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Research article

An experimental study of colony-founding in pine saplings by queens of the arboreal ant, *Crematogaster ashmeadi*

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Summary. Newly mated queens of the arboreal ant *Crematogaster ashmeadi* initiate colonies in old beetle galleries in the dead branches of longleaf pine trees. In a study by Hahn (1996), a number of tree characteristics were correlated with the number of newly-mated queens in those trees, with branch length the best indicator of queen presence. Three of these characteristics, tree height, dead branch length, and the number of dead branches were tested in an experiment to see which, if any, the queens were using to choose a tree. Both tree height and the number of dead branches significantly influenced queen choice: shorter trees (4–5 m) had more queens than tall ones (7–9 m), and trees with 8 branches had significantly more queens than trees with 2 branches. Branch length had no effect on the number of queens. These findings suggest that newly mated *Crematogaster ashmeadi* queens search for founding trees on the basis of the height of a sapling and its number of dead branches. Modes of searching are discussed.

Key words: Pinus palustris, *Picioides borealis*, haplometrosis, forest entomology, Formicidae.

Introduction

Crematogaster ashmeadi is the dominant ant species of the arboreal ant community inhabiting the longleaf pine forests of the southeastern United States (Tschinkel and Hess, in press). It shares this forest with the endangered red-cockaded woodpecker for whom it serves as a primary adult food. The largest population of this woodpecker is found in the Apalachicola National Forest near Tallahassee, Florida (Hess and James, 1995), making knowledge of the biology of *C. ashmeadi* of potential importance to the conservation of the red-cockaded woodpecker.

As in the majority of ants, newly mated queens of *C. ashmeadi* start new colonies without the aid of workers, drawing on metabolic reserves stored in their bodies (independent founding) (Hahn and Tschinkel, 1997). Mating and colony founding of *C. ashmeadi* occur between June and August (Deyrup and Trager, 1986). Newly mated *C. ashmeadi* queens initiate colonies in the dead branches of the longleaf pine saplings, using abandoned beetle galleries as an initial nest chamber and requiring about one month to produce minim workers (Hahn and Tschinkel, 1997). As the tree grows, the colony grows with it and the bark thickens. The colony eventually takes up residence in vacated chambers made by termites or wood-boring caterpillars within the bark of the trunk (unpubl. data). Because the ants never leave their tree even to forage, each tree is a habitat island (Tschinkel, unpubl.).

Hahn (1996) demonstrated that a number of tree characteristics, including tree height, dead branch length, and the number of dead branches were positively correlated with the number of newly-mated queens that settled in a tree. The strongest correlation was with branch length, and Hahn hypothesized that once queens find a cluster of saplings, they search hierarchically first for tall trees, then for those with the longest branches.

Because tree height, branch length, and branch number are all correlated with tree age and size and with each other (older trees are taller and have more dead branches), a manipulative study was needed to determine which trait(s) the queens used to choose their founding tree. Therefore, we varied each of these tree characters in a factorial design and quantified subsequent queen founding decisions.

Methods and sites

All manipulations were conducted at one study site in Compartment 222 of the Munson Sandhills of the Apalachicola National Forest 11 km southeast of Tallahassee, Florida. This forest is characterized by an overstory of longleaf pine (*Pinus palustris*) interspersed with deciduous

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clonal oaks, such as turkey oak (*Quercus laevis*) and bluejack oak (*Q. incana*), and a ground cover consisting of perennial grasses, especially wiregrass (*Aristida stricta*) and a few woody shrubs (Meyers and Ewel, 1992).

Setting up the experiment: Six areas, or clusters of saplings, were chosen within the study site. Beginning in early May, 1996, 186 saplings were classified into one of two height categories – short (4–5.5 m) or tall (7–9 m). This included 24 extra trees in case of loss or destruction of the experimental trees. Each tree was labeled with an aluminum tag identifying the tree by number (1–186) and area (1–6). Because tree height was a selected, not a manipulated variable, each tree was randomly assigned to one of the four treatment groups within tree height (see below).

As the first step in setting up the experiment, all dead branches were removed from the saplings. This was done to remove any pre-existing *C. ashmeadi* colonies which might otherwise interfere with the subsequent colony-founding attempts by newly-mated queens, and to assure that the only dead branches on the trees were those experimentally reattached in a particular design.

Dead branches (those having brown or no needles) were cut from trees in a longleaf pine plantation outside the study areas. No fallen branches were used so that the branches were all approximately the same age and state of decomposition. Each branch was checked for pre-existing colonies and only branches with 1–2 woodboring beetle exit holes were used.

The experimental treatments were tree height (tall, short); branch length (20 cm, 80 cm); branch number (2, 8) in a crossed design. This yielded a total of 8 treatment groups, each with approximately 20 trees, or replicates.

The collected dead branches were then cut to either 20 cm or 80 cm in length. Either 2 or 8 of these branches were attached to each experimental tree according to the assigned treatment group. To attach the branches, a headless nail was driven into the tree, a branch was pushed onto the nail and supported with a guy wire from the branch to the trunk. Branches were attached at two heights on the trees (125 cm and 155 cm from the ground), with either one or four branches per height, such that half the branches on a tree were at each level. A total of 930 branches were attached and were monitored throughout the summer to ensure they were still on the trees.

In order to detect existing colonies of ants on experimental trees, the trees were baited immediately after setup of the experiment (late June, 1996), and again at the end of the experiment (mid-August, 1996). Baiting consisted of dipping a small piece of filter paper into a slurry of two parts Alpo Ocean Whitefish Treat cat food to one part water and 1/2 part vegetable oil, and then placing it on the north and south sides of the saplings at approximately breast height. Baits were checked after 20–30 minutes and the number of ants foraging on the baits was recorded by species.

Evaluating the experiment: The branches from 167 trees were retrieved during the last week of August 1996. Founding queens were detected by peeling off all bark to expose founding galleries and by splitting the branch to expose queens in the pith (Hahn and Tschinkel, 1997). The following data were recorded for each retrieved branch: the tree identification number, treatment type, diameter of the branch at base (cm), visual estimate of the percentages of subcortical gallerying and pith removal by wood-boring beetles, the number of *C. ashmeadi* founding queens and whether they had brood, and the presence of any preexisting colony with adult workers. Mortality of the queens was not recorded because the recollected branches were kept in the cold room until they could be processed, and whether queens died from cold exposure or were dead in the field could not be determined with confidence.

Exploratory data analysis was used to determine relationships between queens and tree characteristics. Data were analyzed using log-linear analysis and the generalized linear models procedure in S-plus (Version 3.4) because the data were non-normal; i.e., many trees and branches had no queens. These procedures were run first on the full model, followed by the elimination of characters until the best fit was achieved. One of the six areas was dropped from the analysis, because only two of ten trees were recovered. Chi-square tests were also used where appropriate.

Results

Distribution of colony-founding queens: A total of 257 queens was found distributed among 96 trees (57 %), with 41 (25 %) of those trees having one queen, and with the extreme values of 8–12 queens in four trees. Of the 930 branches used in the study, 187 branches (23%) were found to contain queens. One queen was found in 144 of those branches, and one branch was found with six queens. Queens were never found together in chambers, confirming that this species is entirely haplometrotic.

Preliminary analysis showed that there was no significant difference in the relationship of treatment to queen number in the five areas (glm: $F_{4,27} = 0.85$, $p = 0.54$) allowing us to pool the data. When percent pith removed was used as a covariate, it did not have a significant effect on the number of queens per tree ($F_{4,27} = 0.15$, $p = 0.70$).

The number of queens was prorated so that each cell of the $2 \times 2 \times 2$ table represented the number of queens in 20 trees (actual n varied from 18 to 23). The effect of the three experimental factors on the number of queens was then tested with a log-linear analysis. Branch number had the greatest effect: approximately three times as many queens were found on trees with eight branches than those with two branches (partial association $X^2 = 75.73$; $p < 0.000001$) (Fig. 1). Tree height had a significant but smaller effect on the number of queens per tree (partial association $X^2 = 7.09$; $p < 0.008$). Short trees averaged about 40% more queens than did tall ones (Fig. 1). Surprisingly, branch length had no significant effect on queen number (partial association $X^2 = 0.144$; $p > 0.5$). However, the lower number of queens in long branches on tall trees as compared with long branches on short trees gave rise to a small but significant interaction (partial association $X^2 = 4.99$; $p < 0.05$) (Fig. 1).

If each unit of dead branch length represented an equal founding opportunity, queen number should simply be proportional to the total (summed) dead branch length. Trees with two 80 cm branches or eight 20 cm branches both have

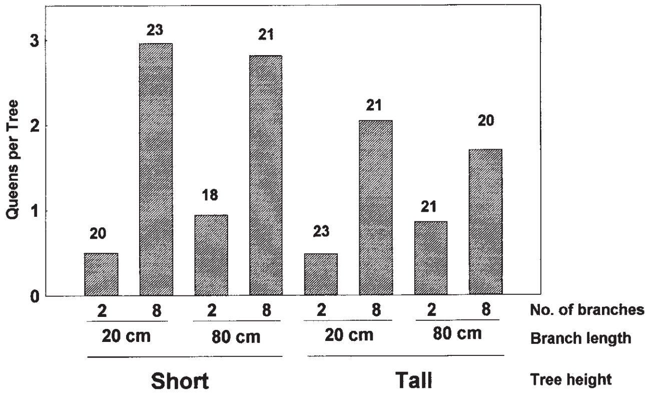

Figure 1. Queen number in relation to all treatments. The manipulated experimental treatments were branch length (20 cm, 80 cm) and number of branches (2, 8). Tree height (tall, short) was selected. Treatments were crossed, with approximately 20 trees in each treatment. The number above each bar represents the sample size for each treatment

Table 1. Queen number in relation to total branch length. Although two treatments have the same total branch length (160 cm), trees with 8 branches have more than twice as many queens as trees with only 2 branches. Queen number was adjusted to an equal sample of 40 trees in all treatments. Tall and short trees were pooled for this table

Branch length	20 cm		80 cm	
Branch number	2	8	2	8
Total branch length	40 cm	160 cm	160 cm	640 cm
Queens per 40 trees	20	101	36	91
N (trees)	43	44	39	41

160 cm total branch length and might be expected to attract similar numbers of queens. However, this was not the case (Table 1). Furthermore, the number of queens on trees with two 20 cm branches (40 cm total branch length) and two 80 cm branches (160 cm total branch length) was fairly similar, and the number of queens on trees with eight 20 cm branches (160 cm total branch length) and eight 80 cm branches (640 cm total branch length) were also fairly similar (Table 1), despite a four-fold difference in total branch length in both cases. Trees with 8 branches consistently had 3–5 times more queens than trees with 2 branches, regardless of whether those branches were long or short.

Baiting and adult worker presence: Of the 167 experimental trees, 37 trees (22%) showed no ants on the baits at either sampling period. Of the remaining 130 trees, 34 trees (26%) had only *Crematogaster ashmeadi*, 10 trees (8%) had both *C. ashmeadi* and various ground-nesting species such as *Camponotus nearcticus, Pheidole anastasii,* and *Solenopsis invicta* at the same time, 7 trees (5%) had either *C. ashmeadi* or terrestrial ants, but at different baitings, and 79 (61%) trees showed only terrestrial ants. The number of trees with *C. ashmeadi* was significantly greater at both baitings for tall trees than for short trees ($X^2 = 13.15$, df = 1). Terrestrial ants were distributed equally between trees of both heights ($X^2 = 0.317$, df = 1).

Colonies or colony fragments of *C. ashmeadi* were also found in some branches on 15 of the trees during processing at the end of the experiment. Workers of only 11 of these were detected by baiting. Seven of these worker-containing trees had no founding queens, but eight did. Of these eight, four had one founding queen on the same branch with an active colony (but in a different chamber), and four had 2–10 queens, but on different branches than those with colonies.

The presence of founding queens on trees was not affected by the presence of *C. ashmeadi* workers on those same trees ($X^2 = 0.384$, df = 1) as detected by baiting, nor was the number of queens affected ($X^2 = 3.257$, df = 5).

Discussion

Cavities suitable for colony founding are associated with pine saplings and, like the nest cavities used by *Leptothorax* ants (Herbers, 1989), occur nonrandomly throughout the forest. It is probably most effective for insects to use a hierarchical search for such resources (Bell, 1990; Baader, 1996; Collett and Baron, 1994; Seeley and Morse, 1978), using larger landmarks or features from a distance and then smaller and smaller features within features as the goal is approached (Tinbergen, 1932). According to Hahn and Tschinkel (1997), it seems probable that newly-mated queens first search for a clump of saplings, then for a sapling within the clump offering suitable founding conditions, based on some tree attribute(s). They may also inspect the available cavities directly, much as honeybees do before moving to a new site (Seeley, 1985). Because several tree traits are correlated (r between 0.16 and 0.87; Hahn and Tschinkel, 1997), queens may use surrogate traits that predict founding opportunity. Founding opportunities can be estimated as the total cm of subcortical and pith galleries. This variable correlated well with the number of dead branches ($r = 0.62$), suggesting that a sapling with more dead branches should offer a higher chance of finding a suitable founding chamber, as well as providing additional chambers (the other branches) into which the colony can expand as it grows. However, in Hahn's study, branch length predicted founding opportunity even better ($r = 0.70$) (recalculated from Hahn, 1996), and it is surprising that our experiment showed that queens clearly base their choice on branch number while ignoring branch length.

How can these findings be reconciled? It is not obvious why the same total dead branch length would not predict founding opportunity equally as well whether divided among more shorter branches or fewer longer ones. Perhaps predator avoidance plays a role (red-cockaded woodpeckers seek out founding queens in dead branches of saplings), or branch number predicts some future favorable tree characteristic, or dead branches drop from trees in a relatively short time so that multiple dead branches provide some important security. Perhaps the contradictory outcomes are the result of our study being an experimental one, and Hahn and Tschinkel's (1997) being a correlational one with relatively low proportions of explained variance. Other differences between these studies were: (1) we partly uncoupled the tree traits so that they were less strongly correlated. For example, the correlation between queen number and founding opportunity was 0.54 in Hahn's study (1996), but only 0.27 in ours. Each branch on an experimental tree was likely to have originally come from a different source tree so that the branches on an experimental tree may not resemble each other the way they did on the source tree. (2) The majority of the saplings in our experimental areas were part of our study, forcing queens to choose mostly from unnatural trees with either short or long, few or many branches. However, it is not obvious how this might bring about the observed outcome. (3) Two to six branches (modal class 2–3) on longleaf pine saplings often grow at the same height, forming a whorl on the tree. Whorls of 4 branches make up less than 10% of the cases (unpublished data). Our experimental trees, each with 2 whorls of 4 branches, may have acted as a "supernormal stimulus" and overwhelmed the effect of branch length on choice. Approximately 50% of our study contained these "unnatural" trees with eight branches.

It should be noted that the branch placement on tall trees was unnatural, between 1.5 and 2 m above ground, whereas natural dead branches were usually at mid-height or higher. With our current knowledge, it is not possible to determine whether this influenced queen choice of tall trees.

Do queens first land on a tree before assessing its suitability or do they make this assessment from the air before landing? If queens search visually, what might be the nature of their search image? Our results suggest that the complex mid-trunk silhouette of trees with many-branched whorls may best fit a search image based on radial patterns. Bees are able to detect radial symmetry (Free, 1970), and they also show strong preferences for certain complex patterns (von Frisch, 1993; Hertz, 1930). Queens could also have a preference for shorter trees because these are less likely to have been previously colonized.

Having landed on a sapling, the foundress may then search for other cues to make her final choice. Jander (1990) found that ants search differently on branches than on the ground. When an ant arrives at each branch point, she always makes the same choice of direction (right or left or up or down), thus covering the entire branch in a systematic manner. Although this is generally employed in a search for food, queens may also use this method for an efficient search of branches for colony founding opportunities.

Ants make decisions about continuing to forage on a plant by associating the quality of the first inflorescence they visit with the rest of the plant (Ganeshaiah and Veena, 1988). Similarly, *C. ashmeadi* queens may visit one branch and make a decision on whether to stay on that tree or leave by associating the quality of the first branch with the quality of the tree as a whole. This might account for the lower correlation between queen number and founding opportunity in our experiment than in Hahn's correlational study.

Up to one-third of all the trees had *C. ashmeadi* workers on the baits, even though all pre-existing colonies were thought to have been removed from the trees with the original dead branches. The most likely source of workers is probably from undetected colonies or colony fragments in the transplanted experimental branches. It is also possible we were not able to eliminate all colonies by cutting off all natural dead branches. In any case, although baiting did not always detect the presence of ants, the presence of workers had no effect on the settlement of founding queens, either in our study or in Hahn's (1996). This is somewhat surprising because *C. ashmeadi* is very aggressive, both against members of other *C. ashmeadi* colonies and against other species of ants (Tschinkel, unpubl. obs.). Perhaps the resident workers had not yet detected the queens, or perhaps they reserve their aggression for incipient colonies, not queens.

The presence of the brood gives insight into the timing of mating flights. Samples of branches in June contained newly-mated queens without brood, indicating recent mating flights. In contrast, branches collected in August contained queens with minim workers, queens with larvae and pupae only, and queens without brood. Brood development takes about a month. Altogether, this confirmed that mating flights occurred throughout the summer, as reported by Hahn (1996) and Deyrup and Trager (1986).

Finally, even though the effect of the number of dead branches and tree height were highly significant, much of the variance in queen number remained unexplained. This suggests that other biological and physical factors remain to be discovered.

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