The Old Ladies of the Seed Harvester ant *Pogonomyrmex Rugosus*: Foraging Performed by Two Groups of Workers

Jan Oettler · Robert A. Johnson

Abstract We examined temporal polyethism in *Pogonomyrmex rugosus*, predicting a pattern of decreasing age from foragers to nest maintenance workers to individuals that were recruited to harvest a temporary food source. Nest maintenance workers were younger than foragers, as indicated by their heavier mass and lower mandibular wear. In contrast, recruited foragers were similar in mass to foragers but they displayed higher mandibular wear, suggesting that they were at least as old as foragers. Longevity estimates for marked individuals of these two latter task groups showed mixed results. Higher mandibular wear of recruited foragers suggests that they did not follow the normal sequence for temporal polyethism, but rather that they functioned as seed-millers, which should more quickly abrade their dentition. This would be the first demonstration of specialist milling individuals in a monomorphic seed-harvester ant.

Keywords Age polyethism · forager · *Pogonomyrmex rugosus* · nest maintenance workers · recruited foragers

Introduction

Division of labor is considered to be one of the cornerstones for the phenomenal success of social insects (Traniello and Rosengaus 1997). Such division of labor

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occurs via morphological differences among individuals within a colony (size polyphenism or castes) or via temporal polyethism, which involves age-related changes in tasks performed by workers independent of variation in size. Division of labor via morphological castes is uncommon given the low percentage of social insects that exhibit substantial size variation or physical castes (Wilson 1953; Hölldobler and Wilson 1990). Alternatively, temporal polyethism is common among social insects, and is best studied in honey bees (Seeley 1982; Seeley and Kolmes 1991; Robinson 1992).

In general, temporal polyethism follows the pattern of young workers performing inside the nest tasks while older workers perform outside the nest tasks such as foraging and defense, such that the most hazardous tasks are postponed until late in the life of the worker (Porter and Jorgensen 1981; Seeley 1982; Hölldobler and Wilson 1990). Physiological changes and shifts in activity of exocrine glands often accompany these behavioral changes (Hölldobler and Wilson 1990). However, this age-related progression of tasks is flexible because individuals can advance, delay, or reverse tasks in response to changing colony conditions (Robinson 1992). For example, removal of foragers results in recruitment of new foragers that are younger, while removal of brood care individuals results in foragers regressing to brood care tasks (Robinson et al. 1992). There are also exceptions to foragers being the oldest individuals in the colony, as exemplified by the leafcutter ant *Acromyrmex subterraneus* where fungus garden workers within the middens are the oldest individuals in the colony (Camargo et al. 2007).

As in most other social insects, ants in the genus *Pogonomyrmex* display age-related shifts in task allocation (Robinson 1992; Tschinkel 1999). The normal sequence for these ants involves young workers performing tasks related to brood care near the reproductive center of the nest. As the workers age, they move to the periphery of the nest and progress to nest maintenance tasks, with brief trips out of the nest, then to patrolling tasks with short forays from the nest, and finally to foraging. Several independent morphological characters correlate with this age-related shift in task: dry mass and fat content decrease with this progression, while mandibular wear increases (Porter and Jorgensen 1981; MacKay 1983; Tschinkel 1998; Smith 2007). Moreover, foragers are the oldest workers in the colony and they generally display a shorter longevity than workers from other task groups, possibly because of their lower energy reserves (Porter and Jorgensen 1981; Gordon and Hölldobler 1987). In addition, foragers in *P. barbatus*, the sister species to *P. rugosus*, have a differentially expressed *foraging* gene compared to individuals performing other tasks (Ingram et al. 2005), which might be related to different threshold responses to cues associated with this task. Foragers constitute a relatively small portion of the colony (ca 10–15% of individuals) (Golley and Gentry 1964; Rogers et al. 1972; Erickson 1972; Porter and Jorgensen 1981), but numerous reserve workers are available to harvest temporary resource surges (Rissing 1989; Gordon 1989). These reserves are presumed to consist of younger interior workers that are recruited from the nest.

This study tests the hypothesis that workers of the seed-harvester ant *Pogonomyrmex rugosus* Emery exhibit temporal polyethism. We examined three task groups (foragers, nest maintenance workers, and individuals recruited to forage) predicting that age increases as individuals move from interior to exterior nest tasks,

i.e., individuals recruited to forage are younger than nest maintenance workers who are younger than foragers. We tested this prediction by comparing age-related characters that included dry mass, mandibular wear, and longevity.

Methods

Morphometric Variation Between Task Groups

We made morphometric comparisons between nest maintenance workers and foragers and between foragers and recruits within colonies of *P. rugosus* (pure lineage strain with environmental caste determination, K.E. Anderson pers. comm.) at the Salt River Recreation Area, Maricopa County, Arizona (33° 33′N, 111° 38′W; elevation 412 m). Study colonies were chosen based on presence of a large disc mound and a trunk trail that extended >15 m from the nest entrance; mature colonies of *P. rugosus* contain about 12,000–15,000 workers (Wildermuth and Davis 1931; MacKay 1981). Nest maintenance workers consisted of individuals that carried material from the nest entrance to the refuse pile, foragers collected seeds from the distal end of the trunk trail, and recruits were individuals that foraged at the distal end of the trunk trail after baiting. Baiting involved placing a patch of seeds, tuna, and cookie crumbs across the distal end of the trunk trail on three consecutive mornings (i.e., >15 m from the nest entrance), which provided time to recruit very high numbers of workers. Recruits consisted of individuals that harvested food on the fourth morning, and thus the original foragers were a subset of our recruits.

We collected 20 nest maintenance workers and foragers from seven colonies and 100 nest maintenance workers and foragers from two additional colonies. We also collected 30 foragers and recruits from seven colonies and 100 foragers and recruits in two additional colonies. For each individual, we measured head width (only for the comparison between nest maintenance workers and foragers), dry mass, and number of teeth. Head width is a standard morphological measure that provides an index of body size (Wilson 1978) that acts as a covariate to control for overall body size. Head width was measured to the nearest 0.01 mm with an ocular micrometer at 20× using a binocular microscope. Individuals were then dried at 50–55°C for >72 h and weighed. We counted mandibular teeth under a binocular microscope; the number ranged from 0 (no obvious teeth) to 14 (a full set of unabraded teeth), with the exception that individuals were scored as 13 if they possessed 14 teeth that showed clear signs of wear. We compared our morphometric measures between each pair of task groups using a two-tailed Wilcoxon signed-rank test with colony as the replicate. We assessed within colony patterns using a *t*-test (Sokal and Rohlf 1981).

Longevity of Foragers and Recruits

We compared longevity of foragers and recruits by monitoring marked individuals in each of three colonies. Foragers (*n*=202–226 per colony) were collected and marked on the first day, and recruits (*n*=155–210 per colony) were marked on the fourth day after baiting (see above). Ants were marked on the mesosoma and gaster with

Testor's gloss enamel paint, then returned to the nest entrance after 30 min. We used a different color to mark the two task groups within each colony; individuals in each colony were also marked with different colors to minimize possible affects due to color.

We aspirated all marked foragers and recruits in each colony every 3–4 days using 10 min searches that alternated between the foraging column and areas near the nest entrance. Searches continued until no marked ants were observed in a 10 min period. Following the capture period, all marked individuals were counted, then released near the nest entrance. Due to rapidly declining numbers of marked individuals, colonies were again baited after 20 days in order to recruit all possible individuals to the surface. After 38–39 days we partially excavated two of the colonies (colonies 2 and 3) to assess if marked individuals occurred within the colony. Survival of foragers and recruits was compared within each colony using a general linear models technique with task group as the treatment and time as a covariate. The dependent variable was percent of originally marked individuals that were recaptured at each interval (ln transformed; SPSS 1990). The analysis standardized survival to the first day that each task group was marked.

Results

Morphometric Variation Between Task Groups

Head width was similar for nest maintenance workers and foragers of *P. rugosus* (two-tailed Wilcoxon signed-rank test, $n=9$ colonies, 538 workers, $P>0.20$; Fig. 1a), indicating that the two task groups consisted of similarly sized individuals. In contrast, dry mass ($P<0.005$; Fig. 1b) and number of teeth ($P<0.02$; Fig. 1c) were significantly higher for nest maintenance workers than for foragers. Differences also occurred within colonies as nest maintenance workers were significantly heavier than foragers in five colonies and possessed a significantly higher number of teeth in four colonies (*t*-test, $P<0.05$; Fig. 1).

Foragers and recruits also displayed significant morphometric variation, but in a pattern different from that predicted by temporal polyethism. Across all colonies, foragers and recruits were similar in mass (two-tailed Wilcoxon signed-rank test, $n=$ 9 colonies with 820 workers, $P>0.20$), but foragers possessed significantly more teeth than recruits ($P<0.04$; Fig. 2). Differences in tooth wear extended to individual colonies as foragers had significantly more teeth than recruits within five of the nine colonies (*t*-test, $P<0.05$). The only colony in which number of teeth was higher for recruits than for foragers (colony 9) involved a case in which colonies of other seed-harvesting ants (*Solenopsis xyloni* and *Pheidole tusconia*) appeared to actively interfere with recruitment by *P. rugosus*.

Longevity of Foragers and Recruits

Longevity of workers and recruits showed an inconsistent pattern across the three colonies. Longevity was significantly higher for recruits than for foragers in two colonies (colony 2: $R^2=0.82$, $F_{1,15df}=13.1$, $P=0.003$; colony 3: $R^2=0.86$, $F_{1,15df}=$
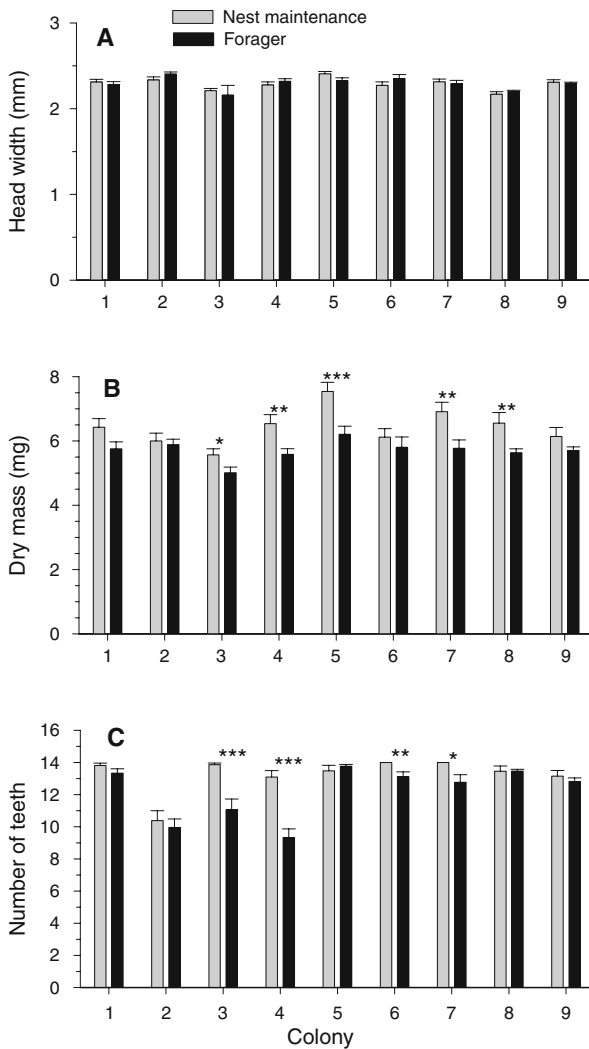
Fig. 1 Head width (**a**) dry mass (**b**) and number of teeth (**c**) for nest maintenance workers and foragers from nine colonies of *Pogonomyrmex rugosus*. Values are presented as means±SE; $n=20$ individuals per group for colonies 1–7, and $n=100$ per group for colonies 8–9. Head width was similar for nest maintenance workers and foragers across colonies (Wilcoxon signed-rank test, $n=9$, $P>0.20$). However, dry mass ($P<0.005$) and number of teeth ($P<0.02$) were significantly higher for nest maintenance workers than for foragers. *Asterisks* denote significant differences within colonies based on a *t*-test; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

19.6, $P<0.001$), while the two task groups displayed a similar longevity in the third colony (colony 1: $R^2=0.93$, $F_{1,17df}=0.34$, $P>0.50$). The second baiting after 20 days resulted in a slight increase in number of recruits in two of the colonies, but did not affect the number of foragers. Our partial colony excavations resulted in finding several marked recruits (four and five in colonies 2 and 3, respectively), but we did not locate any marked foragers (Fig. 3).
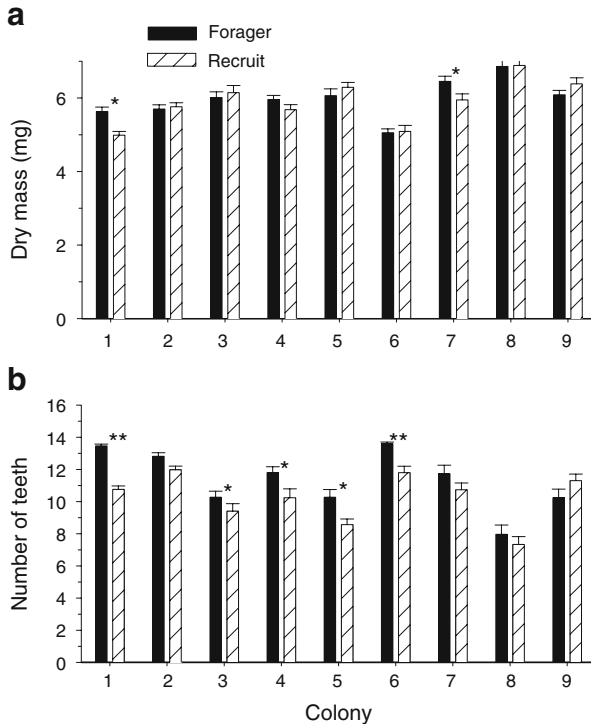
Fig. 2 Dry mass (**a**) and number of teeth (**b**) for foragers and recruits from nine colonies of *Pogonomyrmex rugosus*. Values are presented as means±SE; $n=30$ individuals per group for colonies 1–7, and $n=100$ per group for colonies 8–9. Dry mass was similar for foragers and recruit across colonies (Wilcoxon signed-rank test, $n=9$, $P>0.20$), but foragers possessed significantly more teeth ($P<0.04$) than recruits. *Asterisks* denote significant differences within colonies based on a *t*-test; $*P<0.05$; $**P<0.01$.

Discussion

Temporal Polyethism in *P. rugosus*

Mandibular wear is a good predictor for age on a large scale (young versus old) and task performance (within old ants). The higher body mass and lower mandibular wear for nest maintenance workers compared to foragers supported the hypothesis that nest maintenance tasks are performed by younger ants (see also Porter and Jorgensen 1981, MacKay 1983, Tschinkel 1998) that will soon become foragers. However, our comparison of foragers and recruits demonstrate that the shift from interior to exterior worker does not always follow a simple age-related progression in *P. rugosus*. It appears that the foraging labor is divided in specialists and non-specialists. The similar body mass for foragers and recruits suggests that these two task groups were similar in age, while the increased mandibular wear suggests that recruits were either older than foragers or specialized to perform tasks that resulted in abraded mandibles. Overall, differences in mandibular wear between foragers and recruits were very robust given that the effect occurred across eight of nine colonies with the increased wear being significantly higher for recruits than for foragers in
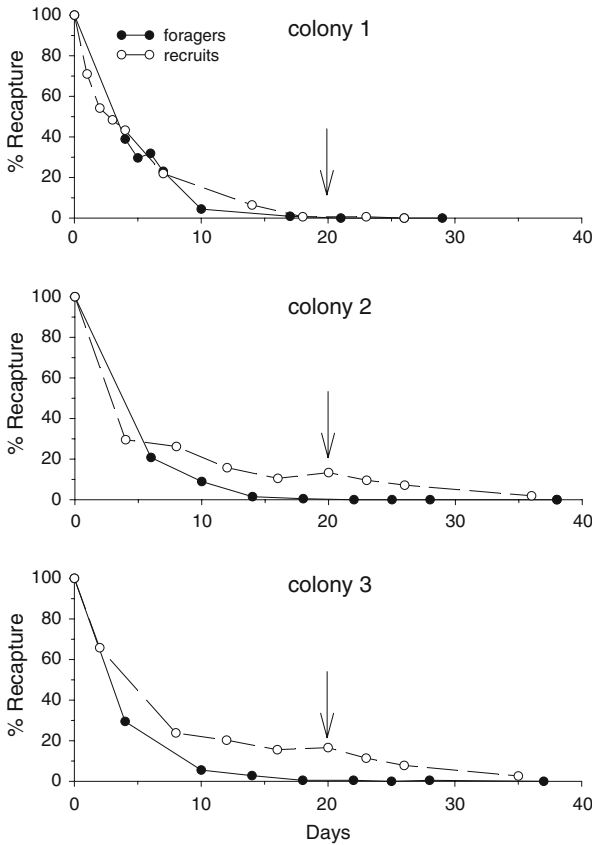
Fig. 3 Longevity of marked foragers and recruits in three colonies of *Pogonomyrmex rugosus*. Data are shown as the proportion of originally marked foragers and recruits that were recaptured at each interval. The arrow denotes the day at which bait was offered to colonies for a second time. See text for number of marked individuals in each colony. Recruits survived a similar length of time in colony 1, but they survived significantly longer than foragers in colonies 2 and 3 (see text).

five of the eight colonies (Fig. 2). The only colony in which recruits had more teeth than foragers involved the colony in which competitors appeared to interfere with foraging and recruitment (Hölldobler 1976).

In a similar study, Gordon (1989) suggested that recruited foragers were derived from task groups that included patrollers, nest maintenance workers and midden workers. Gordon (1989) also indicated that the total number of patrollers, midden workers, and nest maintenance workers is low compared to the total number of foragers, such that these individuals are insufficient to cause the increased mandibular wear that we observed for recruits. This problem is heightened by the fact that nest maintenance workers, and perhaps others, are younger and have a less abraded dentition than foragers. Moreover, even though individuals from these three task groups might switch to foraging, they can not account for the increased mandibular wear exhibited by recruits, especially given that our observed differences are conservative because the original foragers

comprised a subset of the recruits. To achieve such a strong effect necessitates that colonies of *P. rugosus* contain a large reserve of interior workers that can shift tasks to take advantage of ephemeral food resources (Rissing 1989).

One explanation for the increased mandibular wear exhibited by recruits is that these individuals remained in the nest and functioned as seed-millers, which is likely to more quickly abrade their dentition. Such milling specialists are common in seed-harvester ants in the genus *Pheidole*, in which majors perform the task of milling, but they are also sometimes recruited out of the nest in the presence of abundant food resources. A milling task group is not known to occur in monomorphic seed-harvester ants, but other types of task specialization occur when food resources are plentiful. For example, foragers of *Messor pergandei* that harvested seeds from a large seed patch often stopped at the nest entrance (without entering the nest) and dropped their seed into the nest (R.A. Johnson, pers. obs.). From there, other apparent specialists picked up and removed the seed to lower nest chambers. Such task variation might be correlated with patriline, as occurs for foraging behavior in honey bees (Robinson and Page 1989), given that *P. rugosus* queens mate with multiple males (Gadau et al. 2003).

Age and Survival and Changes in Task Preference

Longevity of foragers and recruits varied across the three colonies, with recruits living up to several days longer than foragers in two colonies, but for a similar length of time in the third colony. One explanation for such longevity differences is that foragers retreated to interior tasks more quickly than recruits. The fact that the second baiting resulted in an increased number of recruits but not foragers, and that we located several recruits but no foragers in the two partially excavated colonies negates this possibility. Similar to *P. barbatus* (Gordon 1989), we observed foragers and newly recruited foragers displaying high task fidelity, which suggests that the two groups did not shift tasks at different rates, i.e., a proportion of both task groups switched to nest maintenance (seed husk removal) in response to food intake and switched back to foraging after the second baiting (data not shown). Overall, our longevities were similar to the approximately 35 days that marked foragers survived for *P. barbatus* (Gordon and Hölldobler 1987).

Our data showing that recruits are more long-lived than foragers (in two of the three colonies) contradicts our mandibular wear data. Applying a reliable method to age these individuals could resolve this paradox in the future (i.e., Newey et al. 2008). Regardless of whether recruits are similar in age or younger than foragers, it remains that recruitable interior workers have experienced a different task history that has increased their mandibular wear relative to foragers.

Benefits of Mass Recruitment

Foraging efficiency in seed harvester ants increases with individual learning and memory of handling seeds (Johnson 1991) but we also regularly observe erroneous gathering of non-food items (C. Smith, pers. com.) and nest-bound trips without food (J. Oettler, pers. obs.) after mass recruitment to a bait. Whether this is linked to behavioral differences of experienced foragers and less experienced recruits is

unclear, but we assume that recruited ants harvest seeds less efficiently. On the contrary, intraspecific competition and colony density in the trunk trail foraging *P. rugosus* is high (Hölldobler 1976), and only one colony remains to exploit a food patch when two colonies encounter the food initially. Consequently, the sheer numbers of ants that are recruited to a food patch ultimately increase foraging success via numerical dominance.

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