Demography, demand, death, and the seasonal allocation of labor in the Florida harvester ant (*Pogonomyrmex badius*)

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Abstract As a self-organizing entity, an ant colony must divide a limited number of workers among numerous competing functions. Adaptive patterns of labor allocation should vary with colony need across each annual cycle, but remain almost entirely undescribed in ants. Allocation to foraging in 55 field colonies of the Florida harvester ant (*Pogonomyrmex badius*) followed a consistent annual pattern over 4 years. Foragers preceded larvae in spring and peaked during maximal larval production in summer (0.37). In spring, proportion foraging increased due to an increase in forager number and reduction in colony size, and in late summer, it decreased as colony size increased through new worker birth and a loss of ~3 % of foragers per day. The removal of 50 % of the forager population revealed that, at the expense of larval survival, colonies did not draw workers from other castes to fill labor gaps. To determine if labor allocation was age specific, whole colonies were marked with cuticle color-specific wire belts and released, and each cohort's time to first foraging was noted. Workers that eclosed in summer alongside sexual alates darkened quickly and became foragers at ~43 days of age, whereas autumn-born workers required 200 or more days to do so. Following colony reproduction, these long-lived individuals foraged alongside short-lived, summer-born sisters during the next calendar year. Therefore, the large-scale, predictable patterns of labor allocation in *P. badius* appear to be driven by bimodal worker development rate and age structure, rather than worker responsiveness to changes in colony demand.

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Introduction

In eusocial insect colonies, behavioral castes are composed of workers that specialize on sets of nonreproductive tasks over varying time scales (Wilson 1968). In order to optimize growth and reproduction, proportional membership in each caste must change in response to seasonal differences in colony need (Oster and Wilson 1978; Fukuda 1983). Although the maintenance of complex caste structure is a key feature of social evolution, whether annual patterns of labor allocation exist and how they arise from the self-organization of thousands of individuals remain unknown for almost all social insect species (Schmid-Hempel 1992; Tschinkel 2011b). An abundance of theoretical work on the topic has emerged over the last 40 years (Robinson 1992; Schmid-Hempel 1992; Beshers and Fewell 2001). Yet, in practice, untangling the processes underlying caste switching has been difficult because worker age, experience, physiological condition, location in the nest, and task set are often highly correlated (Tofts 1993; Franks and Tofts 1994).

In social hymenoptera colonies, aging workers typically move through a sequence of behavioral castes, beginning with brood care and culminating in risky labor roles outside of the nest (Lindauer 1953). The "centrifugal" movement of workers away from the brood pile and toward developmentally demanding tasks suggests that rate and timing of birth, development, and death among workers may drive the seasonal redistribution of labor (Wilson 1976a; Bonabeau et al. 1998; Page and Mitchell 1998). Under these conditions, colony-level selection may shape age–frequency distributions so that appropriately aged workers are aligned with the temporal

availability of resources or other predictable events during the annual cycle (Oster and Wilson 1978). Alternatively, workers have been modeled as flexible generalists, capable of moving between local behavioral roles fluidly when changes in colony need are detected (Gordon 1996; Beshers and Fewell 2001). In these models, correlations between worker age and task set arise from the sequential filling of labor gaps in structured nest space, instead of directly from meeting developmental thresholds. As individuals detect hallmarks of changing colony need and task occupancy, they "forage" for work, moving ever farther from the brood pile (Sendova-Franks and Franks 1993; Franks and Tofts 1994).

In reality, laboratory observations and experiments examining behavioral flexibility have identified a range of colony strategies related to task switching and have found seemingly conflicting frameworks operating in the same nest. In *Pheidole dentata*, for example, the large repertory size of old minors is correlated with increases in structure and volume of the lip region of the mushroom bodies, which govern olfaction (Seid et al. 2005). Although the task set of young minors is constrained by neural capacity, older individuals acquire an increasingly elaborate repertory size and can potentially switch roles as changes in colony need arise (Seid and Traniello 2006). In honeybees, labor is discretized and attended by age classes with almost no overlap in task set (Johnson 2008). Yet, the ability of workers to fill missing labor roles in single-cohort colonies has been demonstrated repeatedly in observation hives, and behavioral reversions are common following swarm events in natural populations (Huang and Robinson 1992; Robinson et al. 1992).

The capacity of workers from many social insect species to accelerate, reverse, or slow development has led to the popular consensus that social insects more often adopt strategies involving behavioral flexibility over adaptive schedules of birth and development (Meudec and Lenoir 1982; McDonald and Topoff 1985). However, when workers fill experimentally induced labor gaps, their capacity to perform the new task set as efficiently as their predecessors may be limited by their developmental age (Calderone 1995). It is therefore possible that behavioral plasticity acts only as buffer during catastrophes for some species, as the ideal ratio of developmentally appropriate age cohorts is slowly restored. For example, in colonies of *Neoponera apicalis*, workers from colony fragments composed of single age classes were capable of filling missing labor roles but, when recombined with their parent nest, resumed their previous age-specific tasks (Lachaud and Fresneau 1987). Therefore, although behavioral flexibility is likely a critical component of colony resiliency, it may be best considered within the framework of a colony's age–caste structure (Robinson 1992).

Laboratory studies

While the adaptive role of morphological caste ratios has been described by manipulating the proportions of worker size-frequencies and measuring resultant brood success (Porter and Tschinkel 1985; Beshers and Traniello 1994), natural ratios and functions of age-correlated labor groups remain almost entirely unknown. Instead, most studies of division of labor have focused on the behavioral repertories of individual ants or age cohorts in a laboratory setting. While these studies have produced valuable ethograms that define behavioral caste boundaries, repertory sizes, and sequences, they do not describe colony-level strategies in the context of the environment that shaped them (Wilson 1976a, b; Mirenda and Vinson 1981; Pratt 1994; Santos et al. 2005; Holbrook et al. 2011). For ground-nesting ants, laboratory studies also eliminate the spatial relationships of workers, forcing task switching to play out in single-chambered, two-dimensional, soil-free observation nests (Bourke and Franks 1995; Gordon et al. 2005). Under these conditions, the capacity of workers of different ages to perform tasks may be inconsistent with their chance of encountering them within the architecture of a natural nest (Tschinkel 2004).

Laboratory studies of labor allocation also force worker development to occur in the absence of annual temperature and food cycles and, most importantly, natural worker death rates. If individuals "forage for work" and are pushed or pulled into new roles based on current occupancy, the absence of externally induced mortality on old workers could increase forager tenure while delaying the entry of younger workers into that role (Franks and Tofts 1994). Alternatively, if labor allocation is actually age specific, the reduction of mortality among old workers could produce an ever-growing population of foragers, limited only by natural life-span. In either case, worker nest–space interactions and age–task distributions are subject to distortion under laboratory conditions and may obscure an understanding of how selection has shaped patterns of adaptive demography across the annual cycle, with realized worker life-span at the helm.

Field studies

Within social insect colonies, striking differences in life-span are realized by individuals of the same genotype. While queens may live decades, sterile workers, the "soma" of the colony, may live only a fraction of a year (Keeler 1982; Tschinkel 1987; Porter and Jorgensen 1988; Wiernasz and Cole 1995; Meyer et al. 2009). Alternative adult phenotypes and size-related differences in life-span among sterile workers are most frequently the product of larval nutrition (Wilson 1953; Calabi and Porter 1989). Yet, unlike worker morphology, hallmarks of chronological age are subject to environmental influence in adults. Proxies for relative age within a nest

such as cuticle color and mandibular wear are not accurate predictors of chronological age in the field because their rate of change varies with factors such as temperature, humidity, and experience (Oettler and Johnson 2009). As a result, tests of age-related division of labor in ground-nesting ants must rely on permanently marking newly eclosed cohorts, a task which is often too destructive to attempt.

In non-nomadic ants, only behavioral castes that appear on the nest surface can be accessed without destruction of parent nests. Several studies have used mark–recapture techniques to describe the population size of surface castes (foragers, defenders, etc.) in the field, but only a handful have excavated, and performed, whole colony censuses to determine the proportions of the total adult population represented (Golley and Gentry 1964; Erickson 1972; Porter and Jorgensen 1980; Nobua-Behrmann et al. 2013). Fewer still have described annual or environmental changes in caste ratios, which offer more than a snapshot in time of the annual cycle of proportional allocation (Herbers et al. 1985; Calabi and Traniello 1989; Sendova-Franks and Franks 1993). Using estimates of forager population size and total colony size, Tschinkel (2011b) was the first to identify seasonal and size-related differences in proportional allocation to foraging in an ant species. He demonstrated that small *Solenopsis invicta* nests contain proportionally more foragers in the autumn than their large counterparts, owed in part to differential investment in sexual and worker production in spring. Because small colonies invest in growth instead of reproduction in the spring, their large, autumn-time forager populations are capable of expanding territory boundaries at the expense of neighbors, increasing access to resources that will build alates the following spring (Tschinkel 2011b). Thus, it seems that, like morphological castes, behavioral caste size serves an adaptive function with respect to annual patterns of growth and reproduction.

Study species

The Florida harvester (*Pogonomyrmex badius*) is a dimorphic, diurnal, seed-harvesting ant characteristic of the coastal plain in the southeastern USA. Each colony contains a single, multiply mated queen and reaches maturity with a minimum adult population of approximately 700 workers, releasing its own sexual alates in June of each year thereafter (Golley and Gentry 1964). In northern Florida, colonies are active between March and November of each year and may consist of as many as 11,000 workers that excavate nests more than 2.5 m in depth (Tschinkel 2004). Within each nest, workers are vertically stratified by cuticle color, with brood and seeds appearing in proportion to colony size, at specific depths. Unlike colonies of western sister species, which are nearly impossible to collect, a large *P. badius* can be neatly exhumed, with all colony members vacuumed and sorted by

strata in 4–9 h by one enthusiastic worker and a small shovel. The relative ease of collection, along with the existing body of sociometric data relating to the annual cycle of fat storage and brood production, makes *P. badius* an ideal candidate for studies of labor allocation (Golley and Gentry 1964; Tschinkel 1998, 1999; Smith and Tschinkel 2006). Our studies describe the proportion of workers foraging in each of 55 colonies of the Florida *P. badius*, for four annual cycles of colony growth and reproduction, across a full range of colony sizes. This work is one of the first field-based, colony-level analyses of labor ratios that integrate season, colony size, worker death rate, and chronological worker age. It represents a missing component in the current literature on adaptive demography and the organization of labor in social insects (Oster and Wilson 1978; Tschinkel 1991, 2011a, b; Nakata 1996).

Methods

Study site

Studies were conducted from 2009 to 2012 in a 23-ha, sand hills habitat of the Apalachicola National Forest, 16 km southwest of Tallahassee, FL (latitude 30.35, longitude −84.41). The site was characterized by well-drained deep sand and an overstory of 40-year-old long leaf pine (*Pinus palustris*), a midstory of turkey oak (*Quercus laevis*), and a ground covering that included dwarf huckleberry (*Gaylussacia* spp.), pricklypear (*Opuntia* spp.), beard grass (*Andropogon* spp.), gopher apple (*Licania michauxii*), and catbrier (*Smilax* spp.). *P. badius* colonies appeared at an average density of one nest per 670 m^2 alongside *Trachymyrmex septentrionalis*, *Solenopsis geminata*, *Forelius pruinosus*, *Dorymyrmex bureni*, *Aphaenogaster floridana*, and a number of less common ant species. All *P. badius* colonies were returned to their original territories following census.

Proportion foraging

Allocation of labor with respect to season, demography, and colony size was estimated as the proportion foraging in 48 active season and 7 winter colonies of the Florida harvester ant (*P. badius*). Colonies were sampled in all months and seasons, and focal nests were selected haphazardly based on mound diameter so that the full range of colony sizes was represented across each annual cycle. Forager population size (N) was calculated using the Lincoln index mark–release–recapture method, where the proportion of the total forager population marked in an initial sample (m) was expected to be equivalent to the proportion of a recapture sample (n) that was marked (Lincoln 1930). The Lincoln–Peterson correction was not applied to population estimates, because it did not significantly alter these estimates, given the high proportion of workers

recaptured in each sample (Bailey 1952). Each estimate of forager number was followed by the immediate excavation and census of the focal colony, thereby allowing calculation of the proportion of the total adult population that foraged.

$$M/N = m/n$$

where M = number initially marked
N = total population
m = number marked in recapture sample
n = number recaptured.

For each colony, recapture followed the initial capture and marking by 24 h to allow sufficient mixing of foragers and to reduce the effects of forager death and recruitment into the population. On day 1, a band of bird seed was positioned 150 cm from the margin of the focal nest mound so that the mound was completely encircled and all established trunk trails were intersected. Foragers were defined operationally as individuals that traveled 150 cm or more from the nest mound, collected bait, and began a return trip to their nest. To encourage continued recruitment to baits, the foraging area was shaded by a beach umbrella, and individuals were gathered at approximately 15-min intervals until fewer than five were captured in 30 min. Following this 3- to 6-h collection period, a perfume sprayer was used to apply two "spritzes" of 10 % fluorescent printer's ink in diethyl ether to all foragers simultaneously (Gan's Ink, Supply Co., Los Angeles, CA; Risk Reactor, Santa Ana, CA; Porter and Jorgensen 1980). Individuals were not anesthetized for marking and resumed normal behavior immediately following mark application. The quality and uniformity of each mark were checked using an ultraviolet flashlight, and foragers were released en masse onto their nest mound.

The following day, each colony was baited in the same manner as on day 1. However, because colonies rejected the same bait on consecutive days, they were offered shortbread cookie crumbs instead of birdseed. Foragers attending baits were gathered by hand as described above and taken to the laboratory where they were placed in a tray and counted individually by removal with an aspirator. Then, marked individuals were separated and counted under ultraviolet light. All foragers were returned to their nest mound later that same day.

Nest excavation

On day 3 of each sampling event, a large pit was excavated adjacent to the focal nest, and chambers were revealed by making thin, horizontal slices into the nest area. Exposed ants, seeds, and brood were gently vacuumed from their chambers using a Dewalt D500 vacuum cleaner and sorted into separate boxes at every 20-cm depth increment. The numbers of marked foragers, unmarked adults, larvae, and pupae per stratum were counted individually by removal with an aspirator in the laboratory. In 2011 and 2012, the number of adult workers belonging to each of three, cuticle color-specific groups was also tallied. For consistency, worker cuticle color was scored against a PANTONE[TM] color scale palette derived from the myPANTONE application for iPhone (Pantone Inc., copyright 2012). The youngest workers were identified as PANTONE 602 C and PANTONE 121 C (light tan to butter yellow, with even coloration), middle-aged workers as PANTONE 144 C and PANTONE 1375 C (orange to pale red, with uneven tanning), and older workers as PANTONE 490 C or darker (deep, reddish brown). Foragers were always PANTONE 490 C or darker.

Every effort was made to excavate nests in the early morning before foragers departed, but in cases when less than 100 % of marked foragers were recovered during nest excavation, an estimate of the number of marked and unmarked foragers afield was calculated from the proportion of marked workers absent at the time of excavation. The number of missing workers was then added to the total number of adults in each colony. To determine the proportion of adults allocated to foraging, the estimated forager population size was then divided by the total adult population size, including foragers absent during colony collection. Interannual patterns of forager allocation were assessed both by calendar date and by aligning the dates that colonies first foraged following winter dormancy. Following colony census, most colonies were released in their original territory where they reexcavated nests.

Change in forager number

Seasonal variation in percent foraging may result from a change in the total number of adults in a colony or a change in the number of adults that forage, or both. For example, a gradual decrease in proportion foraging over time could be a consequence of an increase in colony size and maintenance of forager number, maintenance of colony size, and a decrease in forager number, or a decrease in both colony size and forager number. To determine how each population influenced proportion foraging, repeated monthly estimates of forager number were made for a set of ten colonies in two size classes, throughout the annual cycle (five in 2010, five in 2011). Maturity was inferred from initial colony size estimates (>800 workers) and confirmed by the appearance of alates during mating flights (Smith and Tschinkel 2006). On each sample date between May and November, foragers were marked with fluorescent printer's ink, released, recaptured after 24 h, and then returned to their mound following mark inspection. Though capture events were separated by a minimum of 2 weeks, one of three unique ink colors was applied to foragers so that surviving, previously marked individuals would not confuse the most recent estimate of population size.

Weather permitting, each focal colony was sampled monthly between mid-April and late October. Foragers were baited with a millet-based birdseed mixture and cookie crumbs. These items were chosen because unlike natural seeds which the ants cache, seeds in the commercial mixture germinated within days of collection and were discarded by the ants, while cookie crumbs had disappeared from nests within 24 h. It is therefore unlikely that baiting colonies 6 h per month significantly influenced larval production in these colonies. In order to compare foraging between years, each estimate of forager number was divided by May's estimate and expressed as a proportion of May's population size for the focal year.

Estimates of number foraging were also divided by date-specific, proportion foraging estimates (see above) and used to approximate focal colony size on each sample date. The expected proportion foraging on each sample date was determined by the neighbor-weighted interpolation of points between sample dates by Kriging (Beers and Kleijnen 2004). In each year, mature and immature colony values for proportion foraging were Kriged separately to account for differences in forager number resulting from alate production. Focal colony growth was calculated by dividing estimated colony size in October by initial colony size in May of each year to obtain a factor by which colony size changed. For each year, the average change in colony size was obtained by summing these factors and dividing by the total number of colonies sampled.

Chronological forager age

In 2012, six *P. badius* colonies were excavated, and workers were divided into three demographic groups by using the aforementioned PANTONE™ color scale values. Each demographic group was marked with a different color of 38-gauge copper wire and fluorescent printer's ink and released (Fig. 1). In order to detect seasonal differences in the length of time between worker eclosion and foraging, as well as the length of time required for each initial demographic group to appear in the forager population, colonies were marked and released at three different periods during 2012: February (one colony), following mating flights in June (three colonies), and prior to winter dormancy in October and November (two colonies).

Wire marking consisted of tightening an overhand knot of colored, 38-gauge copper wire around the petiole of each lightly anesthetized ant (Mirenda and Vinson 1979), while ink was applied to the cuticle in the manner described above. Though more time consuming to apply, the permanence of wire marks allowed us to verify the longevity of concurrently applied ink marks. Aside from an initial period of self-inspection, wire belts did not appear to alter the behavior of ants. During the wire application process, each colony was housed in a plaster laboratory nest for a maximum of 48 h and offered frozen crickets (*Acheta domesticus*), water in cotton-plugged test tubes, and access to their original seed cache. Mortality during this period was negligible.

Ice nests

During colony collection, the chamber depth, number, and area were recorded. To spare each colony energetic costs associated with reexcavation, a nest with similar features was constructed out of ice, buried, and allowed to melt in the location of the original nest. Ice chambers of the appropriate dimensions were frozen in species-typical, copper molds, transported to the field on dry ice, and sequentially packed in place in the nest pit (Tschinkel 2013). Chambers were linked together by threading plastic tubing through semicircular grooves filed into the ice. After the ice nest was buried and had melted, the tubing was gently removed by pulling the free end at the surface. Following marking, ants and their original seed cache were poured into a 20-cm×20-cm enclosure surrounding the new nest entrance. All colonies settled in readily, and enclosures were removed within 48 h of worker release. Colonies were revisited twice monthly, and the color of the wire and ink mark of each forager captured in a 3-h period were recorded. The longevity of the fluorescent ink

Fig. 1 (**a**) A wire-marked *P. badius* forager. Whole colonies were sorted into three cuticle color groups according the PANTONE™ color scale values (**b**) and given one of three wire belt colors depending on relative age
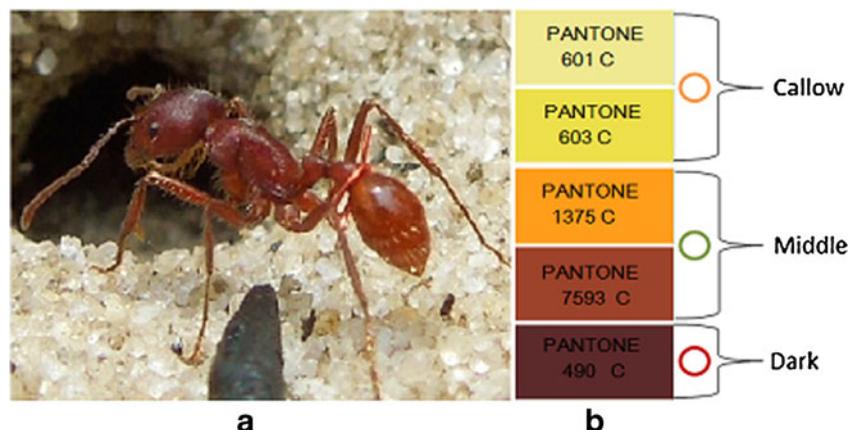
mark was verified by noting its presence with the permanent wire mark in subsequent months. Appearance of workers without a wire mark indicated the movement of the first unmarked cohort into the role of forager.

An additional five colonies, containing between 312 and 2,314 workers, were excavated between December and March of 2010. Each colony was censused, and all adults were marked with 10 % orange fluorescent printer ink in ether (Gan's Ink, Supply Co., Los Angeles, CA; Risk Reactor, Santa Ana, CA). For each colony, a subsample of the lightest-colored (and presumably, the youngest) workers was marked with an additional coat of blue fluorescent printer's ink in diethyl ether. Though naturally inactive during winter months, colonies were released on warm days with their seed stores and successfully reexcavated nests in their original territory following mark application.

Beginning in mid-April, samples of 20 foragers were taken approximately every 30 days and checked for mark presence and quality until no more marked ants appeared. Marks were scored visually by anesthetizing ants with ether and counting the number of ink blotches and dots per dorsum under a microscope outfitted with an ultraviolet light. Foragers were returned to their nests following mark assessment. The appearance of workers marked with blue ink indicated the movement of the youngest cohort into the role of forager. Having calibrated the permanence of ink marks with the wire mark study, these five ink-marked colonies were combined with the six wire-marked colonies for analysis of forager age.

Behavioral flexibility

To determine if forager death drives the movement of workers from other behavioral castes into the role of forager, colony response to the experimental reduction in forager population size was measured in the field over 7-day increments throughout the annual cycle. Number foraging was estimated for ten colonies using the Lincoln index mark–release–recapture method. For half of the nests, 50 % of the estimated forager population was removed. After 1 week, forager number was estimated again in both experimental and control colonies. Because this experiment was conducted at several different points in the annual cycle, each experimental nest was sampled concurrently with a single control colony. The change in forager population size for all colonies was determined by subtracting the initial forager number from the final forager number after 7 days. The estimated proportion of the final population added per day was determined by dividing the number of new foragers by the number of days (7) from the initial mark in control colonies. This rate of forager addition was used to determine whether the entry of foragers in experimental colonies occurred at a higher daily rate than control colonies.

Data analysis

Data were analyzed using regression and T tests in Statistic 7.0 or R (package GeoStats). Proportion data were arcsine-transformed to stabilize the variance.

Results

Location and catchability of foragers

A full series of immature and mature colonies, ranging in size from 157 to 9,656 adults, were sampled throughout four annual cycles. In all colonies, foragers were found in the uppermost strata of the nest, showed fidelity to the role of forager on successive days, and represented only a fraction of the total adult population of each colony. Sampled nests contained between 40 and 1,865 foragers, and up to 1,651 foragers were captured and marked for each colony. On day 1 of each sampling event, as many as 93 % and no less than 35 % of a colony's total estimated forager population was marked (one outlier at 23 %). A mean of 44 % (SD 15.5 %) of foragers captured on the first day of each sampling event were captured foraging the following day, representing 59 % (SD 16.5 %) of the recapture sample (n =48).

Though foragers were afield from some nests during early-morning excavations, a mean of 83 % (SD 16.7 %) of marked foragers were recovered in their nests on the third day of each sampling event. Nests ranged from 40 to 270 cm in depth, with an average depth of 135.8 cm (SD 54.2). Foragers were not the only individuals near the nest's surface and only represented a mean of 46 % (SD 20.1 %) of the ants found above 20 cm. For five nests sorted by chamber during excavation, marked foragers were found a maximum of 12 cm below the nest surface. In these and the remaining nests sorted in 20-cm increments (n =42), 96 % of marked foragers were recovered 0 to 20 cm below the nest surface, indicating that the forager population is a discrete group distributed nonrandomly within the nest and the larger adult population of the colony (Fig. 2).

Seasonal and size-related patterns of forager allocation

The percent of each colony allocated to foraging was highly seasonal, and a distinct pattern of proportional labor allocation was conserved across years (Fig. 3). Foraging began in early March or April and increased to a maximum of 35–41 % foraging midsummer before declining to zero in December. In comparing across 4 years of this study, calendar date was a better predictor of percent foraging than the number of days from the start of foraging (cubic polynomial fit, arcsine-transformed adjusted R^2=72.0 %, $F_{3,\ 45}$=42.9, p <<0.0001). In 2009, 2011, and 2012, foraging began within 5 days of
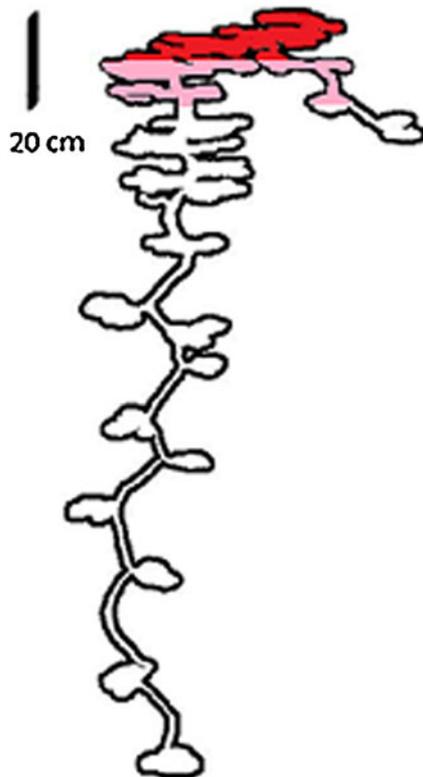
Fig. 2 Foragers were always found in near-surface chambers. For five nests sorted by chamber, marked foragers were discovered at a maximum depth of 12 cm (represented in *red*, on a nest 145 cm in depth). *Pink shading* indicates the maximum depth of 96 % of marked foragers from 42 nests excavated in 20-cm increments (image modified from Tschinkel 2004)

March 1st, while in 2010, foraging did not commence until a full month later (Table 1). In 2010, larval production, mating flights, and peak in proportion foraging were aligned with other years, but proportion foraging reached a higher maximum. This indicates that the survival and accumulation of old workers during the additional month of dormancy may have increased the number of available foragers during subsequent, active months.

Although the general pattern of foraging was conserved regardless of colony size, immature colonies (<800 workers) reached a higher maximum proportion foraging than large, mature colonies each year (mean residuals, mature=−0.019,
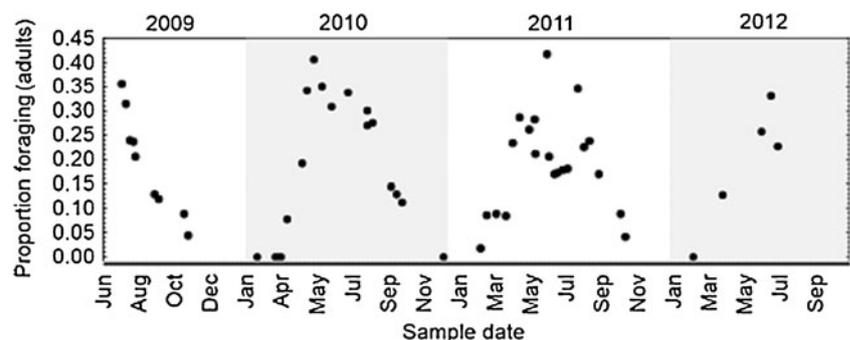
SD 0.058; immature=0.050, SD 0.092; t_{53}=3.31, $P<0.03$; Fig. 4), and a higher proportion of dark-colored workers were foragers in immature colonies (mature mean=0.35, immature mean=0.60, T_{22}=4.05, $P<0.01$). This difference likely resulted from investment in growth in lieu of reproduction by immature colonies in the early summer, so that a larger forager population and proportion foraging were present by midsummer. These findings confirm that estimates of colony size based on forager population size should be avoided unless a patterned relationship between number of foragers and total number of adults is first identified across a full range of dates. For example, *P. badius* colonies composed of 10,000 workers may contain anywhere from 100 to 3,500 foragers during the active period, depending on the date.

Forager relationship with larvae

Foragers are a colony's only means of acquiring food for developing larvae. Despite the presence of cached seeds, percent larvae and percent foraging were positively correlated (r = 0.759) and changed in parallel from May until October of each annual cycle with a mean of 1.64 foragers per larva (SD 0.99; Fig. 5), regardless of date and adult population size. Foragers preceded larvae by 30 to 40 days in all years and decreased in abundance more slowly than larvae in late autumn (Fig. 6). The appearance of foragers more than a month before the year's first larvae suggests that larval cues do not stimulate workers to forage. In 2011 and 2012, larvae continued to appear in nests well into autumn, while no pupae or new callows were discovered after October. In 2011, a record drought year, failure of pupation occurred a full month earlier than in the three surrounding years. The presence of these "doomed" larvae in the midst of a healthy forager population and seed cache suggests that provisioning stops either due to environmental cues or lack of appropriate nonseed, food items before winter.

Wire and mark results

For workers from 11 wire- and ink-marked colonies, the age of first foraging ranged from 43 to more than 300 days. This dramatic difference in the chronological forager age was

Fig. 3 Foraging began in March or April of each focal year, reaching a proportional maximum between May and June before declining to 0 %, prior to overwintering. Each *point* represents a single colony

Table 1 Important dates and events related to foraging across the annual cycle from 2009 to 2012

Year	Sample date range	Number of samples	First date foraging (f.f.)	Max % foraging	Peak foraging period	Observed at time of excavation			Date of first mating flight (m.f.)	No. of days btw. f.f. and l.p.	No. of days btw. f.f. and p.p.	No. of days btw. f.f. to first m.f.	No. of days btw. f.f. and c.w.
						Larvae present (l.p.)	Pupae present (p.p.)	Callow workers present (c.w.)					
2009	Jul. 5–Oct. 25	9	Mar. 1	—ᵃ>35.7 %	NA–Jul. 5	May 4–Oct. 25	NA–Oct. 25	NA	Jun. 21	NA	NA	113	NA
2010	Apr. 10–Oct. 22	14	Apr. 5	40.6 %	May 13–Jun. 7	May 5–Oct. 20	May 11–Oct. 22	May 25–Oct. 22	Jun. 22	30	36	77	50
2011	Mar. 2–Nov. 3	21	Feb. 28	41.8 %	May 6–Jun. 21	Apr. 13–Nov. 1	May 22–Sept. 17	Jun. 2–Oct. 25	Jun. 14	44	83	106	97
2012	Mar. 6–Dec. 2	6	Mar. 6	—ᵃ>33.2 %	NA	Apr. 12–Dec. 2	May 15–Oct. 19	NA–Oct. 19	Jun. 14	37	70	100	NA

ᵃ Maximum not recorded due to less frequent sampling

strongly related to birth month and produced two distinct worker life-spans. The first cohorts of workers, which eclosed in June and July of each focal year, sclerotized and passed through other temporal castes quickly to appear as foragers at an average adult age of only 43 days (SD 1.78, $n = 10$ colonies, sampled at 15-day intervals). Conversely, autumn-born workers darkened and sclerotized over a period of months, overwintered, and did not appear as foragers until 210 to 360 days of adult age ($n = 9$ colonies; Fig. 7).

In addition to moving through behavioral roles at an advanced rate, adults that eclosed in early summer showed an increased rate of physical development relative to autumn sisters. *P. badius* colonies do not overwinter with brood, as October of each year is the latest possible date of pupal eclosion (Tschinkel 1998). When all years were pooled, sampled colonies contained an average of 4 % callows (SD 4.64 %) and 54 % midcolored (SD 18.9 %) prior to overwintering (October and November, $n = 7$). During the first 2 months of postwinter foraging (March), no workers were still in the callow-color phase, but 64 % (SD 2.2 %) were scored as midcolored ($n = 5$). Surprisingly, colonies excavated before the appearance of new callows in June still contained more than 30 % midcolored workers that could only have eclosed in November or earlier the prior year (Fig. 8). This is an important observation because all foragers that eclose in June are fully darkened and sclerotized by the time of first foraging, which occurs only 1 month later, in July.

The youngest workers in winter (December–February) appeared as foragers the following July, a full 8 months after the last observed eclosion date. The darkest-colored and oldest workers marked in colonies excavated during winter months began foraging in early spring. These slow-developing individuals were proportionally dominant in the forager population until July and present in small proportions until September, 11 months after an October eclosion date. Workers that eclosed in early summer during the focal year became proportionally dominant in the forager population by late August. Individuals born the year prior were replaced by early September as successive cohorts graduated into the role of forager. Therefore, long-lived individuals born the year prior foraged alongside their short-lived, summer sisters, co-occupying the forager population following sexual alate production and release in June and July.

Like their autumn-born sisters, workers that eclosed in June and July declined in number and disappeared from the forager population by September of the same year in all but one wire-marked colony ($n = 3$). For colony 357 (marked on July 23rd, 2012), workers scored as "dark" and "midcolored" during excavation still dominated the forager population by October and even appeared in low numbers the following April (Fig. 7). This was puzzling because the "dark"-colored

Fig. 4 Foraging began in early spring and reached a proportional maximum in early summer, before declining to zero in late autumn. A higher proportion of adults foraged in immature colonies than mature colonies during midsummer. The *graph* is based on 55 colonies of varying size, sampled from 2009 to 2012, with 95 % confidence bands (cubic polynomial fit, $Y = -0.5194 + 0.0109x - 4.5466^{-5}x^2 + 5.2176^{-8}x^3$, arcsine-transformed adjusted $R^2 = 72.0$ %, $F_{3,\,45} = 42.9$, $p < 0.0000$)
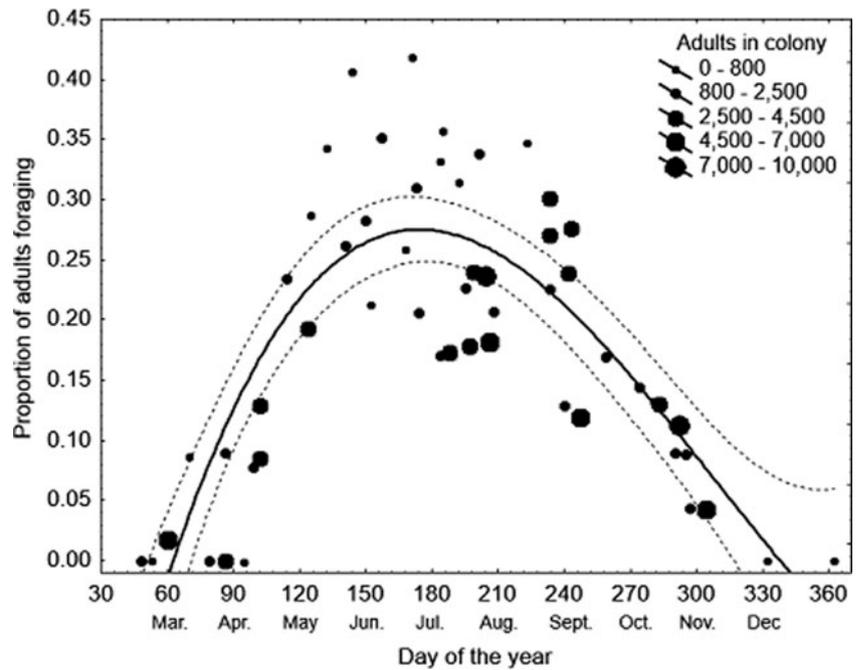
workers could only have entered adulthood in June of the focal year or in autumn of the previous year. Although this colony is an anomaly, it invites questions about natural and induced forager life-span and whether local environment could influence longevity and replacement of foragers.

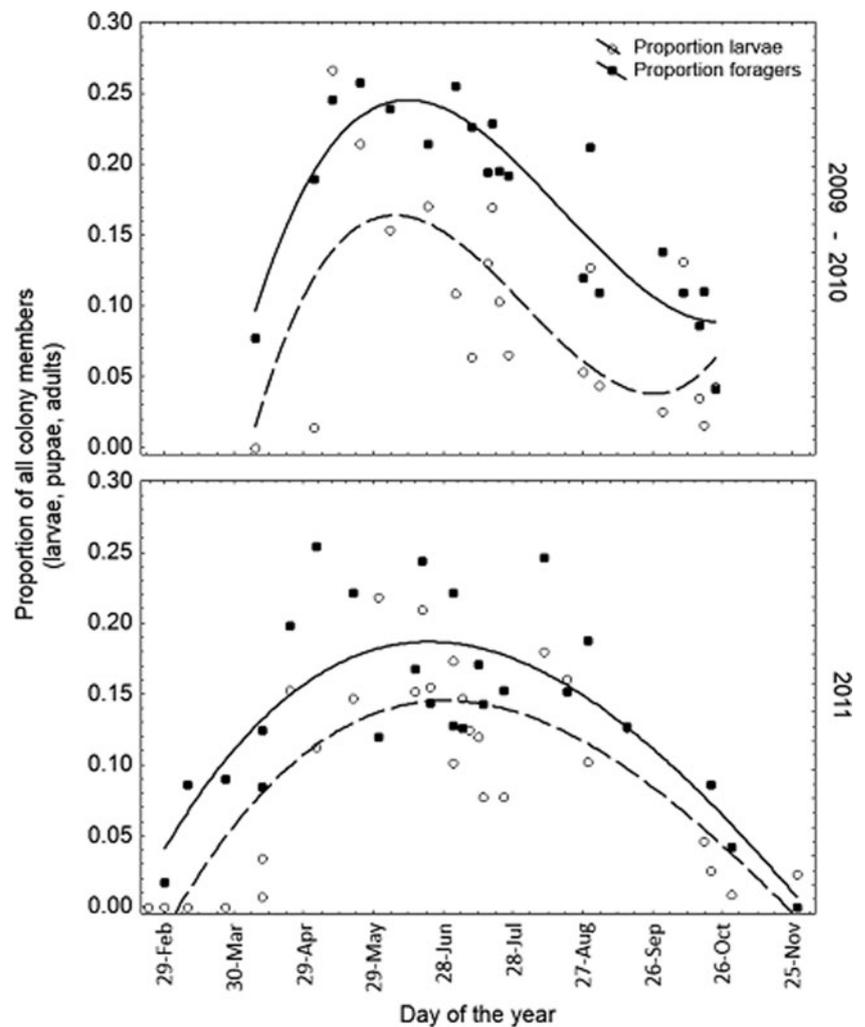Forager population size and colony size

Foraging began in March or April of each year. Overwintering workers populated labor roles without replacement until June, when the first callows appeared. Therefore, as foragers were lost, colony size declined for 4 months following winter

dormancy. Monthly estimates of forager population size revealed that the percent of each colony allocated to foraging increased in spring and early summer due in part to an increase in total forager number alongside a reduction in total colony size from forager death (Fig. 9). In mature colonies, a sharp decrease in number foraging followed in midsummer and likely resulted from investment in sexual production earlier that same year. The production of sexual larvae in lieu of additional worker larvae in the spring could result in a demographic gap, reducing the number of workers of foraging age for a period the following month. This is a reasonable interpretation, because the year's first cohort of workers enters the

Fig. 5 The ratio of foragers to larvae (1.6:1) is not significantly related to date between May and October of each year despite a dramatic change in the proportion of the colony allocated to foraging (*gray dotted curve* and *right Y-axis*). Variability in the ratio of foragers to larvae results from low larval abundance just before and after winter dormancy (*gray bands*)
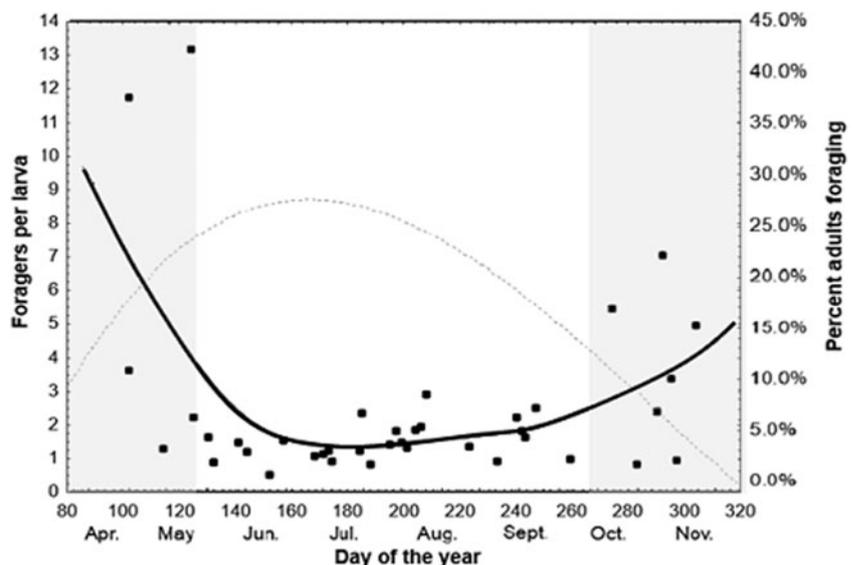
Fig. 6 The proportion foragers and larvae (as a fraction of all colony members) change in parallel, so that their ratio is constant from May to October. The years 2009–2010 differed from 2011 and are shown separately

forager population at approximately 43 days of age (mid-July) and is reared concurrently with sexual brood.

In June of 2010, the bulk of colony growth took place from July to October following the period of stasis and loss of colony size. The decrease in percent foraging between July and November resulted from an increase in colony size through new worker birth and, ultimately, forager death without replacement prior to overwintering. Between May and October of 2010, colonies grew a mean of 4.64 (SD 1.93, $n=$ 5) times their original size. Surprisingly, the mean growth of colonies monitored between the same months in 2011 was 0.996 (SD 0.73 $n=5$), representing a maintenance of adult population size for that annual cycle (an extreme drought year; Fig. 9). In the same year, foraging also reached a lower proportional maximum, though it followed the same general pattern. Therefore, the decrease in percent foraging during autumn of 2011 was the result of maintenance of colony size and an ever-shrinking forager population size. The result for immature colonies would certainly be continued immaturity the following year, as total colony size did not reach the minimum 700-ant mark before winter.

Forager life-span and death rate

While the rate of entry into the forager population varied considerably across the annual cycle, marked groups of foragers sampled in 2011 were lost at a mean rate 3.35 % (SD 1.32) per day. Foragers sampled between March and July of each year survived an average of 38 (SD 10.1) days in the forager population and represented individuals born the autumn prior. Conversely, colonies sampled after July contained both short-lived summer workers and autumn-born workers, which survived an average of only 26.9 (SD 7.93) days after marking (T test, $t_{12}=2.28$, $P<0.041$). Without further subdivision of the mixed-age caste that appears from July to September, it is impossible to know whether the slight reduction in forager longevity arises from a change in environmental conditions or a reduction in worker quality later in the year. Because colonies did not grow in 2011, reduced forager survival may have had a measurable impact on larval survival. However, sampling across multiple years would be necessary to

Fig. 7 At the time of excavation, workers of each cuticle color category were marked with a different colored wire belt. Marked ants appeared as foragers in the order of their relative ages at the time of marking, as shown for five sample colonies marked throughout 2012 (labeled *a*–*e* with excavation dates). Workers that eclosed in June or July of the focal year began foraging at an average age of 43 days, while those eclosing in autumn were 210 or more days in age
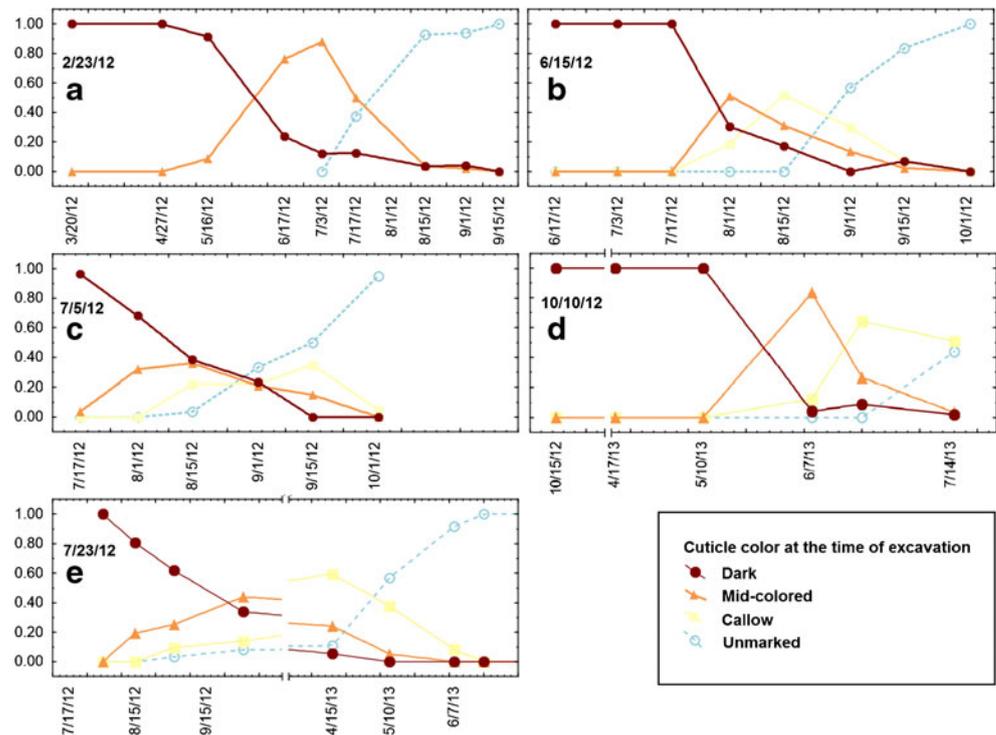
determine the typical range and forager longevity necessary for colony growth to occur.

Behavioral flexibility

In experimental nests, forager number was estimated 7 days after reducing the initial forager population by 50 %. Colonies did not replace experimentally removed foragers by drawing from other castes, and nearly half of the population was still absent at the end of the 7-day period (mean proportion of original population absent=0.48, SD 0.05) (Fig. 10). The daily rate of addition to the forager population did not differ significantly between paired control and forager removal colonies, indicating that colonies did not draw workers from other castes to fill the induced labor gap (T test, control mean=−0.010, experimental mean=0.002, t_6=−1.42, P=0.21).

Seven days after forager removal, the ratio of foragers to larvae in experimental colonies also did not differ significantly from that of paired control colonies (paired T test, t_3=1.75, P=0.18; mean difference between pairs=0.24, SD 0.27). This indicates that half of the anticipated larval population was missing in nests where foragers were removed. These results demonstrate that (1) larvae depend on foragers for survival and (2) colonies do not replace workers in missing labor groups by drawing individuals from other behavioral castes. Therefore, *P. badius* colonies appear inflexible with respect to large-

scale reallocation of labor over a period of a week, and the "foraging for work" model can be rejected in this case (Franks and Tofts 1994).
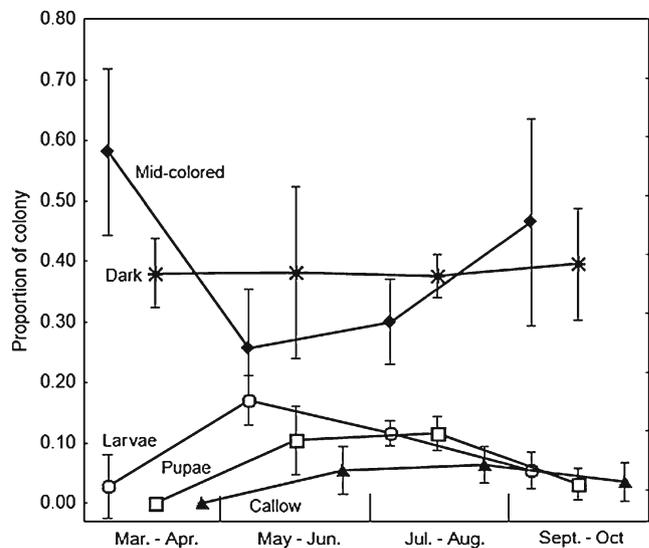
Fig. 8 The mean proportion of each colony represented by larvae, pupae, callows, and middle or dark workers at four points during the annual cycle, averaged across years (means with *standard error bars*). The large cohort of middle-aged workers present in March moves into the forager population in late spring and early summer to produce the highest annual proportion foraging
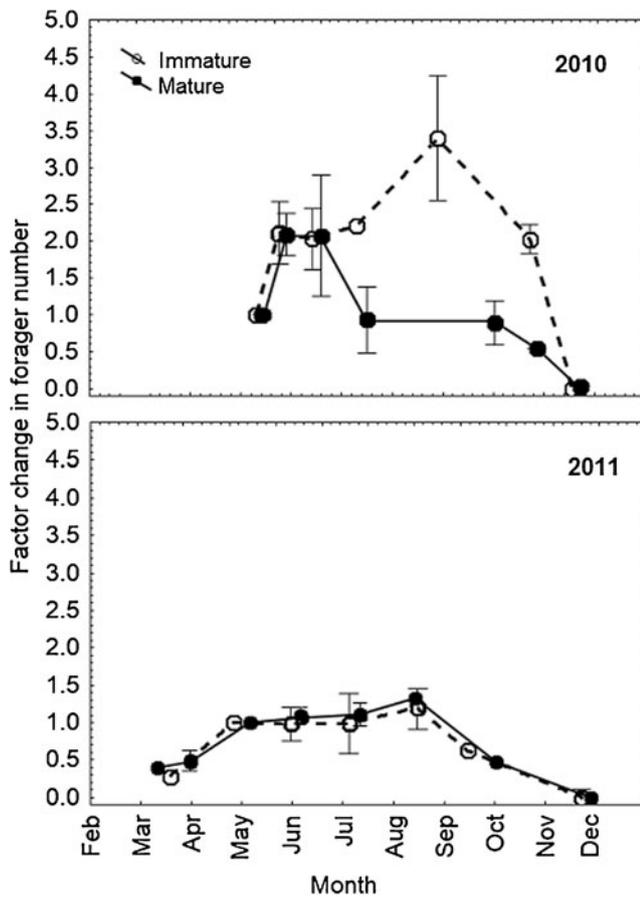
Fig. 9 For each focal colony, forager number on each sample date was divided by the May's estimate to obtain a factor of change in forager number (means with *standard error bars*). In 2010, forager population size increased in immature colonies and decreased in mature colonies when the year's first cohort of new workers entered the forager population following mating flights (July). In 2011, forager population size did not differ between May and August for mature or immature colonies

Discussion

Four important results emerge from this study. First, in *P. badius*, proportional allocation to foraging follows a seasonal

schedule that is conserved across years. Second, there is a fivefold difference in the age at first foraging, life-span, and rate of cuticle darkening between summer-born and autumn-born workers of identical body size. Third, when foragers are experimentally removed, colonies do not replace them by drawing workers from other castes, and larvae die in proportion to the number of foragers lost. Finally, seasonal allocation to foraging maximizes colony fitness and is correlated with colony age structure. Fitness is maximized by colony age structure because the annual production of alate larvae during May and June coincides with the annual peak in forager and old worker number in mature colonies, generated by the absence of new worker births and forager deaths during the 4 months of winter dormancy. Because forager abundance is directly responsible for larval abundance, this seasonal age distribution aligns the greatest number of old workers (foragers) with alate production, maximizing colony fitness. Although the same relationship could arise from behaviorally flexible workers responding to more numerous and nutritionally demanding larvae, we demonstrated that allocation to foraging is not driven by larval cues. In all years of this study, foragers appeared more than a month before larvae and larval population size shrunk in response to the removal of foragers.

It is unclear whether the annual pattern of demography that aligns the maximum number of old foragers and alate brood is a response to pressures that drive mating flights to occur in early summer, or an epiphenomenon that has shaped the temporal occurrence of mating flights. Though all mature colonies have a similar proportion of foragers at this time, larger colonies have a greater number foraging and therefore a higher reproductive potential (Mackay 1981; Tschinkel 1993; Cole and Wiernasz 2000). Investment in worker production from August to October increases colony size and forager population at the time of sexual production and during mating flights the following year. For immature colonies that do not produce alates in spring, springtime investment in worker larvae produces a relatively larger forager population later in the summer, which allows for colony growth through

Fig. 10 a When half of the forager population was removed, it was not replaced 7 days later (means with *standard error bars*). **b** The ratio of foragers to larvae did not differ in control and experimental colony pairs after 1 week, indicating that larval number is directly related to forager number, and larval population size was reduced as a result of forager removal
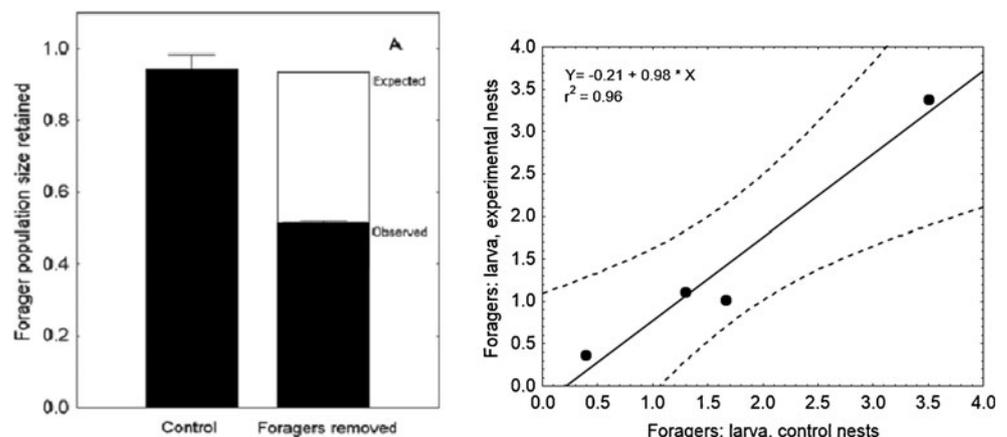
increased larval production. In either scenario, springtime production is owed in part to the sequestration of fat by overwintering, mid-aged workers, which peak in abundance early on during alate production (Tschinkel 1999; Smith and Tschinkel 2006).

Behavioral plasticity and age to first foraging

The dramatic difference in the age to first foraging, life-span, and development between summer-born and autumn-born *P. badius* workers is not unheard of among social insects. In honeybees, behavioral plasticity is socially mediated, so that the removal of foragers stimulates precocious foraging in younger cohorts, while the presence of older bees inhibits movement into the forager caste (Huang and Robinson 1996). Seasonal development rates in larvae and pupae have been demonstrated to vary by orders of magnitude due both to exogenous factors (temperature, photoperiod) and apparent, endogenous factors (Kipyatkov and Lopatina 2003, 2009). For *P. badius*, proportion foraging decreased precipitously during summer months, while the number foraging (2010) changed little, suggesting that a stable caste size is maintained when the rapid development of summer-born workers facilitated replacement of lost autumn-born foragers. It appears that replacement is achieved by an adaptive annual colony cycle, not a flexible response to worker demand.

If caste membership *were* socially regulated in *P. badius*, then the decreased age to first foraging in summer-born workers could arise from an increased forager death rate or demand for food beginning in July. However, we found that death rate is only slightly higher in midsummer, and new foragers do not enter the population at an accelerated rate when 50 % of the standing population is removed. Gentry (1974) also demonstrated that the prolonged removal of foragers from field nests did not result in a redistribution of labor; instead, it caused colonies to become inactive as foraging ceased for a period of months.

Though brood care is typically associated with very young workers, it is accomplished by *P. badius* workers no younger than 6 months of age following winter dormancy. The year's first callow workers do not appear until late May or June, indicating that all alates and the year's first cohorts of workers are reared by undarkened, autumn-born workers. These workers come to dominate the forager population in July and still appear as foragers as late as September. As only a small difference in death rate exists between individuals once they begin foraging, autumn-born workers are not simply entering the forager population en masse and outliving summer-born sisters.

Instead, it appears that autumn-born workers remain in interior labor roles for an extended period of time, as summer-born sisters move through at an accelerated rate. The result is that behavioral castes are comprised of both veteran and transient individuals that develop on different schedules. By the time the final autumn-born workers are fully darkened and become foragers, they will have been passed by hundreds or thousands of sisters born in the new calendar year. The occurrence of "mixed" behavioral castes invites questions about how experience and learning may influence task performance and the capacity of labor groups (Farris et al. 2001; Tripet and Nonacs 2004; Ravary et al. 2007). More importantly, it indicates that response thresholds differ for workers in the same behavioral caste and are not likely to be driven by the absence of workers in a subsequent labor role.

Seasonal characteristics of foragers

Although this study does not address the mechanisms underlying seasonal variability in age to foraging onset, several important correlates of colony investment could produce a caste structure that promotes colony growth, while inhibiting the replacement of foragers when loss exceeds a normal rate. The first factor is that all foragers, regardless of birth month, share a low body fat content. Tschinkel (1998) demonstrated that dark-colored (older) workers in *P. badius* colonies have the lowest relative fat content of all adults, averaging just 10 % of their total body weight. Additionally, fat content in all ages of workers was shown to be lowest in July, following the production and release of sexual alates. If summer-born workers develop more quickly as adults to become foragers at 43 days, then it follows that their relative fat content is lower than that of autumn-born sisters of the same age. It has been suggested that physiological correlates of starvation may actually drive the rapid movement of summer-born workers into the role of forager (Toth and Robinson 2003; Robinson 2009). In *Temnothorax albipennis*, worker fat content governs task allocation, and though age and physiological condition may be correlated, task attendance can be induced by supplemental feeding or starvation of workers (Robinson et al. 2009).

In the 48 colonies we censused, the ratio of foragers to larvae was conserved from May to October, indicating that there is no seasonal difference in the ability of foragers to provision the colony. This relationship seems puzzling in light of the large seed caches housed in most colonies, but as Smith (2007) demonstrated, at the expense of larval production, colonies do not tap into those stores when experimentally starved. It is possible that the age of foraging onset is driven by food quality. Selective feeding of foods with a higher ^{15}N signature occurs between alate and worker larvae in early summer (Smith and Suarez 2010). Presumably, insect protein is available throughout the annual cycle, but in the absence of sexual alate larvae late in the year, it is possible that autumn-born workers receive higher-quality food, which may contribute to their adult fat content, higher physiological "quality," and relatively longer life-span.

Colony growth and size depend in part on a queen's oviposition rate and the continued survival of eggs into adulthood (Nakata 1996). In *Solenopsis invicta*, egg volume and resultant worker size, which is correlated with longevity, are related to follicle residence time in queens (Tschinkel 1988). The factors that stimulate egg laying in *P. badius* queens are still a mystery, but if forager death rate and larval hunger offer any cues, their reduction in autumn months could slow egg deposition rate, producing larger more well-provisioned eggs and longer-lived adults, contributing to the observed patterns in worker life-span and labor allocation.

Worker location within the nest

P. badius foragers were found primarily in the top 12 cm of nests which can reach depths of 250 cm or more. The position of foragers near the nest entrance likely makes them available for recruitment by returning foragers while decreasing the probability that they interact with seed stores, brood, or excavation tasks deeper in the nest. The spatial localization of foragers has been observed in other species and implies that behavioral discretization may be influenced by access to task-specific nest space. Localization may also be adaptive because it diminishes the transmission of externally acquired pathogens (Wilson 1980; Naug and Camazine 2002). It also suggests that foragers deposit seeds in superficial chambers and do not shuttle them down to seed chambers, 40 or more centimeters below the surface themselves. In our study of *P. badius*, the observed number of workers present in the top 20 cm of the nest far exceeded the estimated number of foragers in those strata, implying that additional castes reside there or are transient workers that transport seeds downward or move sand and trash upward from lower chambers. It follows that nest architecture may serve as an additional factor driving the discretization of tasks (Tschinkel 2004). In species that occupy cavities or shallow nests with a simple architecture, the rigid form of labor allocation demonstrated in *P. badius*, which may produce nests in excess of 2.5 m, may not exist.

Death rate

It has been suggested that environmental predictability is capable of driving specialization and discretization of castes and that colonies organized more heavily by "inflexible" processes, like absolute worker age, are likely to live in predictable environments (Seeley 1989). If age to first foraging is not driven by a response to losses in the standing forager population, it is possible that entry into labor groups evolved to match predictable rates of extrinsic mortality, which occur on an annual schedule. Colonies sampled in 2010 achieved forager replacement in summer, while many of those sampled during the extreme drought of 2011 failed to do so, resulting in

an ever-decreasing forager population following mating flights. In both cases, the most important factor in determining colony growth was the ability of rapidly developing summer-born workers to, at minimum, replace lost autumn-born workers (present from March until September the year following their birth). This is so because forager number was shown to be directly related to larval abundance, with the experimental removal of foragers resulting in predictable larval mortality. If labor in *P. badius* colonies can be redistributed in response to need, one would expect forager replacement in 2011 to have increased to meet forager loss and prevent larval death; but it did not (Fig. 9).

On warm mornings, *P. badius* foragers deploy on trunk trails and make multiple forays in pursuit of seeds, insects, fungus, and "decorative" charcoal. Foraging pauses only under exceptionally hot, cloudless conditions or during heavy rain and concludes approximately 1.5 h before sunset. Although the age at first foraging differs for summer-born and autumn-born workers, both die approximately 1 month after entering the forager population. This indicates that either foraging itself is a fatal role or death is programmed to occur (based on intrinsic factors) upon entry into the forager caste. Rueppell et al. (2007) demonstrated that the life-span of foraging honeybees cannot be extended by a reduction in exposure, once individuals join the forager caste. The same may be true for *P. badius* workers, but an experimental reduction in extrinsic mortality factors would be necessary to find out.

If death in *P. badius* is not programmed, it may be owed, but not limited, to heat and desiccation, policing during mating flights, predation by mound-visiting *Apiomerus* reduviids, and most obviously, tussles with neighboring conspecifics (CLK, personal observations). It is not uncommon to see a forager ambling about with the decapitated head of a conspecific permanently clamped onto her petiole, as fatal encounters between neighboring nests occur frequently during the peak of foraging in spring and early summer. It is therefore possible that death rate is density dependent, so that populations in relatively empty "neighborhoods" see a reduction in forager mortality and an increase in forager life-span.

Inflexible workers and allocation to labor

Marked foragers were observed performing a variety of nonforaging tasks on the nest surface, such as trash and sand removal. We also observed daily, weather- and resource-related variation in foraging intensity, suggesting that "foragers" have a complex task repertoire and that task switching does occur locally within behavioral castes. Detailed observations of these fine-scale shifts in task attendance have been made for other seed harvesters, such as *Aphaenogaster cockerelli* and *Pogonomyrmex barbatus* (Gordon 1991; Sanders and Gordon 2002; Schafer et al. 2006; Greene and Gordon 2007; Pinter-Wollman et al. 2011). However, it is

apparent that colony allocation to task sets is limited by colony age structure and worker development rate in *P. badius*. This study shows that while workers may move fluidly between local tasks, individuals performing a task set are drawn from a finite group, structured by worker development rate. Seasonal patterns in forager allocation unfold from an interaction between two seasonally distinct development rates, life-span and cohort size, and individuals are not supplanted from other castes once the forager population is exhausted.

Seasonal differences in worker investment are probably common among ant species that produce alates from worker fat following winter dormancy, and may be the basis for generating annual patterns of labor allocation. The occurrence of seasonally distinct development rates, which produce behavioral castes of mixed age, is probably not unique to *P. badius* and may have important implications for caste performance. Future studies of labor allocation in the field will likely reveal that seasonal caste ratios, arising from predictable rates of birth, death, and development, are elegantly intertwined with annual patterns of growth, reproduction, and resource availability for many ant species.

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