An Experimental Study of Pleometrotic Colony Founding in the Fire Ant, Solenopsis invicta: What Is the Basis for Association?
Author(s): Walter R. Tschinkel
Source: *Behavioral Ecology and Sociobiology*, Vol. 43, No. 4/5 (Oct., 1998), pp. 247-257
Published by: Springer
Stable URL: http://www.jstor.org/stable/4601515
Accessed: 03-11-2015 17:28 UTC

Behav Ecol Sociobiol (1998) 43:247–257

Original article

Walter R. Tschinkel

An experimental study of pleometrotic colony founding in the fire ant, *Solenopsis invicta*: what is the basis for association?

Received: 20 November 1997 / Accepted after revision: 14 March 1998

Abstract Many benefits and risks of cooperative colony founding (pleometrosis) have been identified, but rarely have the proximate factors that lead to association been considered. This study examined the choices queens make during the first few hours after mating, and some of the correlates of those choices. Queens had a strong affinity for preformed holes in the soil and readily used these as their initial founding chambers. This affinity was so strong that in a field experiment, the dispersion pattern of preformed holes controlled the final dispersion of colony-founding queens. Attraction to partially formed holes is thus an important cause of pleometrosis. The excavation of complete founding chambers incurred no measurable cost on the subsequent reproductive output of queens, suggesting that the primary benefit of using preformed holes is to remove the queen quickly from exposure to predation and desiccation. In the field, pairs of queens offered five equivalent preformed holes in soil were more likely to share the same hole if the holes were shallow and close together. In these experiments, queens modified preformed soil holes so that the test holes were no longer equivalent, causing the choice of queen and hole to become confounded. Laboratory experiments in plaster arenas with unmodifiable holes confirmed the field experiments: queens were more likely to share a hole when the holes were shallow than when they were deep. Because queens entering adequately deep holes seldom reemerged, this suggested that the likelihood of sharing increased with increasing contact between queens, that is, when queens were readily and frequently detected. Such contacts will also predict the future competitive environments to be experienced by incipient colonies, and may temper the tendency of queens to associate. However, experiments in which queens were exposed to high and low densities before pairing in the choice arenas failed to show an effect on the choice to join the resident queen. Queens that joined a resident queen differed in their robustness from queens that did not join. Queens choosing their own partners did no better reproductively than those assigned partners at random. Overall, this study suggests that (1) newly mated queens are under strong selection to leave the soil surface and do so by using any available holes, whether dug by another queen or of some other origin; (2) they are attracted to other queens, and are more likely to cofound as contact with the potential cofoundress becomes more frequent and (3) they choose whether or not to cofound partly on the basis of their own reproductive characteristics.

Key words Formicidae · Cooperation · Reproductive success · Cofounding

Introduction

Colonies of many ant species are founded by newly mated queens who rear the first brood of workers, in isolation, from body reserves. In a wide variety of species, such independent, claustral foundresses may associate into cooperative groups which share brood care through the founding period (pleometrosis) (Hölldobler and Wilson 1977, 1990). Such cooperative founding is the norm for many wasp species (Strassmann 1989). Generally, the benefits of pleometrotic over haplometrotic (solo) founding include one or more of the following: greater brood production, improved survival of the founding period, greater success during brood raiding of neighboring incipient colonies, improved group defense against workers from mature colonies (Bartz and Hölldobler 1982; Tschinkel and Howard 1983; Rissing and Pollock 1986, 1987; Mintzer 1987; Tschinkel 1992a,b, 1993a; Jerome et al., in press).

W.R. Tschinkel (✉)
Department of Biological Science, Florida State University,
Tallahassee, FL 32306-4370, USA
e-mail: tschinkel@bio.fsu.edu, Tel.: +1-850-6444489,
Fax: +1-850-6440481

Counterbalancing these mutualistic advantages is the cost of competition within the group of queens for ultimate survivorship. In spite of the identification of several relevant factors, the balance sheet of credits and debits has not been completed for any species, and the relative importance of each component is unknown. When mature colonies are monogyne, all but one queen are killed or expelled, either by queen fighting or by the workers (Mintzer 1979; Bartz and Hölldobler 1982; Tschinkel and Howard 1983; Rissing and Pollock 1986, 1987; Balas and Adams 1996). Nonacs (1989, 1992) has addressed the competitive and mutualistic factors that should shape the choices queens make in joining other queens. He showed that when forced to make a choice, queens chose to join lighter queens that were likely to be less keen competitors in the future.

Most accounts imply that joining with other queens results from attraction among *queens*. In some cases, however, it seems more likely that the attraction is not to the other queen, but to the burrow in which she resides. For example, Pfennig (1995) found no fitness benefit from pleometrosis in *Veromessor pergandei*, and suggested that rapid entry of an existing burrow reduces predation mortality and water loss. If queens are strongly attracted to existing burrows, the clumping of queens in burrows (Tschinkel and Howard 1983; Rissing and Johnson 1986) may not be the result of queens preferring to cofound with other queens, but simply the outcome of several queens preferring the same burrow. If this were true, the literature on pleometrosis might need to be reinterpreted. In reality, this literature offers little information on the proximate factors which cause queens to join other queens. In this paper, I tested the relative attractiveness of holes versus queens, the cost of excavating a nest chamber, and the reproductive attributes of joining and non-joining queens.

Methods

Collection of newly-mated queens

Mating flights of *Solenopsis invicta* take place on warm days after heavy rains during the months of May, June, and July. Between about 1:00 p.m. and 4:00 p.m. on such days, newly mated fire ant queens were collected in and around Tallahassee, Florida. All field experiments were initiated before sundown of the same day and, when necessary, were checked the next day. Most laboratory experiments were set up on the same day, but in a few cases, queens were stored at 4 °C for up to 3 days.

Field experiments

Two field sites were used. The first was a field that had lain fallow for several years, parts of which were cleared of vegetation in preparation for the experiments. The second was a construction site which had been stripped of topsoil several years earlier, and which supported only sparse, weedy vegetation separated by large areas of bare soil. Experiments were laid out in these barren areas.

Arenas were fashioned by sawing 11.5-cm PVC pipe into 10-cm-long sections. The insides of these rings were painted with Fluon (Northeast Chemical Co., Woonsocket, R.I.) to prevent queen escape and pressed into contact with the soil. From 50 to 100 such rings were placed in closely spaced arrays to reduce the effect of spatial variation in conditions. Within each ring, either two or five evenly spaced 3-mm-diameter holes were made in the soil with a metal rod. Newly mated queens placed into these arenas could choose among the holes and or other queens, and generally disappeared belowground within a short time. Their final choice was usually assessed the next day by noting which of the offered holes was plugged from below with soil. In experiments with two queens, two soil plugs in different holes indicated that the queens resided separately. All other cases were assessed by excavating the holes to locate the queen(s). The specific conditions of hole depth and spacing are noted in the results.

Laboratory experiments

Laboratory experiments were carried out in arenas fashioned from 9-oz plastic drinking cups (top diameter = 9 cm, bottom diameter = 5 cm, height = 7 cm), the bottom of which contained a layer of orthodontal plaster with five evenly spaced, drilled holes. The holes were 3 mm in diameter, and either 0.5 cm deep (shallow treatment), or 3 cm deep (deep treatment). Prior to experiments, the plaster bottoms were removed and soaked in water, the sides of the cups were talced to prevent queen escape, and the damp bottoms returned to the cups. The cups were placed in arrays of 50 or 100 in a room with fairly even overhead fluorescent light. Queens were released in the centers of these arenas either singly, or in pairs, either first held until calm within a small fluoned ring, or simply dropped into the arena. They were allowed to choose a hole and enter it, something they almost invariably did quickly. Occupancy of holes was noted after one to several hours, or overnight.

Data analysis

In two-queen experiments, pairing of queens was tested against an expected fraction of 0.5 for two-hole experiments, and 0.2 for five-hole experiments, using either the chi-square or the binomial test. All experiments were also checked for directional preference by the queens, which could influence the rate of pairing. No bias was found in field experiments. Bias of laboratory arenas is discussed below.

Results

Post-settlement exploration by newly-mated queens

After mating, each queen alighted on the ground and explored a limited area on foot. Typically, she broke off her wings within seconds or minutes of landing, but in rare cases, she took wing again. Exploration usually involved a looping walk, during which the queen inspected any depressions, holes, and shelter she encountered (Fig. 1). Eventually, she began digging, either enlarging or modifying an existing hole, or started one fresh. Suitable pre-formed holes are undoubtedly a valuable resource for newly mated queens, and queens that encountered them vanished quickly from the surface, reappearing occasionally to deposit soil from their subterranean modifications. Queen-dug founding chambers were usually 5–10 cm deep (mean = 7.3 cm, SD = 1.6 cm, n = 143), with a small chamber at the bottom. In some cases, the queen abandoned a partly finished hole after some time and moved on.

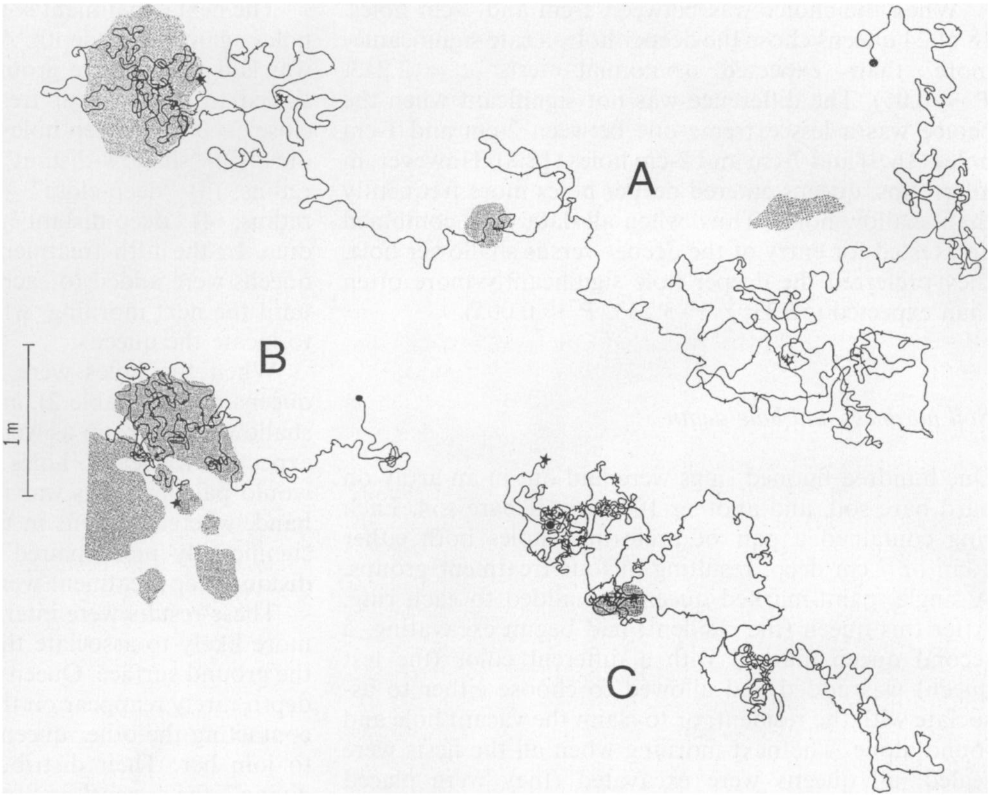

Fig. 1 Three representative tracks (*A*, *B*, *C*) of newly mated queens searching for a nest site on foot after landing and breaking off their wings. The landing point is shown as a *filled circle*, the queen's path as *a line with tick marks every 30 s*. *Open stars* show any holes inspected by the queen and the *filled star* shows the final position in which the queen remained for at least 30 min. Observations ceased after this time

In an area of sparse lawn, 19 queens were visually tracked from the moment of landing, and their paths recorded on graph paper. The characteristics of their searches are summarized in Table 1. The straight-line distance between the landing point and the final founding chamber was usually less than 2 m, and never more than about 13 m. The scale of their search was typically a few square meters in area, a few meters in path length, and less than an hour in duration. During this search, queens inspected most of the preformed holes they encountered (from 0 to 18). Altogether, it appears that queens make the general site choice from the air, choosing only the exact location of the nest chamber on foot. Although queens may have left their last hiding places once my observations ceased, it is clear that they do not move far from their points of landing.

Table 1 Characteristics of the pedestrian searches by newly mated queens immediately after landing from the mating flight. Observation ceased when a queen remained hidden for more than 30 min. *Time observed* refers to the time elapsed before the queen remained stationary for 30 min or more ($n = 19$)

	Median	Minimum	Maximum
Time observed (min)	38	5	113
Distance between initial and final points (m)	1.75	0.5	12.8
Approximate area searched (m^2)	3.9	0.2	88
Number of holes inspected	1	0	18
Path length (m)	10.5	1.6	50.3

When queens encountered other queens, they usually paused to antennate one another and to engage in a calm, mutual head-to-gaster inspection that could last for many seconds. These inspections were often accompanied by slow movements of the head and antennae.

Field arena experiments on factors that promote pairing

Choice of hole depth

Experimentally provided holes were usually discovered and used quickly, allowing the queen to remove herself from exposure on the soil surface with little effort. If the hole indeed represents a valuable resource for newly mated queens, then queens should show preferences for holes with favorable characteristics, such as adequate depth and bore. I tested for such preference in the following field experiment.

In an area of bare, firm soil, 106 fluoned rings were laid out in an array. Within each ring, I made two holes of different depth, so that there were three treatment groups: (1) a 1-cm and a 2-cm hole; (2) a 1-cm and a 7-cm hole; (3) a 2-cm and a 7-cm hole. One newly mated queen was placed into each ring and allowed to choose which hole to enter. Her choice was confirmed the next morning by recording which hole contained a dirt plug or, if no plug was apparent (sometimes holes were plugged below the surface), both holes were excavated to find the queen.

When the choice was between 1-cm and 7-cm holes, 18 of 24 queens chose the deeper hole, a rate significantly more than expected (binomial test, $z = 2.243$, $P < 0.05$). The difference was not significant when the choice was a less extreme one between 2-cm and 1-cm holes (16:9) and 7-cm and 2-cm holes (18:8). However, in all groups, queens entered deeper holes more frequently than shallow holes. Thus, when all data were combined and tested for entry of the deeper versus shallower hole, they preferred the deeper hole significantly more often than expected (52:23; $z = 3.233$, $P < 0.005$).

Soil hardness and hole depth

One hundred fluoned rings were laid out in an array on hard bare soil, and another 100 on soft bare soil. Each ring contained a pair of preformed holes both either 1 cm or 7 cm deep, resulting in four treatment groups. A single, paint-marked queen was added to each ring. After this queen (the resident) had begun excavating, a second queen marked with a different color (the test queen) was added and allowed to choose either to associate with the resident, or to claim the vacant hole and found alone. The next morning when all the nests were sealed, all queens were excavated (they were placed singly into nest tubes and allowed to rear brood whose quantity was assessed in an experiment described below).

Of the four treatment groups, only those provided with 1-cm holes in hard soil joined the other queen at higher than expected rates (30 pairs of 40 trials; binomial test, $z = 3.469$; $P < 0.001$). When digging was easy, or holes were of adequate depth, queens did not pair with greater than expected frequency. When digging was hard and holes shallow, they did.

Hole depth and spacing

It seemed likely that queens would be more likely to pair if the holes were less adequate. This was tested with two replicates of the following experiment. An array of 100 4-in fluoned rings was laid out on bare soil, each with five evenly spaced holes. In half of the arenas, the holes were 1 cm deep, in the other half, 7 cm. On the afternoon of a mating flight, two queens were placed into the center of each arena, and allowed to assort until the next morning. Location of the queens was determined by excavation. Two replicates were carried out one day apart.

Queens offered 1-cm-deep holes were paired significantly more often than expected (binomial test, $z = 3.895$, $P < 0.001$), whereas those offered 7-cm holes were not ($z = 1.240$, n.s.). Furthermore, in a subsequent experiment in which queens were placed into 50 arenas lacking any holes whatsoever, all queens formed pairs.

The next experiment sought to play off spacing of the holes against their depth. An array of 250 fluoned arenas was laid out on bare ground. Five-hole arenas were assigned to one of four treatment groups: (1) "shallow-close" – 0.5-cm deep holes evenly spaced on a 1-cm radius; (2) "shallow-distant" – 0.5-cm holes on a 3.5-cm radius; (3) "deep-close" – 5-cm-deep holes on a 1-cm radius; (4) "deep-distant" – 5-cm holes on a 3.5-cm radius. In the fifth treatment, there were no holes. Two queens were added to each arena and allowed to assort until the next morning, when all arenas were excavated to locate the queens.

When the holes were shallow and distant, all the queens paired (Table 2), and this was almost true of the shallow-close group as well. From this result, one might expect that when no holes were provided, all the queens would pair, and this was indeed the case. On the other hand, whereas queens in the close-deep treatment were significantly more paired than expected, those in the distant-deep treatment were not (Table 2).

These results were interpreted as follows. Queens are more likely to associate the more contact they have on the ground surface. Queens that enter a hole of adequate depth rarely reappear on the surface, have less chance of contacting the other queen, and therefore are less likely to join her. Their distribution therefore reflects "hole diving", the queens' first (usually random) choice, rather than any assessment of neighboring holes and queens. When the holes are shallow, queens return to the surface more often, both to dump excavated dirt and to explore, and are thus more likely to contact the other queen and join her. No matter whether holes are deep or shallow, closer proximity increases the chance of contact and therefore the chance of joining.

Hobbled queens

Clumping within holes could be due to mutual attraction between queens, or to preference for the same hole. If the former, a queen immobilized in a hole should cause a test queen to choose that hole more frequently than expected. A fine wire was tied around the petiole of newly mated queens such that when the queens were

Table 2 Queen pairing under different conditions of hole depth and spacing in field arenas. Observed values were compared to the expected proportion of pairing (0.2) using the binomial test corrected for large samples (Zar 1974)

Hole depth (cm)	Hole spacing (cm)	Number paired	Number separate	Total	Z	P-value
0.5	1	23	2	25	12.90	$\ll 0.001$
0.5	3.5	20	0	20	∞	0
5	1	19	25	44	2.95	<0.005
5	3.5	8	37	45	0.195	n.s.
No holes	No holes	13	0	13	∞	0

pushed down a hole, the ends of the wires engaged the walls of the hole and prevented the queen from being able to leave again. Five-hole fluoned arenas were set up in an area of bare, level soil, and a hobbled queen was pushed into one of the five 4-cm-deep holes in each arena. A single test queen was then added to each, and 12–14 h were allowed for the free queens to make their choice of holes and seal themselves in. Ambiguous cases were excavated. Three replicates were completed, and were combined for analysis ($n = 147$).

The free queens did not choose to associate with the hobbled queens more frequently than expected (binomial test, $z = -0.021$, n.s.). Thus, the simple presence of a tethered queen in the hole is not sufficient to cause a free queen to associate. Perhaps the contact must be made on the ground surface, as in the experiment above. It is also possible that hobbled queens emit alarm signals which counteract the attraction seen in other experiments.

Association with free queens

In this experiment, 100 arenas were set up as above. Fifty of these received a single 4-cm hole and a single queen who was allowed to dig until shortly before dusk, whereupon four additional, evenly spaced holes of similar depth, and a second queen were added. The first queen changed holes in only one case, so that the second queen can be considered a test queen making the choice of joining or founding separately. These results were compared to those from the other 50 arenas which received five evenly spaced 4-cm holes and a pair of simultaneously added queens shortly before dusk. Thus, the two treatments differed in whether both queens, or only one, searched among a set of preexisting holes.

In both groups there was a significant tendency to join another queen, with higher rates of joining (22/39) in the sequentially added group than the simultaneously added group (17/43) (binomial test: sequential addition, $z = 4.424$, $P < 0.001$; simultaneous addition, $z = 2.464$, $P < 0.05$). The proportions joining were not quite significantly different (Fisher exact test, $P = 0.095$). It appears that when a queen was actively engaged in digging a largely completed nest, another queen was likely to prefer joining the first queen rather than using an equivalent preformed hole.

However, a weakness of this experiment is the lack of certainty that the four holes added later to complete the circle of five holes may not have been equivalent in depth or quality to the queen-modified hole, even though pains were taken to estimate the queen-hole depth with a straw. Thus, in spite of the clear preference for the hole with the queen in both treatments, there is some doubt that the choice was made for the queen herself, rather than her hole. Whenever queens can modify the holes, or holes are not exactly equivalent for other reasons, the choice to join another queen will be confounded to some degree with the choice to use the same hole, independently of the other queen. Queens are clearly capable of discriminating hole depth, as shown in the experiment above. The choices "hole versus queen" can be separated only under the more rigidly controlled conditions of a laboratory experiment in which the holes are identical and cannot be modified, as below.

Can the dispersion of queens and holes affect the dispersion of subsequent queens?

To the degree that other queens and preformed holes may represent valuable resources to founding queens, the dispersion of later-settling queens should be affected by the dispersion of earlier queens, their excavations, and other preformed holes. This was tested in a factorial field experiment using preformed holes and tethered queens.

Thirty-six 5 × 5 m plots were laid out in a sparsely vegetated, level area. A mixture of crude oils was poured in a line along all perimeters to prevent newly mated queens from leaving the plots (Tschinkel and Howard 1983). Treatments consisted of one of two dispersions of 25 tethered queens, either overdispersed (1 tethered queen in the center of each square meter) or clumped (25 tethered queens in a regular array in the central square meter). Each plot was also treated with one of two dispersions of 25 preformed, 7-cm-deep holes, either overdispersed (1 hole in the center of each square meter) or clumped (25 holes in an array in the central square meter). Addition of plots lacking either tethered queens or holes or both brought the number of plots in one replicate set up to nine. The experiment was replicated three times resulting in 27 plots in all. Formation of the holes was accomplished on the morning before a mating flight was expected and tethering of the queens (these were glued to a fine 10-cm wire and staked with a pin) was done in the early afternoon.

Each plot then received 25 test queens who could move and associate freely within the plots, responding or not to the tethered queens and preformed holes. The next morning, founding chambers could be recognized by the plug of dirt pellets, and were marked for later excavation. All founding nests were excavated over the next several days. Data consisted of the map location of chambers and the number of queens in the chamber. In most cases, fewer than 25 test queens were recovered.

For each plot, the dispersion of the test queens among nests, and the dispersion of test queens and occupied nests within the plot were estimated from a dispersion index calculated as the ratio of the variance to the mean of queens/nest, queens/m^2, and nests/m^2, respectively. A ratio significantly greater than 1.0 indicated clumping, less than 1.0, uniform dispersion. The individual indices were tested for significance by comparing the test statistic $(k-1)(s^2/x)$ to the chi-square distribution with k degrees of freedom. In addition, the three dispersion indices were log-transformed and subjected to two-way MANOVA, using the dispersion of tethered-queens and of preformed holes as the independent variables.

The dispersion of preformed holes caused parallel changes in the dispersion of test queens and nests within the plot (Fig. 2) (MANOVA log dispersion index of queens/m^2, $F_{2,18} = 13.02$, $P < 0.0003$; log dispersion index of nests/m^2, $F_{2,18} = 17.65$, $P < 0.00006$), but not of queens among occupied nests ($F_{2,18} = 0.613$, n.s.). Thus, when the preformed holes were clumped, the dispersion indices showed that both nests and queens were significantly more clumped within plots than the no-holes control (Duncan's multiple-range test, $P < 0.01$). When the preformed holes were uniformly dispersed, queens and nests were significantly less clumped than the controls (Duncan's multiple-range test, $P < 0.01$). When the preformed holes were clumped and located in the central square meter, both queens and occupied nests were much more likely to occur in this central square-meter than in the surrounding 24 m (Table 3), indicating that the queens used these preformed holes. Similar clumping of queens in the central square meter had no such effect on either nests or test queens.

On the other hand, the dispersion of tethered queens had no significant effect on the dispersion of queens among nest chambers, queens within plots, or nests within plots (MANOVA: all $F_{2,18} < 1.4$, n.s.). There was also no significant interaction between queen and hole dispersion.

How can these results be reconciled with the fact that queens chose to join other queens in the five-hole choice experiments? First, this tethering experiment manipulated only distribution of queens on the ground surface, the tethered queens being incapable of digging holes. Second, it is possible that tethered queens were too artificial a condition to cause a response in free queens. Third, the likelihood of association is greatly reduced when a fully adequate preformed nest hole is provided, as it was in this experiment.

Plaster arena experiments in the laboratory

Field experiments do not clearly separate the two possible reasons for the choice a queen makes when she joins another queen – she may be responding to the queen directly, or to the hole the queen has excavated or modified, or both. In soil, a queen enters a hole and almost immediately begins deepening or modifying it. If this activity makes her hole more desirable than the remaining four unmodified holes, then when a second queen joins the first, the experiment confounds, to varying degrees, whether the second queen chose to join the first queen or simply chose the most suitable hole independent of the presence of the first queen. Alternately, the tool-made holes may not have been exactly equivalent because small differences in depth, soil consistency, soil moisture, exposure, or unknown attributes may have made one more attractive than others, leading the two queens to make the same choice on the basis of the hole, and not the queen. The same criticism can be leveled at most of the field experiments in which queens could modify the starter holes provided. Only by forcing queens to choose among identical, unmodifiable holes in a homogeneous medium can an experiment truly sepa-

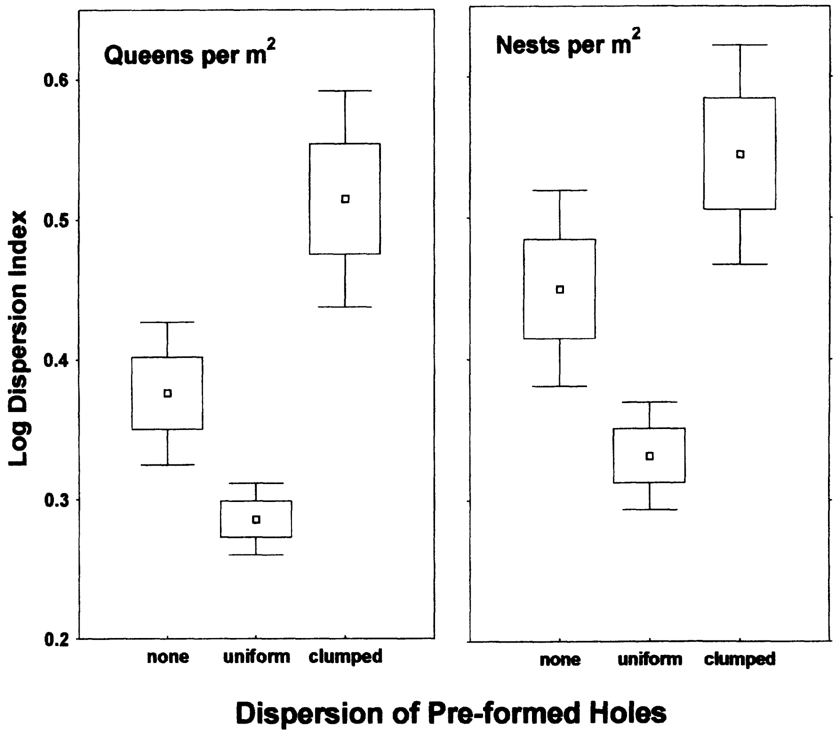

Fig. 2 The dispersion of queens and nests within plots paralleled dispersion of preformed holes. When preformed holes were overdispersed, both queens and occupied nests were significantly more dispersed than controls (no preformed holes). When the holes were uniformly dispersed, queens and occupied nests were significantly more uniformly dispersed than controls (MANOVA, Duncan's test.). *Boxes* indicate 1 SE and *bars* 1.96 SE

Table 3 The expected and observed frequency of nests and queens in the central square meter of the 25-m^2 plots. The three replicates were summed for these calculations. Expected values were 1/25th of the total number of nests or queens recovered from the plot

Treatments		Percent found in central 1 m^2		Number expected		Total number		Chi-square		*P*-value	
Tethered queens	Preformed nests	Nests	Queens	Nests	Queens	Nests	Queens	Nests	Queens	Nests	Queens
None	None	0	0	1.44	1.64	36	41	1.440	1.640	n.s.	n.s.
None	Uniform	3.85	5.9	2.08	2.72	52	68	0.003	0.602	n.s.	n.s.
None	Clumped	32	29	2.00	2.48	50	62	98.000	97.125	<0.0001	<0.0001
Uniform	None	0	0	1.08	1.44	27	36	1.080	1.440	n.s.	n.s.
Uniform	Uniform	4.8	4.4	1.68	1.84	42	46	0.061	0.014	n.s.	n.s.
Uniform	Clumped	25.7	25	1.40	1.44	35	36	41.257	39.690	<0.0001	<0.0001
Clumped	None	6.8	5.5	1.76	2.20	44	55	0.874	0.291	n.s.	n.s.
Clumped	Uniform	4.4	3.9	1.84	2.08	46	52	0.014	0.003	n.s.	n.s.
Clumped	Clumped	18.4	18.6	1.52	1.72	38	43	19.757	22.929	<0.0001	<0.0001

rate the influence of the queen from that of the hole in the decision to associate.

I therefore tested queen choice in five-hole plaster arenas in the laboratory in which queens cannot modify the holes (except perhaps chemically). Experiments in which queens associate in significantly more than 20% of the cases indicate that queens are choosing to associate with other queens.

Testing the arenas for bias

Because the laboratory environment was not homogeneous, the plaster arenas were tested for bias by determining whether queens chose some holes preferentially, and whether such preference could lead to an increase in the expected number of pairs simply as a result of this hole preference. One newly mated queen was dropped (with random body orientation) into the center of each of 100 five-hole plaster arenas. After 1.5–3 h, the hole each queen occupied was noted. All queens were removed, mixed and randomly added singly to the cups again. Position and orientation of the cups were not changed. This was repeated ten times, so that each cup was tested ten times with ten randomly selected queens from a set of 100 queens.

Queens preferred some hole positions over others, with the most preferred drawing 25.1% of the queens and the least 16.7% ($\chi^2 = 26.55$, $df = 4$, $P < 0.00005$). The expected probability of pairing in each hole was calculated as the square of the fraction of queens (over all ten trials) in that hole. The probability of "pairs" arena-by-arena ranged from 0.21 to 0.62 ($n = 100$), indicating that some arenas may have contributed more "pairs" than others. The sum of these probabilities for all five holes estimated the expected probabilities of queen-pairs in each of the 100 arenas, if the pairing had been simply based on hole choice (not queen choice) by the two queens. The queens' hole preference raised the likelihood of pairing in some holes and decreased it in others, but the overall expected probability of 0.204 was only slightly higher than it would have been if hole choice had been completely random (0.2).

The pairing rate expected from unequal hole preference was analyzed further. The ten runs were taken in all possible pairs and analyzed as though the queens had been added simultaneously. That is, if the queens in the two runs had entered the same hole, they were scored as a "pair," if different holes, they were scored as separate. The frequency of such "pairs" over all possible combinations of ten runs was used as an estimate of the "observed" rate of pairing when queens did not interact directly.

The mean "pairing" over all ten trials and 100 arenas was 24.2% ($n = 45$). Queens were somewhat more likely to "pair" in immediately following runs (25.7%) than in later runs (23.5%), but these differences were not significant (one-way ANOVA: $F_{1,43} = 1.94$, n.s.). Each of the 45 combinations was also compared to an expected "pairing rate" of 0.204, using the chi-square test. After applying the Bonferroni correction for multiple tests, only 2 of the 45 combinations showed significantly greater pairing than expected. Both of these were immediately following runs. It remains possible that queens are attracted (an odor?) to holes previously occupied by other queens, but evidence from this experiment was weak.

Tests of pairing in shallow-hole arenas

In five replicate sets of this experiment, a pair of queens (from three different mating flights) was added simultaneously to the center of each of 100 shallow-hole arenas. The position and pair-status of all queens was noted three to six times over up to 18 h. The plaster inserts of all arenas were soaked in hot water between replicates.

For analysis, the number of pairs observed at each reading was compared to an expected value (binomial test, *z*-score). To adjust for the bias of the arenas, the observed proportion of "pairs" (24.2%) from the bias tests was used as a more conservative expected proportion, rather than the value calculated from hole occupancy (20.4%). In 29 of the 37 readings, there were significantly more pairs of queens than expected (binomial test, z-scores between 1.96 and 7.82, P-values from <0.05 to <0.001). Of the 8 non-significant readings, 6 were the first readings of a set, when little time for assortment of queens had elapsed, and queens had dashed into the nearest hole. When averaged over all readings for a replicate set, 42–67% of the queens were paired, as compared to the expected 24.2%. The z-scores for the five replicates were 2.404 ($P < 0.05$), 2.575 ($P < 0.01$), 3.892 ($P < 0.001$), 4.615 ($P < 0.001$) and 6.194 ($P < 0.001$). Thus, when queens were present simultaneously in the arenas, they were much more likely to pair than can be explained from random assortment, even after adjusting for the bias of the arenas.

Pairing in deep-hole arenas

Several experiments used the plaster arenas with 3-cm-deep holes. Local queen density has been shown to play a driving role in the formation of founding associations (Tschinkel and Howard 1983). The following experiment therefore tested whether the density of other queens perceived shortly before being placed in the arena affected the tendency to pair, and whether this tendency was different for deep-hole and shallow-hole arenas. On the day of the mating flight, one group of newly mated queens was collected singly into test tubes, whereas the other was maintained in groups of ten. The former group (low perceived density) never contacted another queen before being placed into the arenas, whereas the latter spent the wait in close contact with other queens (high perceived density). After several hours at the treatment densities, two randomly chosen queens were added to each arena and allowed to assort for up to 18 h.

In 1993 and 1995, the low-density queens paired significantly more than expected (19/47 and 17/46; binomial test $z = 2.705$ and 2.230; $P < 0.01$ and $P < 0.05$), whereas in 1996, they did not (16/48; $z = 1.806$, n.s.). High-density queens did not pair significantly in any year (15/48, 14/48, and 16/49; $z = 1.526$, $z = 1.238$, and $z = 1.736$; all n.s.). When the data were combined by density across all 3 years, both densities were associated with significantly more pairing than expected (low density, $n = 141$, $z = 4.198$, $P < 0.001$; high density, $n = 145$; $z = 2.328$; $P < 0.05$). Pairing was not significantly different for the two densities (Fisher exact test).

When pairing was compared between similar experiments in deep-hole arenas and shallow-hole arenas, queens showed significantly more tendency to pair in the shallow holes than in the deep ones. Because queens in the shallow-hole tests above were kept communally before use, the comparison of these experiments with the high-density deep hole is justified. An average of 51.5% of the queens paired in the shallow holes (mean $z = 3.769$, $P < 0.001$) whereas in the deep holes, only 37% did so ($z = 2.247$, $P < 0.05$), i.e., significantly more did so in shallow than in deep holes (chi-square $= 3.98$, $df = 1$, $P < 0.05$).

These results were interpreted as follows. When the hole was of adequate depth for colony founding, queens showed high levels of "hole diving", i.e., they enter a randomly chosen hole and rarely come back to the ground surface. This reduces their chance of contacting other queens (who are also at the bottom of a suitable hole), and thereby reduces the chance of pairing. On the other hand, when the hole is not deep enough for safe founding, the queen is likely to leave the hole to search further, and when she does so, she is more likely to encounter another queen or to enter an already occupied hole. Such increased contact with other queens allows the attraction among queens to take its effect. Further evidence for this interpretation comes from an experiment in which the entrances to all five holes in the deep-hole arenas were blocked with a small amount of wet dirt, so that queens spent considerable time on the surface and had to dig through the plugs to enter the chambers. In this experiment, almost all of the queens paired (33/36, $z = 15.3$, $P \ll 0.001$). The same was true in the field experiment control in which no holes were provided – in this case all queens paired (Table 2). When queens land on pavement, they cannot dig holes, and often accumulate in large numbers under litter and objects.

The pairing of queens was thus the outcome of the interaction between the attraction among queens and the depth of the holes available.

Reproductive correlates of queen choice

Does digging a founding chamber incur a cost?

It seemed possible that queens use preformed holes because it saves them the metabolic cost of digging a founding chamber, a cost which might be measurable in reduced brood production or survival of the founding period. Thus, queens that use existing holes or share the work of digging would benefit by reducing their individual costs and enjoying higher brood production.

Newly mated queens were divided into three groups: the first was allowed to dig a founding nest within a fluoned ring (dig treatment), the second was provided with a 7-cm-deep hole (no-dig treatment), and the third was held in individual nest tubes in the laboratory (control). After 18–20 h, the queens were excavated from their chambers and were installed in nest tubes like those of the control group. After most queens had produced minims, the brood was counted.

There was no significant difference in the number of brood produced by the three groups of queens (mean number of post-egg brood: laboratory control group = 36.0, digging group = 34.2; non-digging group = 31.9; one-way ANOVA $F_{2,116} = 0.61$, n.s.). Similarly, there was no significant difference in the number of pupae or minims produced. Digging a founding chamber therefore seems to incur at most a minor metabolic cost. It seems more likely that the benefits accruing from using a preformed hole or joining another queen have more to do with reducing desiccation and the risk of predation while on the ground surface.

Do pairing and non-pairing queens differ in reproductive attributes?

It seemed possible that the choice to associate with another queen might be influenced by the reproductive or survival capacity of the queen making the choice. The queens from the two-hole, soil hardness/hole depth experiment above were used to test this possibility. A single, paint-marked queen was added to each ring. After this queen (the resident) had begun excavating, a second queen marked with a different color (the test queen) was added and allowed to choose either to associate with the resident, or to claim the vacant hole and found alone. After excavation the next morning, each queen was placed into an individual nest tube. All paired queens were together with their choices for less than 24 h, and completed the founding period alone. When most queens had produced minim workers, the nest was killed, brood was counted and the dry weight of the queen and brood determined. This design allowed the comparison of the reproductive characteristics of queens that joined or were joined with those of solitary queens.

Possible reproductive differences between joiners and non-joiners were analyzed separately for test and resident queens. The total brood weight was very non-normal and could not be normalized by transformation. Data for queens that were alive at the end of the founding period were therefore analyzed by Kruskal-Wallis ANOVA. Resident and test queens did not differ significantly in the total weight of brood produced ($H = 0.012$, $n = 270$, n.s.), and nor did queens that joined (or were joined) differ from queens that did not ($H = 1.98$, $n = 270$, n.s.).

Queens that joined (test queens) or were joined (resident queens) were significantly heavier (mean weight = 2.13 mg) at the end of the founding period than queens that had not joined or been joined (mean weight = 1.56 mg) (ANOVA, main effect of joining: $F_{1,395} = 13.80$, $P < 0.0002$; main effect of resident/test: $F_{1,395} = 0.90$, n.s.; no significant interactions). Because 400 queens were randomly assigned to be test queens or residents, it seems unlikely that initial weights were significantly different. Because brood production did not vary significantly across treatments, higher final queen weight probably indicates more efficient investment of reserves in brood. Whatever the cause of the higher final queen weight, queens exhibiting this character seem to prefer to join other queens with the same character.

On the other hand, test queens that joined were significantly more likely to survive the founding period (81% alive) than those that did not (65% alive) (Pearson chi-square = 5.875, $n = 200$, $df = 1$, $P = 0.015$). This was not true for the resident group, in which 70% of joined and 58% of non-joined queens survived (chi-square = 3.08, $n = 199$, $df = 1$, n.s.). Test queens made the active choice to join or not, whereas resident queens merely acquiesced to the choice of the test queen (though they could have played an active role in inducing the test queen to stay). Because all paired queens were separated and completed the founding period alone, this means that test queens that made the active choice to join another queen (the resident queen) were more capable of surviving the founding period, even by themselves. In some important way, they were more robust than those test queens that chose not to join, although this robustness was not expressed in greater brood production. The resident queens who were chosen by the test queens showed no significant difference in survival capacity.

Does joining particular queens improve performance?

If queens are choosing other queens in such a way as to optimize the performance of the group, then reassorting these groups randomly should decrease their performance. A large number of newly mated queens were collected and scattered in an area with large expanses of bare soil. The next morning, founding chambers were marked and excavated. If they contained more than one queen, these were kept together in nest tubes. A total of 28 nests contained two queens and 24 had three queens. Half of each group were kept together in the original associations (control group). Queens in the other tubes were traded randomly among tubes until none of the queens were with their original associates (randomized group). All groups were then kept at 30 °C until minims appeared. At this time, the brood was counted, dried, and weighed, and the queen was dried and weighed.

The performance of the randomized associations did not differ significantly by any measure from the self-associated ones, nor did the number of queens (two or three) have an effect (two-way ANOVA of total dry weight of brood: random vs self-selected, $F_{1,48} = 0.362$, n.s.; queen number, $F_{1,48} = 1.282$, n.s.; interaction, $F_{1,48} = 0.528$). The final dry weight of queens from self-associated and randomized groups was not significantly different ($F_{1,119} = 1.09$, n.s.). It thus appears that queens do not choose queens with whom they enjoy a particular synergism.

Discussion

Cooperative colony founding follows aggregation. Many animals aggregate, and the cues that draw them together are as variable as their sensory systems. Aggregation may result when many animals are attracted to the physical conditions at a location, from mutual attraction to one another, independent of location, or both. Once aggregated, animals can reap any mutualistic benefits that grouping may carry, be it e.g., defense, mating, or predator avoidance. In colony-founding ant queens, many mutualistic benefits have been identified, some with a cascading effect as colony-founding proceeds. Studies have addressed the existence, conditions, benefits, and consequences of group founding in *S. invicta* and other ant species (see Hölldobler and Wilson 1990 for review prior to 1989; Mintzer 1990; Rissing and Pollock 1991; Stamps and Vinson 1991; Tschinkel 1992a,b, 1993a; Adams and Tschinkel 1995a,b; Pfennig 1995; Sasaki et al. 1996; Balas and Adams 1997; Jerome et al., in press), but few have addressed the basis on which queens choose to associate with others. This study focuses on the first few hours of a queen fire ant's life after mating, during which she makes the choice of whether or not to join other queens.

After landing from her mating flight, the scale of the newly mated queen's pedestrian search is quite modest, and it is apparent that the general choice of founding site must be made while the queen is still airborne. There is little information on how queens choose their landing sites, but to judge from their abundance on the ground, they appear attracted to disturbed, partially vegetated areas (unpublished observations), as would be expected for a "weedy" species (Tschinkel 1993b). Newly mated queens often settle on the surface of water or other shiny surfaces, perhaps as an aspect of their association with seasonally flooded land.

Colony founding by groups of fire ant queens has long been known (Markin et al. 1972; Tschinkel and Howard 1983), but experiments on the factors that cause a queen to join or not to join are rare (e.g., Nonacs 1992). It is clear from the present study that holes are a powerfully attractive resource. This is true to such an extent that queens will enter a hole of adequate depth and never leave again to explore or meet other queens. Holes are so attractive that the dispersion of queens in space can be experimentally manipulated through the dispersion of nail holes in the ground. Clearly, one of the strongest reasons why a queen might join another is the hole excavated by the resident queen. When empty holes are in short supply, this choice would lead to pleometrosis.

The attraction of preformed holes probably results from the escape from desiccation and predation that they offer, as suggested for the desert ant, *V. pergandei* (Pfennig 1995). Certainly, a newly mated *S. invicta* queen on the soil-surface is easy prey for any sharp-eyed predator. Soil surface temperatures, especially in the more barren sites preferred by *S. invicta*, may reach near-lethal levels, even on the day of the mating flight. Queens released in such sites two days after a mating flight, when the soil surface has dried considerably and thus warms more rapidly, may die of overheating within 5 min (unpublished observations). Together, these observations suggest that escaping underground quickly enhances the chances of a newly mated queen's survival. Preformed holes, be they constructed by other queens or of some other origin, offer a rapid escape from dangerous conditions, and are avidly accepted by newly mated queens.

Experiments that appear to demonstrate that queens in a natural context choose to cofound with other queens, independently of the hole they occupy, must be approached cautiously. Experiments in soil confound any hole modifications that queens make with the presence of the resident queen. Laboratory choice experiments in arenas may be subject to directional and other biases that must be taken into account. Nevertheless, it was possible to overcome most of these challenges to show that queens are attracted to cofound with queens, independently of their holes. The expression of this attraction, however, was conditional on the likelihood of contact. Shallower holes, or holes closer together were more likely to lead to joining, probably because queens were more likely to come to the surface again and/or to contact the other queen in the neighborhood. Under extreme conditions in which no preformed holes were provided, most confined queens associated. The probable importance of queen-queen contact was pointed out by Tschinkel and Howard (1983) who proposed that increased queen density on the ground surface caused increased pleometrosis because queens were more likely to come into contact at higher densities. They also showed that adding queens as a group to a hole-choice arena leads to higher levels of association than adding them singly, and suggested that simultaneous addition results in contact among queens. Under natural conditions, frequent contact with other queens is a surrogate for high local queen density, and predicts a more competitive future environment for incipient colonies and their queens. The winners of the brood-raiding competition among incipient nests are those with the most workers, and these in turn tend to be pleometrotic, making the choice to associate a better gamble at higher queen densities (Bartz and Hölldobler 1982; Tschinkel 1992a,b; Adams and Tschinkel 1995a). Interestingly, keeping queens at high and low densities before pairing them in arenas had no effect on their association rate. Perhaps the effect of density fades quickly under these experimental conditions.

As Nonacs (1992) showed for *Lasius pallitarsus*, newly mated queens of *S. invicta* are not all equally likely to join other queens in pleometrosis. In both species, more "robust" queens (as indicated by body weight in *L. pallitarsus*, and by survival and post-founding body weight in *S. invicta*) were more likely to join. The improved survival of joining *S. invicta* queens is a passive outcome of greater "robustness" – these queens spent less than 24 h in association, completing the founding period by themselves. Under field conditions, 20–50% or more of the queens die (Tschinkel 1993a), and this mortality is not affected by association. A large part of founding success for a queen is thus surviving the founding period. If the improved survival of joiner queens is also expressed when grouped, such queens are more likely to inherit the entire incipient colony reared by an association of queens. Because groups of queens are more successful at colony founding than single queens, more robust queens should be more likely to associate, as observed. Interestingly, the benefits of "robustness" in *S. invicta* derive from survival, not greater brood production. Nonacs (1992) did not test brood production in his queen-choice experiments, but Balas and Adams (1996) and Bernasconi and Keller (1996) reported that queens losing less weight during the founding period were more likely to survive the reduction to monogyny. The choices joiner queens make also do not result in greater brood production by the joined group, suggesting that queens are not judging the reproductive attributes of the queens they join. However, the greater survival of joined queens suggests that robust queens are more likely to join other robust queens, perhaps because the association is more likely to survive. In any case, survival of the queen making the active choice to join is higher than that of the joined queen.

Acknowledgements I am grateful to Tracy Andreae, Dennis Ewing, Natalie Furman, and Stephanie Clark for technical help, to Duane Meeter for statistical advice and to Eldridge Adams for helpful comments on the manuscript. This work was carried out with the support of National Science Foundation grants IBN 9317853, BSR 8920710, BSR 8502969 and PCM 8309198.

References

Adams ES, Tschinkel WR (1995a) Density dependent competition in fire ants: effects on colony survivorship and size variation. J Anim Ecol 64:315–324

Adams ES, Tschinkel WR (1995b) Effects of foundress number on brood raids and queen survival in the fire ant *Solenopsis invicta*. Behav Ecol Sociobiol 37:233–242

Balas MT, Adams ES (1996) The dissolution of cooperative groups: mechanisms of queen mortality in incipient fire ant colonies. Behav Ecol Sociobiol 38:391–399

Balas MT, Adams ES (1997) Intraspecific usurpation of incipient fire ant colonies. Behav Ecol 8:99–103

Bartz SH, Hölldobler B (1982) Colony founding in *Myrmecocystus mimicus* Wheeler and the evolution of foundress associations. Behav Ecol Sociobiol 10:137–147

Bernasconi G, Keller L (1996) Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). Proc R Soc Lond B 263:509–513

Hölldobler B, Wilson EO (1977) The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8–15

Hölldobler B, Wilson EO (1990) The ants. Belknap, Cambridge, Mass

Jerome CA, McInnes DA, Adams ES (in press) Group defense by colony-founding queens in the fire ant *Solenopsis invicta*. Behav Ecol

Markin GP, Dillier JH, Collins HL (1972) Colony founding by queens of the red imported fire ant *Solenopsis invicta*. Ann Entomol Soc Am 65:1053–1058

Mintzer AC (1979) Colony foundation and pleometrosis in *Camponotus* (Hymenoptera: Formicidae) in the western United States. Pan Pac Entomol 55:81–89

Mintzer AC (1987) Primary polygyny in the ant *Atta texana*: number and weight of females and colony foundation success in the laboratory. Insect Soc 34:108–117

Mintzer AC (1990) Foundress female weight and cooperative foundation in *Atta* leaf-cutting ants. In: Meer RK vander, Jaffe K, Cedeno A (eds): Applied myrmecology: a world perspective. Westview, Boulder, Colo, pp 180–183

Nonacs P (1989) Competition and kin discrimination in colony founding by social Hymenoptera. Evol Ecol 3:221–235

Nonacs P (1992) Queen condition and alate density affect pleometrosis in the ant *Lasius pallitarsis*. Insect Soc 39:3–13

Pfennig DW (1995) Absence of joint nesting advantage in desert seed harvester ants: evidence from a field experiment. Anim Behav 49:567–575

Rissing SW, Pollock GB (1986) Social interaction among pleometrotic queens of *Veromessor pergandei* (Hymenoptera: Formicidae) during colony foundation. Anim Behav .34:226–233

Rissing SW, Pollock GB (1987) Queen aggression, pleometrotic advantage and brood raiding in the ant *Veromessor pergandei* (Hymenoptera: Formicidae). Anim Behav 35:975–981

Rissing SW, Pollock GB (1991) An experimental analysis of pleometrotric advantage in the desert seed-harvester ant *Messor pergandei* (Hymenoptera; Formicidae). Insect Soc 38:205–211

Rissing SW, Johnson RA, Pollock GB (1986) Natal nest distribution and pleometrosis in the desert leaf-cutter ant *Acromyrmex versicolor* (Pergande) (Hymenoptera: Formicidae). Psyche 93:177–186

Sasaki K, Satoh T, Obara Y (1996) Cooperative foundation of colonies by unrelated foundresses in the ant *Polyrachis moesta*. Insect Soc 43:217–226

Stamps WT, Vinson SB (1991) Raiding in newly founded colonies of *Solenopsis invicta* Buren. Environ Entomol 20:1037–1041

Strassmann JE (1989) Altruism and relatedness at colony foundation in social insects. Trends Ecol Evol 4:371–374

Tschinkel WR (1992a) Brood raiding in the fire ant, *Solenopsis invicta*: laboratory and field observations. Ann Entomol Soc Am 85:638–646

Tschinkel WR (1992b) Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. Ecol Entomol 17:179–188

Tschinkel WR (1993a) Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. Behav Ecol Sociobiol 33:209–223

Tschinkel WR (1993b) The fire ant: still unvanquished. In: McKnight BN (ed) Biological pollution: the control and impact of invasive exotic species. Indiana Acadamy of Science, Indianapolis, pp 121–136

Tschinkel WR, Howard DF (1983) Colony founding by pleometrosis in the fire ant, *Solenopsis invicta*. Behav Ecol Sociobiol 12:103–113

Zar JH (1974) Biostatistical analysis. Prentice Hall, Englewood Cliffs, NJ

Communicated by P. Pamilo