were threaded onto a wire skewer and placed in a Soxhlet extractor, extracted with diethyl ether for 48 h, dried and reweighed (Smith and Tschinkel, 2009). The difference between the pre- and post-extracted dry weights represents the extracted fat, and together with the dry weight allowed the determination of the percentage of fat stored in each individual ant (modified from Tschinkel, 1993; Seal and Tschinkel, 2006, 2007; Soxhlet, 1879), and by summation, in the colony as a whole. From these data, we determined energy (fat) allocation by life stage and throughout the yearly cycle as well as the relative age(s) of workers at each level within the colony, with the assumption that *O. brunneus* workers decrease in fat content with age similarly to *Pogonomymex badius* (Tschinkel, 1998).

Forager collection and marking

Ten nests were chosen for forager monitoring during July 2009. A foraging area with a diameter of 60 cm was cleared around the nest entrance(s). To ensure collection of only foragers and not maintenance workers, ants were collected upon returning to their nest or exiting past 10 cm, this distance was chosen after observing that ants exiting with debris deposited such items within 4–5 cm of the nest entrance, those moving past this point left to forage. Each ant was then individually marked on the posterior of its head using Testors enamel and replaced near the nest entrance. Foragers were collected for two 20-min periods separated by 48 h allowing time for the marked workers to thoroughly mix within the population (Ryti and Case, 1986). All collection/observation was performed in the morning (8–11 a.m.), while colonies were actively foraging.

Estimates of forager populations based on mark-recapture data

The number of foragers in a colony was estimated by a mark and recapture method (Lincoln Index; Chew, 1959; Kruk-De Bruin et al., 1977; Southwood, 1978). The total foraging population of a colony was estimated from the proportion of marked workers in a recapture sample. The number of marked workers initially released is the same proportion of the total forager population as the number of marked workers is of the recapture sample. This method makes several assumptions: (1) all individuals in the forager population have an equal chance of being caught and marked, (2) the marked ants mix thoroughly with the unmarked ants before resampling, and (3) marking is permanent for the duration of the sampling period and does not affect the behaviors or survival of the marked individuals, and (4) immigration and emigration are negligible on the time scale of the estimate (Chew, 1959; Southwood, 1978). There is little doubt that these assumptions are reasonably met by the forager populations of ants. Variances and standard errors were calculated as per Southwood (1978).

Nest excavation for forager distribution

Nests were hand excavated as described previously to determine how workers were vertically distributed throughout the nest. Workers were collected as each chamber was exposed, and the depth recorded. Within-nest locations were combined at what appeared to be natural breaks; i.e., ants located in the topmost chamber were observed to frequently exit nests and thus were combined with the returning surface foragers. Collection was done in the morning at similar times to marking events so that workers would be engaged in similar daily tasks at the time of excavation.

Using the number of marked foragers (m) in the nest during the excavation and the proportion of recaptured marked foragers (p) from the mark-recapture data, the number of foragers (both marked and unmarked) within the nest during the excavation was determined (F):

$$F = \frac{m}{p} \qquad (1)$$

Equation 1, Foraging population in nest

The number of foragers actively foraging was then found by subtracting the foraging population within the nest (F) from the forager population estimate (N). The total colony size was then determined by adding this value to the number of ants collected during excavation.

Results

Annual cycle

The monthly excavations and census of *Odontomachus brunneus* nests revealed the annual brood cycle of this species (Fig. 1). Brood production began with the arrival of the warmer temperatures in late spring and continued until temperatures were too cool for the brood development in the mid-fall (see Appendix for yearly temperatures). The first larvae appeared in small numbers in late April and increased to a peak in June. Pupal cocoons were present beginning in May and increased through October with only a few pupae left to eclose in November. The presence of wing buds in dissected cocoons (Table 1) revealed the majority of recognizable pupae to be sexuals in the months of May and June. Production of alate brood was recorded as late as the second week of June (6/9/2008), after which colonies produced only worker brood. Initial brood production in early spring was a mixture of both worker and sexual brood, not solely sexuals, although sexual brood predominated. Both sexual and worker pupae were found in the same nests. There was no correlation between colony size and sexual production ($r^2 = 19\%$; $df = 12$; $p = 0.14$), or the total
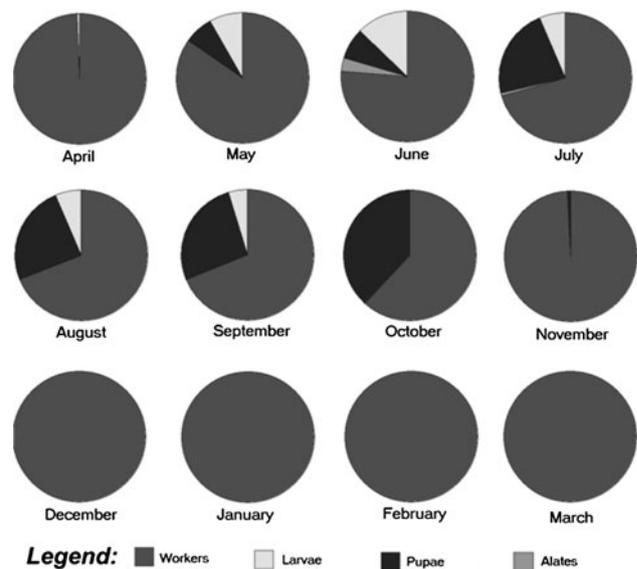
Fig. 1 The annual cycle of production in *Odontomachus brunneus* shown as a mean percentage of the colony population in each category. Nests were broodless throughout half of the year, followed by a period of production of both sexual and worker brood. Sexual brood were produced for only 2 months during the spring, followed by 4 months of worker population

Table 1 Results of cocoon dissections

Month	Total pupae	Worker pupae (%)	Sexual pupae (%)	Unknown pupae (%)	Sexual-to-worker pupae ratio
May	25	12	20	68	1.67
June	81	8.64	16.05	75.31	1.86

number of pupae present and sexual pupae ($r^2 = 21\%$; $df = 12$; $p = 0.12$). Sexual production is slightly related to the relative proportion of fat reserves available to the total colony size ($r^2 = 1.7\%$, $df = 63$; $p = 0.06$).

Adult alates appeared in mid-June (6/16–6/23), with a few female alates remaining as late as the third week of July (7/21/08), suggesting that mating flights are likely to have occurred in mid-to-late June.

The lack of over-wintering brood shows this species to have a heterodynamous annual cycle, with all over-wintering individuals having eclosed prior to the winter inactivity. Of note, in this regard, several colonies moved brood to the surface of their nests in late October as temperatures were beginning to decrease.

Queen presence

Of the 76 colonies excavated, only 26 were queen-right (34%), with one of these colonies actually containing two queens. It is unknown if both queens were reproductively

active as they had been processed for fat extraction and therefore could not be dissected. There was no relationship between the presence of a queen and colony size class (Chi-square test 11.26; $df = 2$; $p = 0.42$).

Seasonal variation on nest depth/architecture

This study revealed changes in the nest depth and structure through the annual cycle, with nests as deep as 60 cm in the summer and as deep at 170 cm in the winter. Fall and spring depths were intermediate between these extremes. In preparation for the winter, the ants extended the final shaft down 60–100 cm from the maximum summer depth. Except on unseasonably warm days, all winter inhabitants of the nest were found in the final, nearly circular chamber at the end of this long shaft.

Regressing the maximum depth of individual nests against the number of ants showed that, while colony size did not change much across seasons, the maximum depth increased with the number of workers in all seasons, except summer. During the summer, no nest was deeper than 60 cm, and nest depth was unrelated to the number of workers (Fig. 2). Winter nests were much deeper than summer nests of comparable size, with spring and fall nests displaying a transition between these two extremes.

On March 23, 2009 an excavation showed evidence of a colony migrating upward in its nest, filling the lower shafts/chambers with loosely packed soil. Around this time, all colonies decreased their depth as the ants moved from their period of winter inactivity into the reproductive period of their annual cycle. In this particular nest, the majority of ants were located in a chamber immediately above this shaft with only a few workers closing the shaft behind the colony.

Dry, lean and fat weights of workers throughout a one-year cycle

Figure 3 displays the monthly means of worker's dry weight, lean weight and percent fat. In April, ants emerging from their winter diapauses ranged in dry weight from approximately 2.14–5.27 mg, a range of 3.13 mg with a mean dry weight of 3.75 mg (COV = 0.15). While the *range* of dry weights remained similar throughout most of the year, the mean dry and lean weights and percent fat changed with season. However, these seasonal patterns of weight and fat content were similar across colony size classes, and are thus shown as monthly means without regard to colony size in (Fig. 3). After their emergence from winter inactivity in April and early May, colonies produced mostly sexual brood (Fig. 1). During this period, fatness of workers declined, so that the annual minimum for fatness occurred in June with the completion of sexual production (Fig. 3). Female alates had a mean of 26% body fat, higher
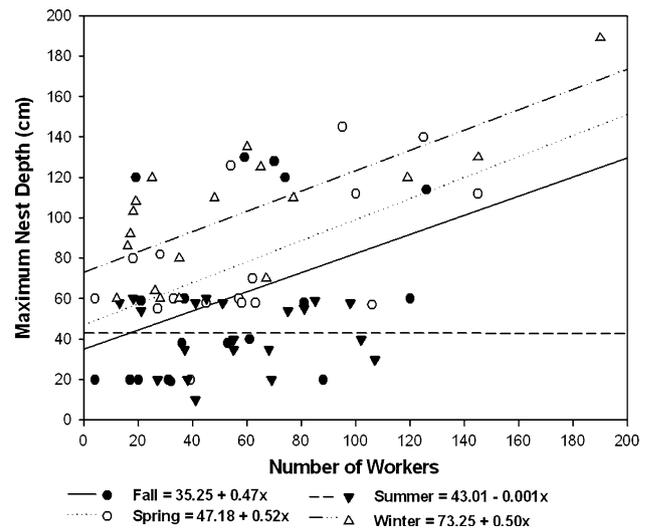
Fig. 2 Correlation of maximum nest depth to number of workers by season. Mean summer nest depth was 43 cm (SD = 16.5) with a little variation, resulting in a *horizontal line*. Winter nests were much greater in depth than summer, with spring and fall occurring in transitional locations between these extremes. Additionally, nests with a larger workforce were capable of digging to greater depths in the cooler seasons

even than overwintering workers, suggesting that they sequestered a large amount of available resources on an individual basis. This spring decline in worker fatness was largely responsible for the initial decline of their dry weight, but their lean weight continued to decline until August, causing dry weight to reach its annual minimum then. Once the colony switched to worker production in June–July, fatness slowly increased as new, young workers replaced old ones that died. When worker production ceased in October and there was no more brood to feed, worker
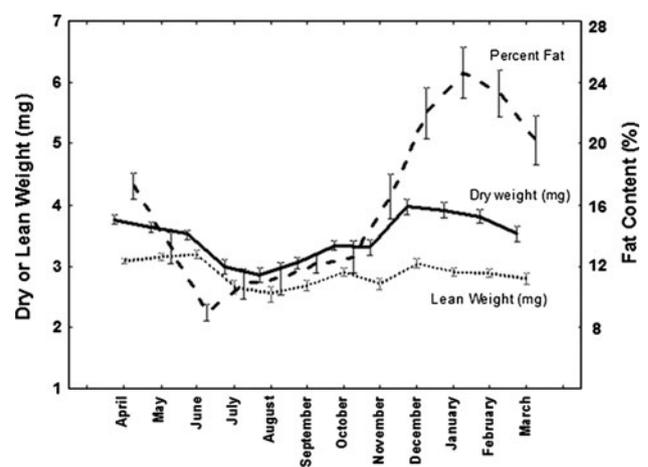
Fig. 3 Lean weight, dry weights and percent fat of workers by month. *Error bars* denote 95% confidence intervals. By decomposing the dry weight into its components, it is apparent that the majority of the fluctuation in mean dry weight of workers is due to a change in the fat content

fatness, dry weight and lean weight increased dramatically in the preparation for winter inactivity and spring alate production. The late winter decline is probably associated with the cost of maintaining workers. Florida winter soil temperatures are not low enough to reduce metabolism to near zero.

This change in weight and fatness can also be expressed in energetic terms. Because fat has about twice the energy content per mg as lean weight, it has a large effect on energy content. Energy content of individuals was computed using 39.33 J/mg for fat and 18.87 J/mg for lean matter. The annual patterns of individual and colony changes in energy content are shown in Fig. 4a, b. In Fig. 4a, the early spring peak in the energy content of larvae and pupae was the result of an initially undetected mixed brood of sexuals and workers. Later dissection of cocoons showed about 64% of them to contain sexuals of both sexes. Because females contain more than triple the energy of males (whose energy content was somewhat less than workers), the brood energy content peaked in June. During brood production, worker energy content declined until about August (Fig. 4). Thereafter, worker energy content slowly built up, peaking sharply in the preparation for overwintering and production of the next spring's brood. Queen energy content varied greatly, but was not related to season. Female alates were present only in June, and were very expensive on an individual basis, as was the queen.

Multiplying the energy per individual times the number of individuals gave the total energy in each type of individual, and summing these gave the total energy contained in the colony. The percent of this total colony energy in each type of ant is shown in Fig. 4b. Figure 4 shows that an increasing amount of energy was found in brood during the breeding season, peaking in October when pupae contain about half of the colonies' energy content. This is probably the result of the replacement of old, lean workers with new, fatter ones that will overwinter. Larvae are not present later than September and pupae than October, so that by November, all energy is found in the workers. Whereas the seasonal pattern of energy in brood is strong and obvious (ANOVA: $p < 0.001$), these patterns do not differ for colonies of different sizes (ANOVA; $p = 0.64$). It is also apparent from Fig. 4 how little energy the ants invest in alates. However, only two colonies contained female alates, so a little can be said about energetic patterns with respect to colony size.

The shifts in the total energy content in Fig. 4b were the result of the changing fat and lean mass in the workers shown in Figs. 3 and 4a, and not of colony growth—colony size did not change significantly across the monthly samples (ANOVA: number of workers by collection month; $p = 0.9$) or even when these were lumped into four seasons ($p = 0.2$). However, it should be remembered that the samples
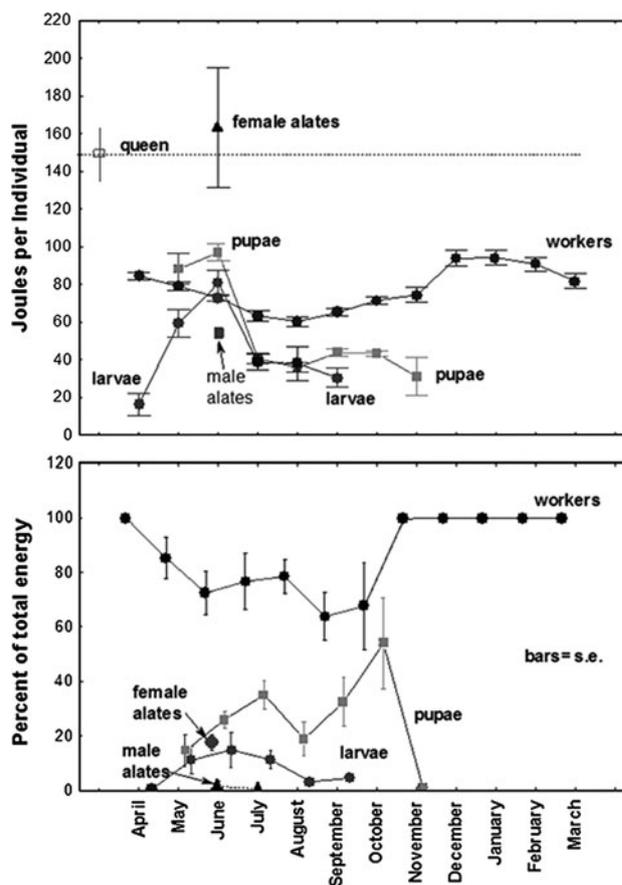
Fig. 4 Seasonal distribution of energy content per individual (**a**) and colony totals (**b**) for 5 types of ants through the annual cycle. The investment in worker pupae gradually increased, peaking in October with the last brood of the year. Alates represent a small proportion of the total energy of the colony when they are present. These shifting investments represent both energy gained from forage, and energy from metabolic reserves

were terminal—no colony was followed across seasons, and the statement of "no change" in colony size applied to the population of colonies through the year. It is possible that individual colonies increase in size during the year.

Vertical distribution of worker fatness within the nest

The fatness of workers was analyzed by nest level within each month (Fig. 5). In 9 out of 12 months, workers were the leanest in the uppermost stratum and the fattest in the lowest (with a few exceptions). This pattern was absent December–February for two reasons. First, the entire worker population becomes fatter, and second the great majority of the colony assembles in the lowest chambers, with only occasional workers occurring in other levels, especially the uppermost (note the large error bars in January and February). This was caused by a small sample size of fatty workers to emerge on warmer days to forage.
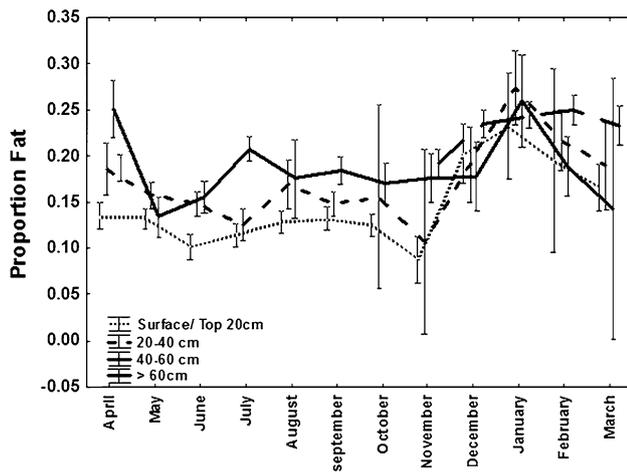
Fig. 5 Monthly within-nest allocation of worker fat by depth. *Error bars* denote 95% confidence intervals. Throughout most of the year, leaner (older) workers were found in the upper chambers of all nests with fatter (younger) workers in the lower regions

Fig. 6 Location(s) of marked and unmarked ants by nest. Marked foragers were distributed throughout nests, but this distribution was unequal, with the majority located in the upper region of the nests

Assuming that the proportion of fat decreases with worker age (as it does in other ants; Porter and Jorgensen, 1981; Tschinkel, 1993, 1998), there appeared to be stratification by age within the nests of *O. brunneus* for the warmer months of the year (Fig. 3).

Estimation of the forager population

Through mark-recapture and excavation, colony sizes and the proportion of foragers per colony were determined. Female alates were included in the population census of workers as they were observed repeatedly foraging in the majority of the nests. Four colonies with large standard errors of the estimate were not used, and the estimates were based on the remaining six. Recapture samples ranged from 8 to 28 workers, of which 27–67% were marked (mean 42%). Colonies ranged in total size from 41 to 107 workers with 51–88% of the workforce participating in foraging, with no relationship to colony size. There is a degree of uncertainty in these estimates that stems from a generous definition of foragers. All workers returning to the nest from a distance and those departing beyond 10 cm were designated foragers. It would include, for example, workers leaving the nest for the first time on exploratory forays, or midden workers venturing farther than 10 cm. These estimates therefore need to be verified by the future studies.

Excavation: location of marked and unmarked workers

Figure 6 shows that ants marked as foragers were distributed throughout the nest upon excavation, but unequally among the levels. A Chi-square test with an expected equal distribution of marked foragers within each nest showed that

the marked foragers were not equally distributed among nest levels (Chi-square = 63.78831, $p = 0.0002$), but were more abundant in the upper levels with a smaller, but varying number in the middle and bottom levels. In all nests, brood was found in the bottom and middle chambers together with both marked and unmarked ants. Brood was also found in the top chambers of nests F10 and F12. It should be noted that the total number of ants collected was not the total individuals per colony as excavations were performed while colonies were actively foraging. An estimate of colony sizes is provided in Table 2.

Discussion

The life cycle of *O. brunneus* is strongly seasonal. Colonies produce brood for 6 months and are broodless for 6 months. Because northern Florida represents the northern range limit of the genus *Odontomachus*, it is possible that this long broodless period is induced by less favorable environmental conditions. For example, in *Myrmica rubra*, brood production is the greatest in mid-range and declines at both range extremes (Elmes et al., 1999). *O. brunneus* sexuals are produced in mixed broods at the beginning of the season, concurrent with a marked decrease in the colony's fat reserves, suggesting a high energetic investment toward the production of these individuals. Reserves recover slowly through foraging during the summer's worker production, and rapidly after brood production ceases in October. This seasonal pattern is generally similar to those of *S. invicta* and *P. badius* in which early spring sexual production is associated with a large decline in worker fat stores (Tschinkel, 1993, 1998). Presumably, this pattern evolved in all three species because it is advantageous to produce sexuals early in the season before much forage is available, but it is probably also driven by the inability to forage

Table 2 Population estimates resulting from mark-recapture and nest excavation

Nest ID	Number marked and released (a)	Recapture sample (n)	Proportion of recaptured ants marked (p)	Forager estimate (N)	Standard error of the mean	Total nest population (excavation)	Total marked ants (excavation)	Total foraging population in nest (F)	Foragers out of nest (N − F)	Estimated total colony size	Percent of colony involved in foraging
F1	31	21	0.67	47	3.33	71	18	27	20	91	51
F4	10	11	0.27	37	6.33	35	8	29	7	42	87
F5	21	20	0.30	70	9.06	76	20	67	3	79	88
F6	18	14	0.36	50	7.95	41	7	20	31	72	70
F8	27	28	0.43	63	5.12	95	22	51	12	107	59
F10	11	8	0.50	22	3.67	25	3	6	16	41	54

during the cold of winter and early spring. With respect to brood rearing, stored fat and forage are probably fungible to a large degree, for that when forage is less available, stored fat can substitute. The extreme of this life cycle is found in *Prenolepis imparis* in which both sexuals and workers are produced from huge fat stores, entirely without feeding (Tschinkel, 1987), thus completely separating the foraging phase of the seasonal cycle during which reserves are stored from the brood production phase when these reserves are used. Considering that these four species are not closely related, it seems likely that the association of declining fat stores with early sexual and worker production is widespread in seasonal ants. How workers convert fat stores into larval food is unknown, but two routes seem possible—the laying of trophic eggs, or the production of nutritional secretions (or both).

The seasonal cycle can thus be understood as a single entity driven by both season and nutritional status. Accumulating data on ant seasonal cycles (Kipyatkov, 1996, 2001; Passera and Keller, 1987; Ricks and Vinson, 1972; Rissing, 1987; Tschinkel, 1993, 1998) is revealing that even for ants in warm temperate zones, the accumulation of metabolic reserves late in the year not only is necessary for overwintering, but also is an integral part of early spring sexual production in the next year. Although sexuals may be produced from overwintered brood in some boreal ant species (Gamanilov and Kipyatkov, 2000; Hölldobler and Wilson, 1990), early production from metabolic reserves is probably widespread among temperate ants for the simple reason that sexual production as early as possible in the spring makes the success of colony founding more likely. Earlier incipient colonies have a competitive advantage over later ones, and can accumulate more workers and reserves before the stress of overwintering sets in. In a number of ant species, as in *O. brunneus*, the early brood is actually composed of both workers and sexuals, with sexuals predominating (MacKay, 1981; Tschinkel, 2006). The reasons for mixed broods are not clear. Candidate reasons include ecological conditions that promote high survival of

founding queens, primitive social organization and worker life span synchronized to the seasons so that there is a high worker turnover at one season. For *O. brunneus*, while worker life span is unknown, it is likely that workers live for at least a year—worker life span generally increases with body size, and *O. brunneus* is a large-bodied ant (Tschinkel, personal observation). The high rate of worker production late in the season also suggests that there is high turnover in workers in late summer. Because we did not track individual colonies through the year, we cannot say with certainty that colonies grew during the warm season. However, mean colony size showed no significant variation during the year suggesting that the colony growth during late summer is modest if not absent.

O. brunneus founds new colonies independently, without the company of workers, and thus female sexuals contain a lot of metabolic reserves, especially fat. Nevertheless, founding queens of this species are semi-claustral, that is, they forage during the founding period (Hölldobler and Wilson, 1990, 2005, 2009). Thus, their 26% fat is lower than the threshold value of about 50% for independent founding reported by Keller and Passera (1989). However, female alate ants gain their metabolic reserves during early adult life, and the females we measured may not all have been flight-ready.

These colony-level attributes are essential to understanding colonies as a unit. It is also important to consider the life cycle of the individual ants that make up the colony. It has been shown in more derived species such as *Pogonomymex badius* (Tschinkel, 1998) that individual ants follow a particular sequence throughout their lives: they eclose on the brood pile where they remain as brood care workers, as they age and new ants eclose, the older ants move away from the brood to perform nest maintenance tasks, and ultimately end their lives as foragers (Bourke and Franks, 1995; Oster and Wilson, 1978; Tschinkel, 1998). This process, termed adaptive demography (Wilson, 1985), is commonly accepted as the core of division of labor of most species. This clear age-related task distribution

appears to be more flexible in *O. brunneus* (Figs. 5, 6): while, there are distinct separations throughout most of the annual cycle, the locations of active foragers (Fig. 6) show that workers can and do migrate within their nests. It is possible that an age-related division of labor is less apparent for this species in natural settings, or that foragers are not as restricted to the upper regions of the nest. Perhaps they deliver food directly to the larvae, rather than to intermediate transport workers. Stratification also appears to be relaxed in the month of January (Fig. 5), during the coolest time of the year. This lack of apparent stratification can probably be attributed to the fact that all workers have gained similar amounts of fat for overwintering. Thus, their ages can no longer be divined through their fat content. It is also likely that the proportion of the workers that are young is higher during this period because most of the overwintering workers were probably born during the summer and fall.

The finding of foraging female alates in both *O. brunneus* and *Neoponera apicalis* (Fresneau and Dupuy, 1988) lends support to either a lack of division of labor or a more primitive version of this distribution. Because nests produce a very few sexuals per season, it seems maladaptive to allow these alates, who represent a large colony energy investment (Fig. 4) to engage in this risky endeavor. Why colonies do not retain these female alates under safer conditions until such time that they mate as is seen in derived species (Tschinkel, 1993) is not yet understood. It is possible that the colonies require the alates to forage in order to maintain sufficient resources not only to nourish the brood but also to maintain the fat stores of the alates until it is time for them to leave the nest. Also, because Ponerine queens found colonies in a semi-claustral fashion, it may just be in the very nature of the female alate to forage during the early portion of her life.

While a few studies of this nature have been performed on primitive ants, there is a similarity in the foraging of *O. brunneus* with *Neoponera apicalis*, a fellow Ponerine species that also engages a large proportion of its workforce, including female alates, in foraging (Fresneau, 1985; Fresneau and Dupuy, 1988). Similar to more derived species, *O. brunneus* displays a spatial partitioning of workers by age, with the youngest residing primarily in the lower regions of nests and the older in the upper region, likely performing primarily as foragers. Due to the diversity of combinations of both primitive and derived traits, Fresneau and Dupuy (1988) suggest that the subfamily of Ponerinae is a potential model for studying the evolution of social organization; such studies could yield powerful insight into how and when the various traits of eusociality were derived within the diverse family of ants.

The high proportion of queenless nests has several possible origins. It is unlikely that we failed to find queens that were present, as the excavation procedure rarely missed ants. True queenlessness is also unlikely, for workers can only produce males. It is more likely that *O. brunneus* is polydomous. If this is the case, then the average colony occupied three nests and average colony size (as opposed to nest size) would be approximately triple our estimate. It would also seem that each nest functions as a largely independent unit. Similar polydomy was found in *Camponotus socius* in which the average colony occupied 2.3 nests (Tschinkel, 2005).

Appendix

Seasonal temperatures and rainfall during April 2008–May 2009

Month	Avg. Temp. (°C)	Avg. Temp (F)	Rainfall (mm)	Rainfall (in)
Apr-08	19	67	3.6	0.14
May-08	24	76	2.8	0.11
Jun-08	27	81	5.08	0.2
Jul-08	28	82	4.1	0.16
Aug-08	28[a]	82[a]	15	0.59
Sep-08	26	79	1	0.04
Oct-08	20	68	4.3	0.17
Nov-08	13	56	4.8	0.19
Dec-08	14[b]	57[b]	1.3	0.05
Jan-09	11	52	1	0.04
Feb-09	11	51	2.8	0.11
Mar-09	17	62	4.3	0.17
Apr-09	19	67	9.7	0.38
May-09	24	76	6.6	0.26

[a] Tropical Storm Fay influenced a decrease in temperatures in mid and late August 2008

[b] Mid-December had several warm days in the high 70s and low 80s

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