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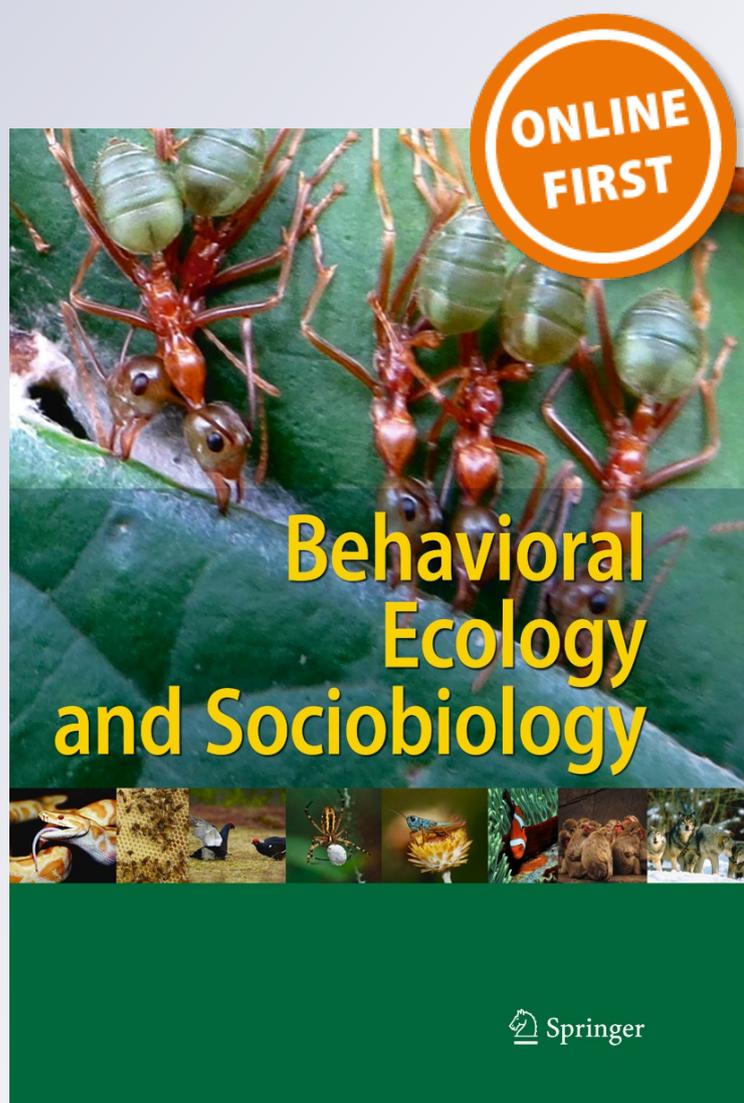
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Limited flexibility and unusual longevity shape forager allocation in the Florida harvester ant (*Pogonomyrmex badius*)

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Abstract The benefits of behavioral flexibility in social insect societies are well known, but the advantages of limited flexibility have seldom been considered. Florida harvester ant colonies maintain a stable forager population size for much of their active season, and despite seasonal variation in chronological age, foragers die within 27 days of initiating foraging. To determine how colonies balance forager mortality and forager replacement, we tested the relative influences of intrinsic and extrinsic factors on forager membership, retention, and longevity. Potential and realized forager longevity differed significantly. Residual lifespan increased by 57 % when colonies were penned for 20 days, and up to 8-fold when foragers were retained in the laboratory. Increased forager longevity *inhibited* the movement of new workers into the forager population. In contrast, increased mortality and starvation did not stimulate the addition of new foragers and forager population size declined when mortality exceeded 4 % per day. Experimental increases in forager number, body fat, and the ratio of larvae to foragers did not induce behavioral reversion in existing foragers. These results suggest an unidirectional allocation strategy, with foragers that are less disposable and less behaviorally flexible than the well-studied honey bee. In

P. badius, forager membership is maintained *not* by young ants detecting increased demand, but by workers developing at rates that allow forager replacement and prevent excessive worker depletion. In the absence of a lifespan matched to predictable risks, opportunistic increases in forager survival may promote colony growth by inhibiting the scheduled and irreversible transitions of younger workers.

Keywords *Pogonomyrmex* · Labor allocation · Lifespan · Extrinsic mortality · Age at first foraging · Behavioral reversion · Social inhibition · Colony size · Behavioral flexibility · Intrinsic development rate

Introduction

In many social insect societies, aging workers progress through a sequence of labor roles, beginning with brood care and ending with foraging outside of the nest. When workers transition from interior to exterior labor roles, their risk of death from age-independent factors increases greatly. In theory, timing of senescence and death should match the lifespan predicted by extrinsic sources, and colonies should position physiologically depleted workers to forage (Medawar 1952; Kirkwood 1977; Kirkwood and Austad 2000). This prediction holds true for honey bees (*Apis mellifera*) (Remolina et al. 2007; Amdam et al. 2009), but growing evidence suggests that actual and potential lifespan differ significantly in some ant species (Schmid-Hempel 1983; Wehner et al. 1983; Giraldo and Traniello 2014).

For social insects with highly integrated societies, the adaptive value of worker life history traits are only realized at the colony level, through their bearing on colony growth and reproduction. Timing of forager senescence and death are of particular interest, because net losses in colony size may occur

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if birth rate and progression through the lifetime-labor-sequence do not adequately anticipate forager mortality. For instance, *Cataglyphis bicolor* loses foragers at a rate of 16.4 % per day yet maintains a stable forager population for the bulk of its active season (Schmid-Hempel and Schmid-Hempel 1984). Colonies of *Solenopsis invicta* are able to persist and grow with turnover rates as high as 50 % of the standing adult population per month (Tschinkel and Porter 1988; Tschinkel 2006 pg.213).

The balance between forager mortality and replacement depends on colony egg production, the number of resulting larvae that can be sustained to pupation, and the transition rates between each labor group. Queens have been shown to adjust egg-laying rates in response to the perceived abundance of pharate pupae (a proxy for larval success; Tschinkel 1988b; 1995) and larval survival has been linked directly to forager abundance (Howard and Tschinkel 1981; Kwapich and Tschinkel 2013). Yet there is a significant delay between pupal eclosion and foraging, so that new foragers are not drawn at random from the adult population, but represent a small age-correlated subset already poised for that transition. The question that remains is whether the age, body condition, and availability of new foragers are driven by real-time changes in demand for foraging or from the bottom up by intrinsic rates of behavioral development. To answer this question, it is necessary to test the relative influences of both extrinsic and intrinsic factors on worker life history and behavioral transition rates and consider the consequences for colony size.

Flexibility and age structure

In honey bee colonies, transitions between age-correlated labor groups are flexible and responsive to social feedback, and both physiological and behavioral acceleration and reversion can be induced within 24 h of manipulating colony demography (Huang and Robinson 1996). While the removal of foragers induces precocious foraging in young bees, the presence of a stable forager population inhibits new workers from transitioning into the forager population (Robinson et al. 1992; Huang and Robinson 1996; Leoncini et al. 2004). Although the honey bee has served as a model of labor dynamics across social insect species, studies of ant species with a temporal division of labor suggest that considerable variation in behavioral flexibility may exist (Gentry 1974; Traniello and Rosengaus 1997; Mersch et al. 2013; Kwapich and Tschinkel 2013).

In the Florida harvest ant (*Pogonomyrmex badius*), proportional allocation to foraging follows a strict seasonal schedule related to demography, and behavioral transitions show limited sensitivity to increased demand for labor. Removing half of a colony's forager population does not stimulate precocious foraging in younger ants (sand carriers, Tschinkel et al. 2015). Instead, new foragers remain absent after one week and larvae

die in proportion to the foragers removed (Kwapich and Tschinkel 2013). Total removal of the forager population results in longer recovery times and extended colony inactivity (Gentry 1974). This finding suggests that selection has not favored mechanisms allowing rapid labor redistribution over a timescale relevant to larval survival and underscores the apparent inflexibility of *P. badius* behavioral development in the forward direction.

In both honey bee and Florida harvester ant societies, chronological age at first foraging varies by orders of magnitude depending on the season (Maurizio 1950; Free and Spencer-Booth 1959; Seeley 1982; Kwapich and Tschinkel 2013). Despite this variation, honey bee workers experience senescence 14–18 days of entering the forager population (Dukas 2008; Münch et al. 2013), and their residual lifespan cannot be extended by eliminating extrinsic sources of mortality alone (Rüppell et al. 2007). For *P. badius*, the relationship between forager mortality rate and the 170-day age difference between early and late summer foragers remains undescribed, but evidence across the genus *Pogonomyrmex* suggests that all workers likely die within a month of entering the forager population (Porter and Jorgensen 1981; Gordon and Hölldobler 1987; Oettler and Johnson 2009). The seemingly disposable nature of the foragers is further accentuated by their low body fat reserves relative to younger nestmates (Tschinkel 1998). Yet whether *P. badius* lifespan is a result of age-dependent or age-independent factors remains untested, and recent evidence suggests potential lifespan may exceed the realized lifespan observed for many ants (Giraldo and Traniello 2014).

Study outline

In light of the honey bee's flexible strategy, the apparent inflexibility of *P. badius* labor redistribution is intriguing. If major behavioral transitions are not socially regulated, then development and functional senescence may have evolved to match a predictable rate of forager loss in the habitat where these ants occur. Furthermore, if the rate at which workers join and leave the forager population is fixed, then experimentally increasing forager longevity should not influence the development of new foragers, despite the presence of an excessively large forager group.

In this study, we investigate the relative influences of intrinsic development rate, large-scale behavioral flexibility, and lifespan on forager allocation in colonies of the Florida harvester ant. Seasonal patterns of forager mortality and forager replacement are described across 3 years, and a series of field experiments addresses how extrinsic risks influence longevity in both slow-developing (autumn) and fast-developing (summer) workers, how forager turnover rate influences the development of younger workers into new foragers, and whether or not forager reversion can be induced by increasing forager number, larval number, and forager body fat.

Methods

P. badius colonies were sampled from a population containing more than 400 nests in a 23-ha, sand hill habitat known as Ant Heaven in Florida's Apalachicola National Forest (latitude 30.35, longitude -84.41). The area was characterized by an over story of a 40-year-old long leaf pine (*Pinus palustris*), a mid-story of turkey oak (*Quercus laevis*), and a ground covering of dwarf huckleberry (*Gaylussacia* spp.), pricklypear (*Opuntia* spp.), beard grass (*Andropogon* spp.), gopher apple (*Licania michauxii*) and catbrier (*Smilax* spp.). *P. badius* nests were spaced an average of 14.6 m apart (Tschinkel 2015) and co-occurred with *Solenopsis geminata*, *Trachymyrmex septentrionalis*, *Forelius pruinosus*, *Dorymyrmex bureni*, *Aphaenogaster floridana*, and numerous less common ant species.

Foragers were defined operationally as individuals that traveled a distance of 150 cm or greater from the margin of their nest mound and collected a food item before beginning a return trip to the nest (per Kwapich and Tschinkel 2013). This distance was selected so that workers encumbered with trash were avoided, and clearly defined trails were intersected. The size of the worker population that could be recruited to forage was defined by the proportion of workers that could be recaptured foraging after mixing randomly overnight. While foraging activity varies on a daily basis, the current study addresses the size of the total worker population from which workers available to forage are drawn.

Method for estimating forager number and survival

In each study detailed below, forager survival and change in forager number were measured between two separate mark–release–recapture events over defined time intervals. On each sample date, the total number of workers that could be recruited to forage was estimated using the Lincoln index mark–release–recapture method (Lincoln 1930). A distinct ink color (orange or green) was used during each event, so that the proportion of workers surviving from the initial marking could be estimated by capturing single-marked and double-marked workers in the final mark–recapture.

On the first day of each mark–recapture event, a ring of bird seed was placed 150 cm from the nest mound 1–2 h after the initiation of morning foraging. To encourage continued recruitment on trails (Hölldobler and Wilson 1971), the area was shaded with a beach umbrella and foragers were gathered in 15-min intervals until fewer than five were captured in 30 min. After the 3–6-h collection period, foragers were marked with a permanent, non-transferable fluorescent printer's ink in diethyl ether, sprayed from a 5-ml atomizer (Gan's Ink Supply Co., Los Angeles, CA; Risk Reactor, Santa Ana, CA; Porter and Jorgensen 1981; Tschinkel 2010; Kwapich and Tschinkel 2013). The quality and uniformity

of each mark was checked using an ultraviolet flashlight and foragers were returned to their nest mound simultaneously. Twenty-four hours later, foragers were captured in the same manner as day 1, and the number of marked and un-marked foragers in the sample were counted.

The proportion of marked individuals appearing in the recapture sample represented the proportion of the total population marked 24 h prior (Lincoln 1930). To maximize the proportion of the forager population that could be identified in subsequent days and weeks, un-marked foragers captured on day 2 were also marked and returned to their nests. The mean proportion of the initial forager population marked per nest was 0.70 (SD 0.10), which yielded exceptionally high confidence in later estimates of survival. During the second sampling event, the percent of marked workers surviving from day 1 was multiplied by the total, initial forager population size to determine the total number of initial foragers represented in the second population.

Seasonal mortality, replacement, and forager turnover

From May to October, the daily rate of forager mortality was estimated from the proportion of foragers surviving between mark–release–recapture events spaced 2 to 31 days apart ($n=29$). Larger, seasonal patterns of forager mortality and replacement were described by mark–recapture events spaced 20 days apart between May and November ($n=22$ colonies, 2012–2014). The estimated number of days until complete forager turnover for each naturally foraging colony was determined by dividing initial forager population size by the number of foragers that died per day, assuming an equal fraction of the total workers were lost per day between the two sample dates (day 1 and day 21).

Experimental reduction of extrinsic risks

To determine if forager longevity was part of a fixed, developmental program or the result of exposure to extrinsic risks, forager survival was compared between penned ($n=15$) and control colonies ($n=14$) after 20 days. Penned colonies were either offered a daily excess of chopped mealworms and native seeds ($n=7$ fed), or denied access to new food for the entirety of the 20-day focal period ($n=8$ starved). Control colonies were divided between natural foraging ($n=8$) and mock enclosures ($n=6$), which simulated experimental enclosures but allowed foragers to exit through four 20-cm flaps cut into the sides. Penned-fed and penned-starved colonies were matched with controls of similar nest-disk diameter and location in the forest and sampled over one 20-day period between May and October (2012–2013). It was not possible to record blind survival data because our study involved focal colonies in the field with visible enclosures.

Each penned colony was enclosed in 60 cm×60 cm×10 cm screen-bottom, open-topped, aluminum box that eliminated interaction with neighboring colonies and reduced foraging distance by an estimated 80 % and area by 99 % (Harrison and Gentry 1981; Fig. 1). At the center of each enclosure, the nest mound was exposed by cutting out a disk of screen flooring. Hanger wire was used to pin the screening to the ground and the screen floor was weighted with a layer of sand and local debris to prevent escape by tunneling. A strip of plastic mesh was affixed to the top of the enclosure to produce a band of shade that projected 5 cm inward from the margins of the enclosure and prevented the unnatural desiccation of foragers that gathered at the enclosure's wall. A band of slippery flouon (a liquid Teflon that prevents climbing) was applied to the inner and outer walls of each enclosure to prevent colony members from crawling out and other insects from crawling in.

On day 1 of each focal period, foragers were captured, marked with orange ink, and released. On day 2, total forager number was estimated from the proportion of marked foragers recaptured, and foragers were returned to their nests. Prior to nest opening on day 3, enclosures were applied to fed–penned, starved–penned, and mock-enclosure control colonies. On day 20, enclosures were removed and colonies were allowed to become reacquainted with their foraging territory. The following morning, each colony's standing forager population size was estimated for the second time by mark–recapture (days 21 and 22). A new ink color (green) was used, so that the proportion of the initial forager population surviving 20 days could also be estimated by double-marking and recapturing individuals from that population, then applying the Lincoln index formula. Due to weather, some colonies were sampled up to 2 days late or early, and their survival estimates were corrected to 20 days by assuming equal daily



Fig. 1 Penned nests (*center*) were enclosed in 60 cm×60 cm×10 cm screen-bottom, open-topped, aluminum boxes that prevented long-distance foraging and neighbor interaction. A strip of plastic mesh was affixed the top of the enclosure to shade foragers that gathered near enclosure walls

loss between marks one and two. Success in capturing old foragers was confirmed by excavating the top 30 cm of four nests and searching for marked workers.

Influence of old forager survival on the addition of new foragers

The influence of forager longevity on the appearance of new foragers was assessed for penned and control colonies from May to August (2012 and 2013). Relative changes in forager population size were calculated by dividing the final number of foragers after 20 days by the initial forager number in each colony.

To determine if the rate of new forager addition differed in penned and control colonies, an *expected* final population size (E) was calculated for each penned nest using the average rate of replacement in control colonies. To do so, the mean proportion of dead foragers replaced by new foragers (r) over 20 days in *control* colonies was multiplied by the initial forager population size (i) in each penned nest. Then, the number of original foragers (day 1) that *would have been* replaced at the control rate was added to the actual number of surviving, old workers (s) in each penned nest ($E=(r \times i)+s$). Each observed (day 21) and expected final population size was divided by the observed, initial forager population size (day 1). These relative forager population sizes were compared to a factor of 1 (complete replacement) and the observed mean in control colonies. One replicate set was removed as an outlier (final $n=17$ colonies).

Energetic reserves and reversion in field foragers

Body condition for penned and control foragers was compared using the percent body fat of lab-starved workers as a baseline. To measure percent body fat, individuals were sacrificed by freezing, then dried at 55 °C in an oven for 48 h, weighed on a microbalance, placed in individually labeled gelatin capsules, inserted into a Soxhlet extractor, and washed repeatedly with diethyl ether (Smith and Tschinkel 2009). The dry, fat-free weight of each ant was then measured and divided by the initial dry weight to determine percent body fat (energetic reserves) at the time of collection.

To determine if longevity and behavioral reversion were influenced by body condition, percent fat was compared between old and new foragers in penned and control treatments from May to November. Eight to 15 “initial foragers” (bearing the ink mark from event 1) and 15 new foragers (bearing only the mark from event 2) were retained from the colony following the 20-day mark–recapture procedure ($n=585$ foragers, 29 colonies). Estimates of “new forager” body condition are conservative because a small portion of individuals labeled as “new foragers” represented old foragers that were not previously captured and marked. In

many control colonies, few individuals marked on day 1 of the study still survived to be captured after 20 days. In these instances, the top 20 cm (where foragers reside) of each nest was exposed to search for marked individuals among uncaptured foragers.

Lab survival and starvation

To reveal potential forager lifespan, and to account for variation in longevity that could result from the 5-fold difference in age at first foraging between autumn-born and summer-born workers, differences in survival were monitored in the laboratory. Two hundred foragers were collected from the same three nests in May and September, and one additional nest in September of the same year. Foragers captured in May eclosed the previous autumn and were a minimum of 240 days old (slow-developing workers). Foragers captured in September eclosed in July/August of the focal year and were 40–50 days old (fast developing workers; Tschinkel 1998; 1999; Kwapich and Tschinkel 2013).

The foragers were taken to the lab and divided into two treatment groups: fed and starved ($n=1400$ foragers). Each group of 100 foragers was housed at 26 °C in a plaster laboratory nest and separate tray. Foragers belonging to fragments in the starved treatment were offered cotton-plugged test tubes containing water only, while those in the fed treatment were offered water, ad libitum mealworm pieces, 20 % sucrose water, and native seeds (gathered from field nests). For all treatments, the number of surviving foragers was recorded approximately weekly up to 39 weeks. To determine the percent body fat of workers that died from starvation, in each season, 10–15 ants were collected after their death from each starved colony and stored at –40 °C prior to fat extraction (sampled $n=80$ starved workers).

Addition of larvae

To determine if forager allocation was driven by colony demand for food, larvae were added to wild colonies and change in forager number was measured after 7 days. In a previous study, we found that *P. badius* colonies maintained a ratio of 0.65 (SD 0.39) larvae per forager over five annual cycles (50 wild colonies). Removing foragers result in a loss of larvae and maintenance of the ratio (Kwapich and Tschinkel 2013). In the current study, forager number was estimated by mark–recapture for six focal colonies at Ant Heaven. Then, using 2nd and 3rd instar larvae from three freshly excavated source nests, one additional larva was added for every forager in each of three focal colonies.

One week after larvae were added, forager number was estimated again in both larvae-supplemented ($n=3$) and paired control colonies ($n=3$). Changes in forager allocation were determined by dividing the final forager number after 7 days

by the initial forager number, to determine the relative change in forager population size. Relative forager population size was compared between larvae-supplemented and control colonies. Brood ratios were also recorded following the chamber-by-chamber excavation and census of all six supplemented nests and compared between pairs.

Like most ants, *P. badius* workers accept and rear conspecific worker brood. To be certain, recaptured foragers were housed with introduced, conspecific larvae overnight. Foragers readily groomed and organized the larvae and brought them inside the nest. Larvae and foragers were released early in the morning, when other broods are typically found warming near the nest's surface. Prior to introduction, larvae from one source colony were fed Rhodamine B-dyed cricket flesh, causing their meconium to appear dark pink. This allowed the larvae to be tracked in their new, supplemented nest following excavation. Forager estimates and excavation occurred well within the larval and pupal periods, which take an estimated 19 days, and 11 days, respectively (Smith and Tschinkel 2006).

Experimental enlargement of forager population size

To determine if the presence of excessive foragers stimulated rapid forager reversion (24 h in honeybees), forager population size was experimentally increased and forager reallocation was monitored over time. For each forager-supplemented and control colony, typical changes in forager number were estimated by mark–recapture over 20 days ($n=4$ supplemented, $n=3$ controls). Then, 50 % of each colony's forager population was removed and retained in the laboratory. Previous work demonstrated that labor recovery is slow, and foragers are not replaced even 7 days after removal (Kwapich and Tschinkel 2013). With complete forager turnover occurring every 26–27 days, recovery was estimated to be complete 1 month after forager removal. To be certain, forager number was estimated 30 days after removing half of each colony's forager population (day 52). Then, surviving foragers that had been retained in the laboratory were returned to four focal colonies (day 53), increasing the forager population to 139.7 ± 5.6 % of the recovered size. Foragers were not returned to control colonies. To determine if younger foragers reverted to interior labor roles after the forager population was enlarged, forager number was estimated one final time for all experimental and control colonies, 1 week later (day 59).

In a pilot study, we found that after 3 weeks, colonies attacked foragers that were removed and retained in the laboratory. Swapping five workers between the field and lab groups each week allowed for maintenance of the necessary recognition cues, and resulted in a peaceable reunion between foragers on day 30.

Analysis

Forager survival was assessed after 20 days using Generalized Linear Models (GLMs) with a logit link function and quasibinomial error distribution to account for overdispersion (R v. 3.3.2 (R Core Team 2013)). GLM1 examined the effect of the season (4 levels) and initial forager number on the proportion of foragers surviving in control colonies only. In the second model (GLM2), differences in forager survival were compared between penned and control colonies using the factors season and treatment (fed–penned, starved–penned, control), and in a third model (GLM3), the main and interactive effects of season and treatment were described. Open and mock-enclosure controls were merged within seasons due to similarity. Tukey's honest significant difference (HSD) tests were used to make *post hoc*, pairwise comparisons between interactive effects (Package *LSmeans*).

Differences in energetic reserves after penning were assessed with beta distributed Generalized Linear Mixed Models (GLMM, package *GLMMadmb*) and *post hoc* Tukey's HSD. Source colony was included as a random effect in all the models. The response variable, "proportion fat," was expressed as the average milligrams of fat per milligram of dry body weight in each age group, within colonies. GLMM1 examined the main and interactive effects of forager age (old or new) and season (May–Aug, Sept–Oct) in control colonies alone. In GLMM2 and 3, the main and interactive effects of forager age and treatment (fed–penned, starved–penned, control) were analyzed separately within each season. Lean (fat-free) weight was excluded because it was not a significant predictor and increased AIC scores.

For foragers retained in the laboratory, the influence of birth season (two levels), starvation, and feeding on weekly survival were described by a Kaplan–Meier survival function. Differences in the weekly risk of death were compared using the Cox proportional hazards model to account for right censored data (observation stopped at 39 weeks) and the continuous change in the number of nestmates already dead on each date as a covariate and source nest, season and feeding treatment as factors (Statistica v.12, StatSoft 2013). The model assumed that ants in the "fed" treatment represented a baseline hazard function, while ants in the "starved" treatment had a hazard proportional to that of the baseline.

In all experiments, relative forager population size was expressed as a ratio between sample dates to account for differences in the initial forager number, and analyzed with two-tailed dependent, independent or single sample *t* tests, and compared to a reference constant of 1, to indicate complete replacement (Statistica v.12, StatSoft 2013).

Results

Seasonal forager turnover and replacement

Colonies sampled in this study were of typical size in the population, with a mean of 328 foragers (SD 221, max.798). Variation in forager number did not influence forager survival in control colonies across the annual cycle (GLM1, $t=0.55$, $p=0.59$, all size:season $p>0.41$). Mortality occurred in a narrow range of 3.0 %–4.9 % of the total forager population per day (avg. 3.7 %, $n=29$, Fig. 2), with complete turnover of the forager population occurring approximately every 27 days between May and October ($n=22$, Fig. 3).

On average, control colonies maintained a stable forager population size for four consecutive months (May–August, Fig. 4). Forager replacement consistently matched or exceeded forager loss when mortality was lower than 80 % over 20 days ($n=35$, Fig. 5B). However, when mortality in the initial forager population was greater than 80 % over 20 days, the proportion of colonies that maintained a stable forager population size declined, so that half lost 20–40 % of their initial forager population size.

In September and October, replacement of dead foragers declined sharply for all colonies. Although colonies continued to lose more than 70 % of marked foragers every 20 days (Fig. 2, *solid line*), only 40 % were replaced (Fig. 2, *dotted line*). By November, death rate also decreased by half, and forager replacement continued to fall to only 20 %, leading to the cessation of foraging during the winter season. The disparity between death rate and replacement in autumn demonstrates that entry into the forager population was not induced by losses in the forager population.

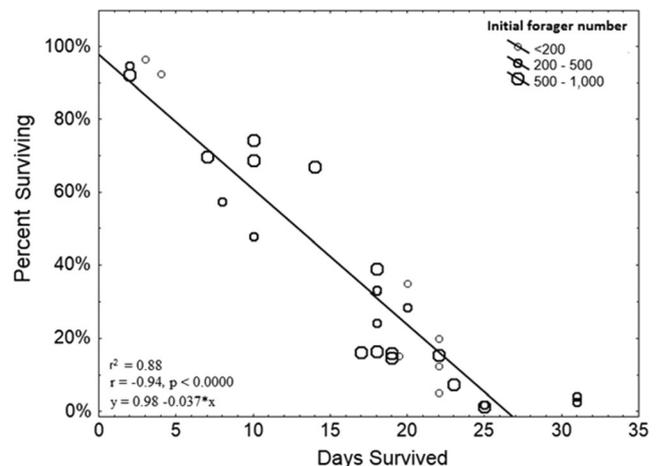
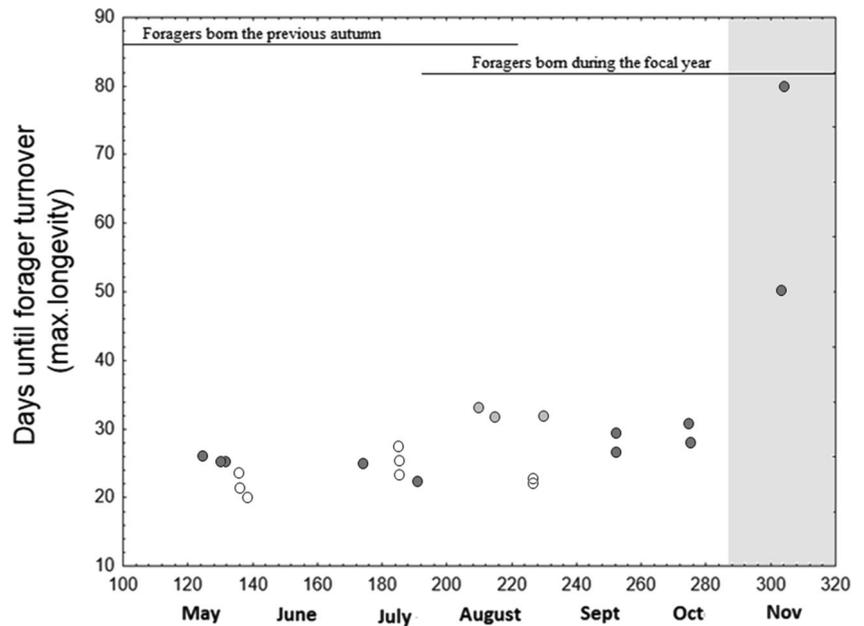


Fig. 2 Forager survival was sampled 2–31 days after an initial mark–recapture event in 29 unmanipulated colonies, spaced at an average of one nest per 14.6 m apart. Approximately 3.7 % of foragers were lost per day from April to October (2010–2014). Each *circle* represents a single colony, scaled by its initial forager population size

Fig. 3 Maximum forager longevity was consistent from May to October, and complete turnover occurred an average of every 26 (SD 3.8) days overall and at 27 days for control colonies paired with penned nests in 2012–2013. Forager longevity increased just prior to winter dormancy (*gray area*). Estimates were calculated from the daily rate of forager loss after 20 days for each of 22 unmanipulated colonies (*light gray*=2012, *dark gray*=2013, *white*=2014)



Extrinsic control of longevity

The percent of foragers surviving 20 days was significantly higher in penned colonies (55 %±4.4 alive, *n*=14) than in control colonies (27 %±2.6 alive, *n*=12), demonstrating that residual lifespan is not fixed when individuals enter the forager population, but reduced by foraging itself (GLM2, fed *t*=5.06, *p*<0.0001; starved *t*=4.40, *p*<0.0003; all Tukey HSD vs. controls *p*<0.0004; Fig. 6). On average, forager turnover took 27±1.39 days in control colonies, 44±3.71 days in penned-fed colonies, and 41±6.24 days in penned-starved colonies. The 57 % increase in residual lifespan for penned foragers likely allowed the same group to provide for more larvae per capita. If each forager made 10 successful trips per day, this would translate into at least 154 additional trips per forager over her foraging tenure.

While forager survival in fed–penned colonies did not differ between months, survival in starved–penned colonies increased from 31 % in May to 82 % in October (GLM3, starved *t*=2.80, *p*=0.01; Tukey’s HSD seasons *p*<0.001; Fig. 6). This was the highest survival rate across treatments and seasons of the study and twice as high as control–forager survival in the same season. As winter approached, the proportion of foragers surviving 20 days in control colonies also increased, but did so a month later, likely due to reduced activity in absence of larvae, and reduced exposure of foragers (GLM1, *t*=5.9, *p*<0.0001).

Social inhibition of foraging

Forager population size was stable across each 20-day sampling increment between May and August (single mean *t* test,

RC=1, mean relative forager number=0.97, *t*₁₆=−0.78, *p*=0.45). Both penned and control colonies replaced dead foragers, but because mortality was higher in controls (Fig. 6), relatively more new foragers were added per day to achieve replacement (single–sample *t* test, mean relative forager population size, control=0.95; fed=0.97, *t*₁₀=−0.22, *p*=0.83; starved=1.01, *t*₁₀=−0.64, *p*=0.54; Fig. 7). After 20 days, 69 %±4.2 of foragers were new in control colonies, while only 43 %±8.7 and 46 %±5.9 were new in fed and starved–

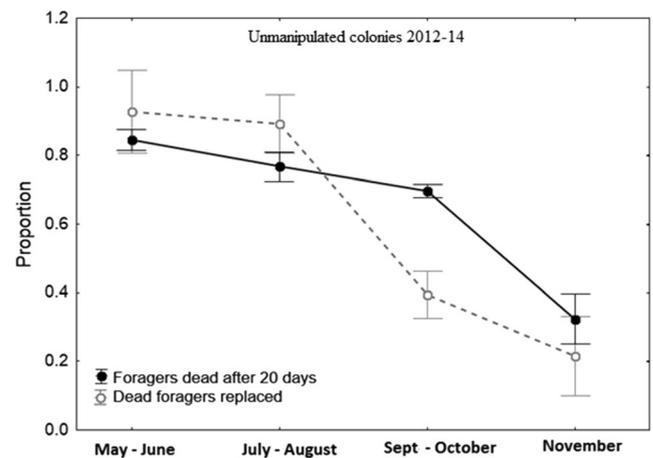


Fig. 4 Forager mortality and replacement were sampled in 20-day increments. On average, more than 90 % of lost foragers were replaced from May to August. Although forager mortality remained high, replacement dropped sharply in September. By November, death rate also decreased (likely due to a reduction in activity) and replacement of dead workers continued to fall (plot shows means with standard errors for unmanipulated colonies, sampled 2012–2014, *n*=22)

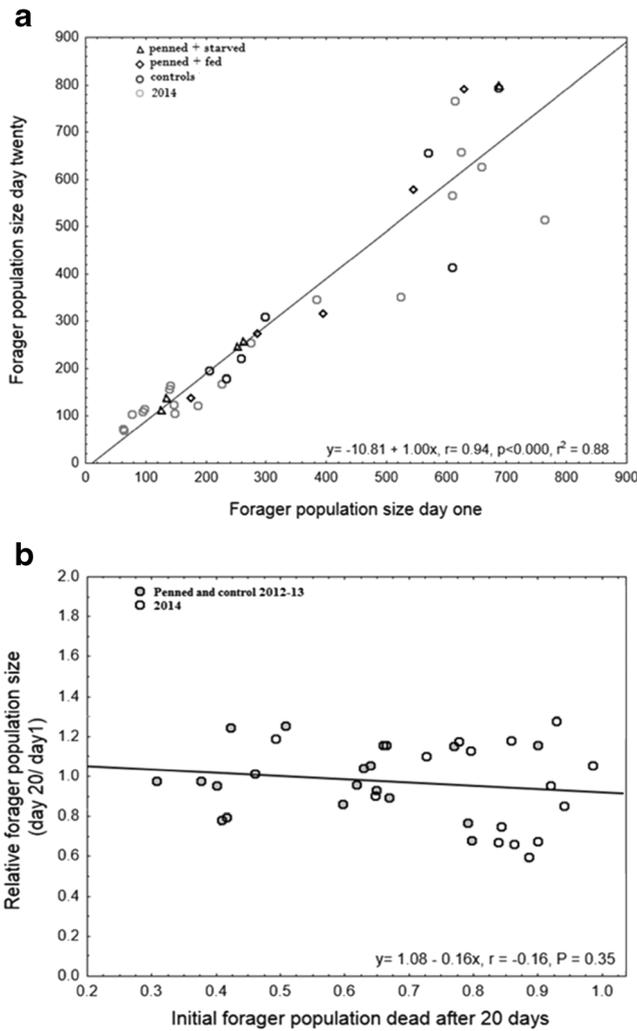
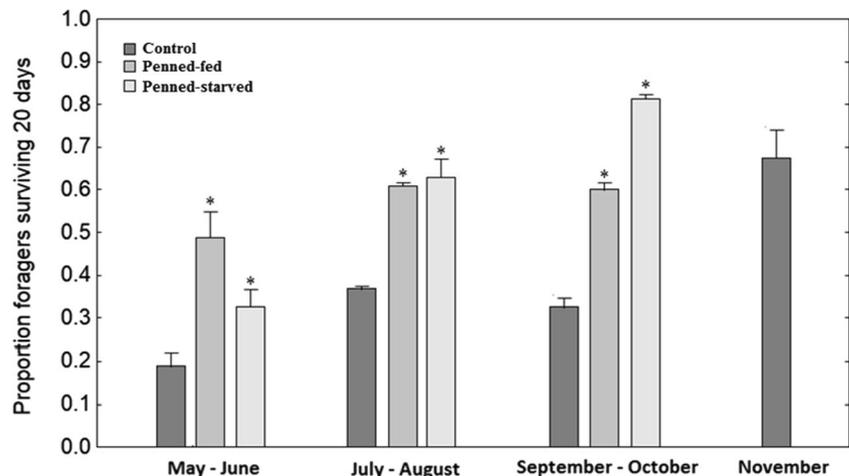


Fig. 5 **a** Each point represents a single colony sampled between May and August (2012–2014, $n=35$). On average, forager number on day 20 approximated forager number on day 1 across years and experimental treatments. **b** Across treatments, mortality after 20 days ranged from 30–98 %. When forager mortality was greater than 80 % over 20 days, the proportion of colonies able to replace lost foragers declined, and half of colonies lost 20–40 % of their forager population size

Fig. 6 Forager survival over 20 days was significantly higher in penned colonies than in control colonies (asterisks denote significance relative to controls within month groups 2012–13; GLM2, fed $t=5.06, p<0.0001$; starved $t=4.40, p<0.0003$). In general, forager survival increased between May and November, as foraging activity declined prior to overwintering ($n=29$)



penned nests. Based on the control rate, the expected relative forager population size in penned nests was 18–23 % higher than the observed value (fed mean, expected=1.21; observed=0.97, $t_8=-2.41, p=0.04$); starved mean, expected=1.19; observed=1.01, $t_8=-2.25, p=0.05$, Fig. 6).

Contrary to our predictions, significant increases in forager survival did not produce a larger forager caste. Forager replacement and mortality were decoupled in autumn, and forager replacement decreased with excessive mortality. Taken together, these results suggest a unidirectional control of labor allocation in *P. badius*, where a stable forager population size is maintained in the face of increased forager longevity, but not when forager loss is excessive. In effect, forager survival inhibited the appearance of new foragers.

Laboratory lifespan and starvation

Despite a 170-day difference in chronological age when collected as foragers, lifespan was not significantly different between summer-born and autumn-born foragers within laboratory feeding treatments (hazard ratio=0.79, $CI_{95\%} = 0.79 - 1.0, p=0.07$). Risk of mortality for foragers in starved laboratory groups was 5.6 times greater than for those in the fed laboratory groups (hazard ratio=5.6, $CI_{95\%} = 4.5 - 6.8, p<0.00001$, Fig. 8). The number of foragers already dead on each sample date did not influence future mortality ($HR=1.00, CI_{95\%} = 0.99-1.01, p=0.13$), although fed foragers from one source nest died sooner than in other fed treatments ($HR=1.26, CI_{95\%} = 1.0-1.5, p=0.003$).

On average, 10 % of fed foragers still survived 180 days after capture, and complete turnover did not occur until more than 250 days in several groups. By termination at 39 weeks, two fed groups had produced one male each and several larvae. In contrast, no broods were produced and only 10 % of laboratory-starved foragers survived after 35 days. Foragers that died of starvation in the laboratory had a mean fat content

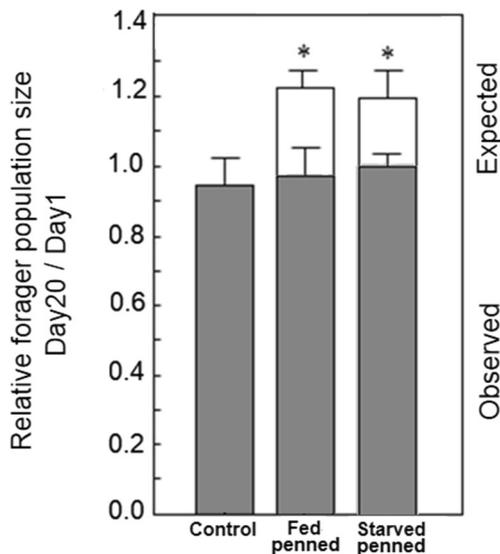


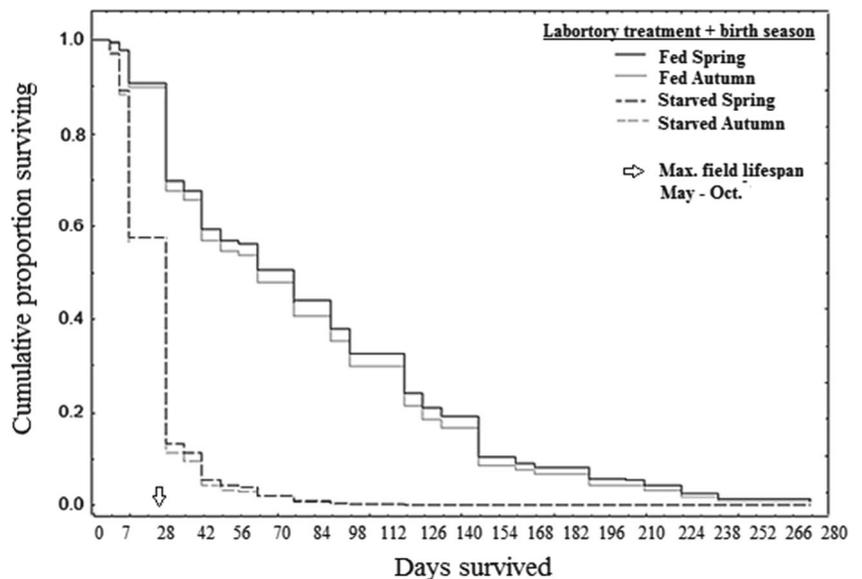
Fig. 7 For each control and panned colony, the observed forager population size on day 20 was divided by forager population size from day 1 to calculate a relative difference in forager number (May–Aug.). Enclosed colony means (with standard error bars) were not significantly different from controls ($t=-0.22, p=0.83$) and not significantly different than “1” ($t=0.78, p=0.45$), indicating that on average, lost foragers were replaced in all treatments. The expected forager number for experimental nests, calculated using the mean proportion replaced in control colonies, was significantly higher than observed, demonstrating the higher relative rate of new worker addition in control colonies (fed $t=-2.41, p=0.04$; starved $t=2.25, p=0.05$)

of $3.1\% \pm 0.3\%$ in summer and $4.0\% \pm 0.1\%$ in autumn (sampled $n=80$).

Foraging tenure and fat content by season

Tschinkel (1998) demonstrated that percent fat increases in autumn across all age classes in *P. badius* colonies. In the

Fig. 8 Kaplan–Meier estimate of probability of survival in the laboratory, at each week for treatments and seasons ($n=1400$ foragers from 3–4 colonies per season, divided between starved and fed treatments). Starved foragers had a mortality rate that was more than 5 times higher than fed foragers (hazard ratio=5.6, $p<0.0000$), and there was no difference in the risk of mortality within treatments or between seasons (hazard ratio=0.93, $p=0.18$)



current study, seasonal comparisons among control foragers confirmed that proportion fat was significantly different between seasons (GLMM1, $z=2.22, p=0.027$, Fig. 9). For control colonies, fat reserves between newly added and 20-day old foragers were not significantly different (GLMM1 age $z=1.23, p=0.22$; all within season Tukey’s HSD, $p>0.53$), and varied by only 3.56 % (SD 2.1) of total body weight within each nest ($n=12$). Assuming a constant rate of loss, it would take summer-foragers approximately 49 days to reach 3.1 % body fat and die of starvation. Therefore, the residual lifespan predicted by a gradual depletion of energetic reserves, is nearly double the maximum (27-day) realized lifespan of foragers from May to October.

Foragers sampled from May–October ranged from 43 (summer-born) to more than 270 days (born the autumn prior) of age (Kwapich and Tschinkel 2013). If each were to live an additional 27 days as a forager (control rate), time spent foraging would represent 39 and 9 % of each lifespan, respectively. Therefore, residual lifespan as a forager was not predicted by fat content or chronological age at first foraging.

Reversal of body condition, not behavior

Forager fat content in controls differed significantly from panned–fed and panned–starved colonies from May to August (GLMM2, fed $z=4.91, p<0.0001$; starved $z=2.7, p=0.0069$). New foragers from both panned treatments entered the forager population with significantly or slightly more body fat than new foragers in controls, suggesting that the transition to foraging is not correlated with an absolute minimum body condition (Tukeys HSD fed $z=-4.90, p<0.0001$; starved $z=-2.70, p=0.075$, Fig. 9).

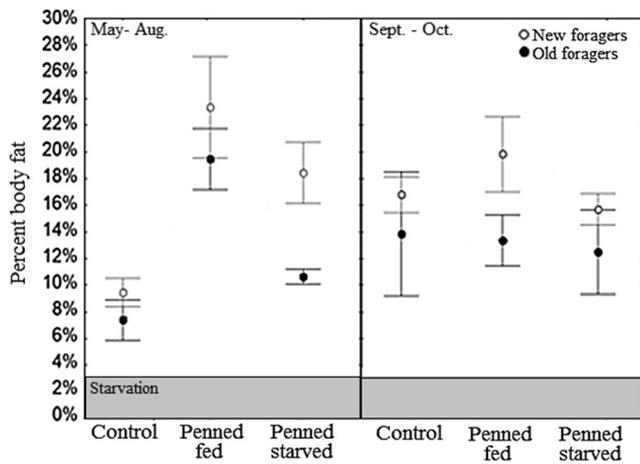


Fig. 9 Treatment means and standard errors for percent body fat, averaged within colonies for old and new foragers (585 foragers, 29 colonies). Old, penned–fed foragers had significantly more body fat than young and old foragers in controls from May to August. (Tukey’s HSD fed $z=-4.91$, $p<0.0001$), indicating that body condition is reversible. Although body condition for fed foragers improved over 20 days, they did not revert to previous labor roles

Notably, 20+-day-old foragers in the penned–fed treatment gained a significant percentage of body fat relative to old foragers in controls (Tukey’s HSD fed $z=-4.91$, $p<0.0001$); indicating that body condition is reversible and foragers will feed opportunistically. Recovered fat was as high as 36 % of total, dry body weight for a few workers in the fed–penned treatment, compared to an average of 8 % in controls. This

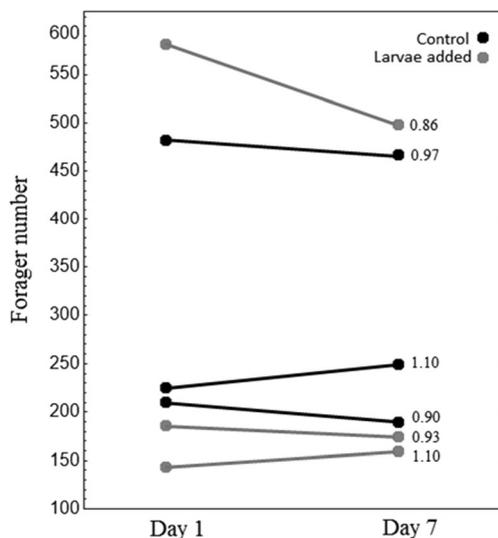


Fig. 10 Seven days after adding one additional larva per forager to three colonies (gray), no significant change in forager number was observed, and relative forager population sizes between sample dates (numbers to the right of plot points) were not significantly different from controls (black). Forager population size did not change in response to more than doubling the number of larvae in each nest

value is well within the range recorded for mid-aged workers deeper in the nest by Tschinkel (1998). Despite recovering energetic reserves, foragers remained recruitable 20 days after penning, suggesting that membership in the forager population is not reversible and not correlated with the maintenance of a minimum body condition.

Absence of behavioral reversion when larvae were added

At the expense of larval survival, forager number did not increase and foragers did not revert to interior labor roles 7 days following the experimental addition of one extra larva per forager (Fig. 10). After 7 days, there was no change in forager allocation for control colonies or colonies supplemented with extra larvae (Independent t test, $t_4=0.27$, $p=0.80$). Relative forager population size between sample dates averaged 0.99 (SD 0.10) in controls and 0.97 (SD 0.12) for larva-supplemented colonies. Excavations revealed that differences in percent larvae between paired nests were minimal, ranging from only 2 to 9 % of total colony members, and that the ratio of larvae to foragers did not differ significantly between experimental and control nests sampled on the same dates (t test on larva to forager ratio, $n=3$ pairs, $t_2=-0.04$, $p=0.97$). However, pupae were 14–16 % less abundant in two larva-supplemented colonies, than in paired controls.

During excavation, 32 % of the Rhodamine B-dyed conspecific larvae added to one experimental nest were recovered. Foreign larvae recovered after excavation still had dark pink meconium, but many other larvae had hints of pink visible in their guts. It is possible that the light pink larvae represented individuals from the natal nest that were fed foreign, conspecific larvae. The low ratio of larvae to foragers and reduction in pupae suggests that instead of redistributing labor or accessing stored seeds, both foreign and natal broods were culled following the influx of excess larvae. These findings corroborate earlier evidence that larvae do not drive forager allocation, and the strong correlation between larval and forager abundance is controlled by foragers in real time, not by stored seeds (Smith 2007; Kwapich and Tschinkel 2013).

Absence of behavioral reversion when foragers were added

Foragers did not revert to interior roles following the addition of extra foragers, suggesting that movement into the forager population is a permanent, one-way transition in *P. badius*. As in earlier studies, colonies maintained a stable forager population size over the 20 days prior to manipulation ($n=7$, relative size days 20/1 = 1.08 ± 0.07 ; Fig. 11a), and all but one pair of colonies recovered an equivalent number of foragers 30 days after 50 % were removed ($n=7$, relative size days 50/20 = 0.98 ± 0.14 , RC = 1, $t_6 = -0.13$, $p = 0.90$). One week after foragers were returned and the recovered forager

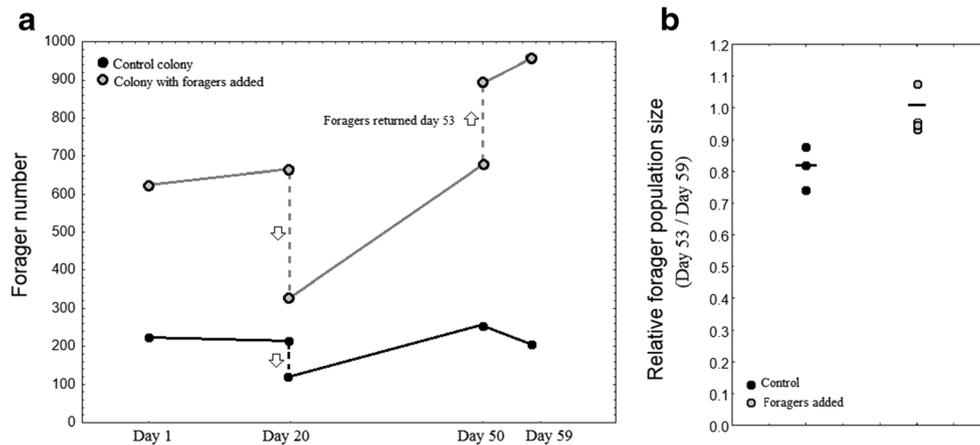


Fig. 11 **a** Representative control and forager-supplemented colonies. For seven colonies, forager mortality and replacement were monitored over 20 days, then half of each forage population was removed and retained in the laboratory. Over the next 30 days, colonies recovered their normal forager number (day 50), and surviving, retained foragers were returned to four colonies (day 53) increasing forager population to ~139 % of the current size. **b** One week later, forager number in these nests was still high

and equivalent to the experimentally increased size, i.e., approximately 38 % higher than typical. Despite excessive labor group size, foragers did not revert to interior roles once they began foraging. Meanwhile, forager number in control colonies began to decline by day 59 due to the demographic gap produced when larvae died following forager removal 39 days prior

population was enlarged by 1.39 ± 0.06 times its present size, forager number *remained* significantly higher than the pre-supplemented size ($n=4$, relative size, days 59/51 = 1.38 ± 0.07 , $RC=1$, $t_3=5.5$, $p=0.001$), and equivalent to the enlarged size (relative size, days 59/53 = 0.98 ± 0.04 , $RC=1$, $t_3=-0.67$, $p=0.55$, Fig. 11b).

Meanwhile, forager number in control colonies began to decline by day 59 ($n=3$, relative size days 59/53 = 0.81 ± 0.04 , $RC=1$, $t_2=-4.8$, $p=0.04$). The age at first foraging is approximately 43 days for workers enclosing in June–August, so this decline may be attributable to the demographic gap produced by larval death following forager removal 39 days prior. These findings demonstrate that while daily foraging activity may vary, membership in the population from which foragers are drawn is not a response to real-time changes in demand for foragers.

Discussion

Limited flexibility

Across social insect species, responsiveness to forager mortality is essential for maintaining colony size. Short-term responses may include accessing stockpiled food (Hölldobler and Wilson 1990) and employing workers whose development can be rapidly accelerated and reversed or whose behavioral repertoires expand with age (Robinson and Huang 1998; Seid and Traniello 2006). Although the connection between behavioral flexibility and colony robustness has been well studied, the benefits of limited flexibility and long-term demographic responses to mortality have seldom been explicitly tested.

Our findings demonstrate that the transition to foraging is irreversible in age-structured *P. badius* colonies, and that excessive forager mortality does not affect the intrinsic rate of development in existing workers by inducing precocious foraging. Workers become foragers at season-specific rates, and the addition of new foragers declines from summer to autumn, despite a consistent forager death rate. This inflexible strategy may benefit colonies by preventing continuous worker depletion when mortality becomes excessive and by facilitating the transition into winter dormancy (Gentry 1974; Whitford and Bryant 1979; Kwapich and Tschinkel 2013; Mersch et al. 2013). When *P. badius* foragers are removed in excess, larvae die despite the presence of thousands of stored seeds and other adult colony members, suggesting that seed processing rate and the intake of fresh insect prey (Smith 2007) may maintain the balance between worker mortality and birth rates.

Potential and realized lifespan

Among the *Pogonomyrmex* species that have been sampled, a realized forager lifespan of approximately 1 month is the rule. Gordon and Hölldobler (1987) described the disappearance of marked, exterior workers by 30 days in *P. rugosus* and *P. barbatus*, while Oettler and Johnson (2009) demonstrated that turnover in marked foragers and recruits occurred at approximately 18 and 35 days, respectively. Forager lifespan in *Pogonomyrmex owyhee* was as brief as 14 days in the field and approximated the 18-day survival of foragers starved in the laboratory (Porter and Jorgensen 1981). In light of the high risk of externally induced mortality that these foragers face (largely from lizards), the necessity of collecting high-value

seeds, and the proportionally small number of foragers, the authors suggest that the relative body reserves of foragers single them out as a “disposable caste” among other adult colony members. The current study finds that although *P. badius* foragers have the lowest relative body fat of all colony members, there is a considerable disparity between potential lifespan and realized residual lifespan, and a two-fold difference in fat content between old foragers and starved foragers.

The perception of foragers in social insects as physiologically depleted and disposable is supported by evidence of high forager turnover rates (Schmid-Hempel and Schmid-Hempel 1984), and the relative differences in the lipid reserves between nurse workers and foragers for numerous species of ants, wasps, and bees (Tschinkel 1988a; Blanchard et al. 2000). Intergeneration (i.e., social) transfer theory predicts that colonies should invest very little in individuals that will be subjected to intense extrinsic sources of mortality, and that dwindling social transfers should increase a worker's chance of entering a risky labor role (Lee 2003; Münch and Amdam 2010).

Several non-mutually exclusive hypotheses could explain why *P. badius* colonies position non-disposable workers to forage: (1) investing resources in workers that can forage twice as long as necessary may not pose a significant cost to the colony, (2) a reduction in function (learning, locomotion, navigation, etc.), or mechanical wear and tear may occur at body reserve levels above starvation; (3) body condition and potential lifespan may simply be emergent products of colony nutrition and the equal dispersion of resources (Smith 2007; Smith and Suarez 2010) (4) lifespan may be the product of a mean response to an environment that is unpredictable or conversely (5) a slow evolutionary response to increasingly powerful extrinsic mortality factors.

Finally, (6) Giraldo and Traniello (2014) suggest that the lack of programmed, demographic senescence observed among ant species may benefit colonies by increasing colony size, which is directly related to resource access and reproductive maturity. Evidence from the current study suggests that when *P. badius* foragers are opportunistically freed from extrinsic sources of mortality, increased survival promotes colony growth by inhibiting new forager development.

An integrated colony strategy

Colony size is an important predictor of reproductive maturity, and mechanisms that promote increases in colony size are expected at both the worker and colony level (Smith and Tschinkel 2006). *P. badius* employs a unidirectional strategy that promotes growth by responding both to decreases and increases in typical mortality rates. Opportunistic increases in forager longevity prevent the irreversible transitions of new workers into forager population and allow the same

group of foragers to support more larvae per capita over time. Likewise, inflexible season-specific development rates prevent new foragers from appearing if foragers die in excess and may allow colonies to avoid worker depletion during rare stretches of unfavorable conditions (Gentry 1974; Kwapich and Tschinkel 2013). For this strategy to be successful, forager mortality rates must fall within a narrow, predictable range (as observed), and catastrophic forager losses should be rare.

The evolution of a colony strategy with unidirectional flexibility suggests the environment itself is predictable and that colony life history does not require labor redistribution. For honeybees, annual fissioning and a disruption of demography are a normal part of colony life history (Robinson et al. 1992), but the multi-year strategy of a harvester ant colony does not include demographic perturbations. Likewise, *P. badius* is restricted to the subtropical, coastal plain of the southern USA, where it finds a dependable food source in an environment with ample moisture and without dramatic fluctuations in temperature. In contrast, the behavioral flexibility of the European honeybee may have contributed to its successful domestication across numerous environments with high seasonality.

Extrinsic sources of forager mortality in *P. badius* may include conspecifics from neighboring colonies, extreme ground temperatures, desiccation, navigational errors, and exposure to disease and predators. Although forager development rates do not respond to excess mortality in real time, whole colonies are capable of matching typical mortality rates. Whole colonies may also respond to excess mortality by moving the location of their nests (up to 3 times a year; Tschinkel 2015) or the location of trunk trails, all without employing workers that accelerate or revert behaviorally in response to changes in mortality.

Worker turnover and the annual cycle

In the Ant Heaven population of *P. badius*, proportional allocation to foraging follows a seasonal schedule related to three distinct phases of colony growth. (1) In the spring, overwintered workers begin foraging a month before the appearance of new worker and sexual larvae. Colony size gradually declines in the absence of worker birth, and proportional allocation to foraging climbs. (2) In mid-July, the year's first cohort of new workers enters the forager population at approximately 43 days of age. From this point, forager number becomes stable, but colony size increases rapidly; in effect reducing the proportion foraging. (3) In autumn, colonies reach their maximum annual size and proportion foraging continues to decline due to a sharp decrease in forager replacement and the abundance of young workers. In each colony, the thousands of accumulated young workers produced at the end of summer remain behaviorally static and unsclerotized. These workers do not forage until the following spring and enter a

3–4-month period of winter dormancy without ever visiting the nest's surface (Kwapich and Tschinkel 2013).

To put these patterns in perspective, a third of each colony's adult population is lost and replaced in under 30 days during the peak of summer forager allocation (Kwapich and Tschinkel 2013), and the proportion of each forager population lost per day occurs within a narrow, predictable range for six consecutive months (average 3.7 %, Fig. 2). Taking into account relative forager population size between months, proportion of foraging (Kwapich and Tschinkel 2013) and seasonal forager replacement rates, a colony with 3000 workers on May 1st will turn over roughly 1.5 times in a single calendar year as more than 4485 workers move through the forager population. This estimate is likely conservative because new workers added between sample dates may die before being sampled, and colonies have a chance to grow each autumn.

In a study of the same *P. badius* population, Smith (2007) determined that pupae eclose at a rate of 3.6 % per day during the summer months. With only 0.73 pupae per forager, and an age of 43 days at first foraging, this production rate seems too low to facilitate complete forager replacement every 27 days. Yet colonies are buffered in the summer by the accumulation of autumn-born workers that slowly enter the forager population, up to 11 months after eclosion (Kwapich and Tschinkel 2013). Together, intrinsic rate of aging and social inhibition produce patterns of labor allocation that are temporally aligned across colonies, on a schedule that corresponds with annual phases of reproduction and colony growth in a predictable environment.

Implications for laboratory and single-cohort studies

Like long-lived unitary organisms, social insect societies adhere to annual and ontogenetic schedules. Seasonally available resources are divided to promote growth through worker production and reproduction through alate production (Tschinkel 2011). The adaptive value of this colony's age structure is lost in the absence of seasonal diets, extrinsic sources of mortality, and nest architecture. Yet studies of age-correlated division of labor in soil-dwelling ant species have traditionally taken place in single chambered, two-dimensional, soil-free observation nests, often under constant thermal and nutrition conditions, all without incorporating natural forager death rates. This study confirms the incredible and unnatural longevity of workers in the laboratory relative to the field (up to 800 %) in *P. badius* (Rüppell and Kirkman 2005). If foragers are not removed at season-specific rates, it may be possible for young workers to accumulate and/or old workers to survive beyond a typical lifespan as a consequence of behavioral inhibition of development (Gordon et al. 2005). This fact may have serious consequences for interpreting data

on aging, foraging, and division of labor in a laboratory setting.

Experiments on worker behavioral development often report the outcome of removing single-age cohorts from whole colonies or examine the emergence of a division of labor from artificial single-cohort colonies (both queenless and queenright). Many studies of single cohorts demonstrate that a division of labor and extreme physiological plasticity emerge without any variation in worker age (Dolezal et al. 2012). However, such workers may not perform their jobs well, and represent a response to catastrophe, *not* a mechanism for regular replacement of labor that is specialized within seasons to match real mortality rates (Calderone 1995). Most ant colonies have a complex demography and workers are lost almost exclusively from the foraging population and not during the sequence of earlier labor roles.

In our study of *P. badius*, foragers isolated from age-structured colonies had an exceptional residual lifespan and participated in male production (Smith et al. 2007). However, it is important to separate the behavior of these isolated workers from those in age-structured, queenright colonies in the wild, where reversion did not occur when demand for foraging decreased. Exceptions may be made for species which are clonal (Bernadou et al. 2015), perform colony fission as a part of their normal lifestyle (as in the honeybee), or where age is unrelated to division of labor and risk of mortality.

Conclusions

Social insect workers are protected from external sources of mortality for the bulk of adulthood, usually do not reproduce, and may develop at rates that vary by orders of magnitude depending on season. These unusual life history characteristics are shaped by colonial living and selection for whole-colony efficiency. Although the honey bee has served as a dominant paradigm for understanding labor dynamics in social insects, it is increasingly clear that worker life history traits, including residual lifespan, are idiosyncratic (Traniello and Rosengaus 1997; Couvillon et al. 2011). While the need to balance mortality, birth, and replacement may be universal, the rules of temporal labor allocation are not fixed across species and are likely as nuanced as colony life history, diet, and predictability of extrinsic risks (Oster and Wilson 1978).

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