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RESEARCH ARTICLE

Forced associations by young queens of the harvester ant *Messor semirufus* during colony founding

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Abstract After landing at the end of their nuptial flight, young queens of the harvester ant *Messor semirufus* search for a suitable nesting site and dig a burrow. After 3 months in the burrow, they start laying eggs, and nurse their first brood of workers alone. Field observations indicate that a few newly dug burrows contain more than one queen. Laboratory experiments were conducted in order to discover why these young queens' associations form. We found that groups do not exhibit any productive advantage over single-founding queens, either with respect to progeny number, or with respect to the time until the first eggs are laid. Groups have a slower rate of nest digging than single queens, and mortality rate is considerably higher for queens in groups than for single queens. From the initiation of the group, queen interactions involve aggression and a behavioral hierarchy, with a prior-residence advantage. The tendency to form groups is stronger if queens density is greater and if digging conditions, characterized by soil hardness, are less favorable. We conclude that foundress associations in *M. semirufus* are in fact the result of nest invasions in an attempt to displace the resident queen. These are motivated by the high cost of the search for a suitable nesting site and of the digging of the nest.

Keywords Colony founding · Facultative sociality · *Messor semirufus* · Pleometrosis · Prior-residence advantage · Queen associations

Introduction

On clear days following the first heavy rains at the beginning of winter, young reproductives of *Messor semirufus*, a common harvester ant in Israel, emerge from mature nests and take off on their nuptial flight. Upon landing, the inseminated queens shed their wings and wander on the ground, exposed to the risks of predation and desiccation, and search for a suitable nesting site (Motro et al. 2016). After excavating an entrance tunnel and cavity, the young queens seal themselves off and rear the first generation of workers on nutrients obtained mostly or entirely from their own storage tissues, including fat bodies and histolyzed wing muscles. This is the claustral mode of nest founding (Hölldobler and Wilson 1990).

When two queens happen to meet during their search for a nesting site, they usually move rapidly away from each other. When a wandering queen comes across a digging site of another queen, she is usually chased away by the resident queen. Indeed, our field observations (carried out in Jerusalem, Israel, during nuptial flights in November, 1990, 1991, 1992 and 1993) show that a single queen inhabits the great majority of burrows. Some burrows, however, are occupied by two or three queens, and we even found a burrow that contained eight queens together. The prevalence of these multi-queen associations depends on the terrain. About 5% of the newly dug burrows in open areas (terra rossa over hard limestone, with Mediterranean scrub) contain more than a single queen, and this proportion can reach 20% in paved areas. These observations led us to conduct

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laboratory experiments in an effort to understand founding queen associations in *M. semirufus*.

Pleometrosis, the cooperative initiation of a nest by several newly mated queens (Hölldobler and Wilson 1977), has been observed and studied in dozens of ant genera [see Bernasconi and Strassmann (1999) for a review], and also occurs in termites (Thorne 1982). In pleometrotic nest founding, the queens in the group cooperate (at least initially) to obtain greater productivity than single queens. In the majority of the pleometrotic species, the co-founding queens live together without any apparent aggression. For example, queens of *Myrmecocystus mimicus* are strongly attracted to each other after they have mated (Hölldobler and Wilson 1990).

In most pleometrotic species, only one of the queens from each founding group survives in the mature colony. In *Veromessor pergandei*, upon the emergence of the first workers, the founding queens start to fight among themselves. These fights result in queens' deaths or injuries. The workers then join in, and direct their aggression towards the injured queens (Rissing and Pollock 1987). In *Lasius pallitarsis*, the number of queens is reduced to one shortly after the appearance of the first workers, by what appears to be the workers choosing to feed only one queen, and starving the others. Heavier queens have a better chance to be fed and survive (Nonacs 1992). In *M. mimicus*, one of the founding queens takes over after the emergence of the workers. The workers then try to conquer and raid the broods of neighboring, weaker nests (Bartz and Hölldobler 1982). In other species, for example, *Atta texana* (Mintzer 1987), *Pogonomyrmex californicus* (Johnson 2004), and *Cardiocondyla wroughtoni* (Michal Motro, personal observations), pleometrosis leads smoothly to polygyny.

For some species, pleometrosis is associated with the density of young queens in the area; the size of the founding group positively correlates with queen density. *Solenopsis invicta* (Tschinkel and Howard 1983) and *V. pergandei* (Rissing and Pollock 1986) illustrate this pattern. The presumed advantage of these queen associations over the single-queen nest founding is in the ability to produce a larger number of workers in a shorter time, before their body resources are exhausted. This ability can be crucial in territorial disputes between conspecific neighboring nests. In the case of *A. texana*, Mintzer (1987) suggests that foundress associations help the queens of this small-sized species survive in areas where larger females of congeneric species coexist. In the semi-claustral seed-harvester ant *P. californicus*, Johnson (2004) reports that queen survival and total number of brood are positively associated with number of queens. In *Formica selysi*, pairs of queens are more productive than single queens (Reber et al. 2010); this difference in productivity is transient, and all types of colonies reach a similar size after 15 months. In the leaf-cutting ant *Acromyrmex versicolor*, single foundresses are less likely to initiate a successful symbiotic fungus garden, which inevitably can lead to colony starvation and death (Cahan and Julian 1999). For the honeypot ant, *M. mimicus*, Bartz and Hölldobler (1982) point out competition with neighboring incipient nests after the emergence of the first generation of workers as a crucial factor governing the success of a new colony. Joining a group can improve a queen's chances of surviving this competitive stage. The intense competition is manifested by inter-colony brood raiding, and by the aggregation in patches of founding nests, which are distant from existing *M. mimicus* colonies (as a result of nests founded in close proximity to existing colonies being quickly destroyed by the resident workers).

The harvester ant *P. californicus* presents two modes of nest founding: populations of haplometrotic and populations of pleometrotic nest founding (with pleometrosis that continues into colony adulthood and the production of reproductives), both in adjacent geographical areas (Johnson 2004). Overson et al. (2014) found that queens from both types tended to aggregate around the brood, but queens from the haplometrotic population showed more agonistic behavior toward other queens, and there is a considerable variation in their aggressiveness. These differences in agonistic behavior between queens from the haplometrotic and the pleometrotic populations persist when queens are placed together in a common environment, suggesting that there is a genetic component to these behavioral differences (Helms Cahan and Fewell 2004; Johnson 2004; Jeanson and Fewell 2008; Overson et al. 2014). An abrupt geographical shift from haplometrotic to pleometrotic populations was found also by Cahan et al. (1998) for *V. pergandei*, suggesting that colony-founding strategy is associated with genotypic variation rather than an ability to respond to changes in environmental conditions.

In this paper, we asked whether queen associations during nest founding in *M. semirufus* are indeed pleometrotic interactions, or rather the occurrence of forced associations by some queens over others. For that purpose, we conducted laboratory experiments to study the nature of interactions within multi-queen founding groups, and the environmental conditions (ant density and soil hardness) that could facilitate such associations. Furthermore, we assessed the possible advantages of these associations either in raising productivity, in increasing queens' survival, or in accelerating the rate of nest digging.

The experiments

We present five different and independent experiments, each aimed at illuminating an aspect of *M. semirufus* queens' associations in nest founding. In the "conclusions" section, the results of these experiments are integrated.

A few statistical remarks common to all experiments: when using Wilcoxon two-sample rank-sum test for data combined from several experiments, the overall p value was calculated by the method suggested in Lehmann (1975, pp 132–141). Unless stated otherwise, given p values are for a two-tailed alternative. There are several theories regarding the presentation of a two-tailed probability in a Fisher's exact test (Agresti 1992). Here, we employed the strictest approach of doubling the one-tailed probability.

Experiment 1: ant density

Aim

To assess how young queens that were collected in the field immediately after landing from nuptial flight, distribute themselves in an open system of available vials.

Methods

In each of nine 36 × 30 × 15 cm plastic boxes, 26 glass vials (2.4 cm in diameter and 10.0 cm long) were arranged, with 13 in each side of the box, so that all the vials opened to the center of the box. The vials were transparent and without soil, and the entire box was covered by a transparent red plastic filter. Queens were collected after landing from their nuptial flight, and placed in these boxes in four different densities: very low (13 queens in a box), low (26 queens in a box), high (52 queens in a box), and very high density (78 queens in a box). The queens were able to enter any of the 26 vials in the box. The number of queens in each vial was recorded daily for 8 days.

Statistical analysis

If N queens are randomly distributed among V vials, the number of queens in any vial has a binomial distribution with parameters N and V^{-1}. The expectation of this distribution is NV^{-1}, and the variance is $NV^{-1}(1 - V^{-1})$, and hence the variance to expectation ratio is $1 - V^{-1}$. Under the assumption of random distribution of queens among vials, the expected number of vials with exactly k ($k = 0, 1, 2, \ldots, N$) queens is therefore $\binom{N}{k}\frac{(V-1)^{N-k}}{V^{N-1}}$.

Using a chi-square test of goodness of fit, we statistically examined the data to see whether or not the actual distribution of queens among vials fits the random scheme. Since these tests involved some expected frequencies that are rather small, the chi-square distribution cannot be a reliable approximation; thus, probabilities were determined using Monte Carlo simulations. Whenever the null hypothesis of random distribution was rejected, we indicated whether the observed distribution was 'regular' (i.e., the queens were more uniformly distributed among the vials than expected under a random distribution) or 'clumped' (i.e., some vials were overcrowded, while others contained less queens than expected under the random distribution). The distinction was made according to whether the observed variance to mean ratio (i.e., the index of dispersion) was smaller or larger than $1 - V^{-1}$.

Results

In most of the cases, the queens did not distribute themselves at random among the available vials (Table 1). At lower densities, the results suggest a tendency to be regularly distributed (possibly due to repulsion or territoriality). Higher densities induced clustering, with some exceptionally crowded vials—for example, in one of the high-density (52 queens) boxes, two out of 26 available vials contained about 35% of the queens (ten and eight in each vial); in another high-density box, two vials contained about 29% of the queens (nine and six in each vial).

Experiment 2: soil hardness

Aim

The aim was to examine the effect of soil hardness on the queens' tendency to cluster shortly after landing from

Table 1 The ant density experiment—self-distribution of queens in an open vials system

Density (queens per box)	Year	Distribution after 1 day		Distribution after 8 days	
Very low (13 queens)	1991	regular	(*)	random	(N.S.)
Low (26 queens)	1989	random	(N.S.)	random	(N.S.)
Low (26 queens)	1989	clumped	(*)	clumped	(*)
Low (26 queens)	1990	regular	(*)	regular	(***)
Low (26 queens)	1990	random	(N.S.)	regular	(***)
High (52 queens)	1989	random	(N.S.)	clumped	(***)
High (52 queens)	1991	clumped	(*)	clumped	(***)
High (52 queens)	1991	clumped	(*)	clumped	(***)
Very high (78 queens)	1989	clumped	(*)	clumped	(***)

Symbols indicate the significance of deviation from a random distribution:

(*) = $0.010 < p < 0.050$; (**) = $0.001 < p < 0.010$; (***) = $p < 0.001$; (N.S.) = not significant their nuptial flight. Our hypothesis was that hard soil facilitates clustering—queens will tend to join already dug holes, and take advantage of other queens' digging efforts.

Methods

Two types of soil hardness boxes were prepared: 16 boxes were filled with a 5-cm-deep layer of loose soil, and 15 boxes were filled with a 5-cm-deep layer of hard soil. The hardness of the soil was measured by the depth of penetration of a 4.5-mm-thick nail, under the pressure of a 500 gr weight: the nail penetrated to a depth of 2.0 cm in the loose soil, but only to 0.6 cm in the hard soil. Queens that were collected immediately after landing from their nuptial flight were placed in each of these boxes, and their location was recorded on the following day. At that time, most of the queens had already started digging, some together and some alone, while a few were still wandering around.

This experiment was conducted in three runs, on different dates. In each run, we compared queen clustering between loose soil and hard soil boxes. The size of the boxes and the number of queens in each box were the same within each run, but slightly differed between runs (Table 2). Each run lasted only 1 day, focusing on how the clustering tendency of queens right after their nuptial flight correlates with soil hardness. We probably missed some rearrangement of queens that could have taken place also after 1 day, as suggested by Overson et al. (2014) experiments with *P. californicus*.

Statistical analysis

Since the number of possible burrows in each box is indeterminable, we could not use the same index of dispersion as in the previous experiment. Instead, for each box, we calculated a clustering index, defined by $\sum_k \binom{m_k}{2} / \binom{N}{2}$, where N is the total number of queens in the box, m_k is the number of queens present in the kth burrow, and the sum is taken over all burrows in that box. Note that the clustering index is, in fact, the probability of finding any two, randomly chosen queens (out of the N queens in the box) dug in together in the same burrow.

Results

Table 2 and Fig. 1 present the clustering index for the boxes in the three different runs. As expected, clustering was greater in the hard soil boxes than in the loose soil boxes (overall $p_{\text{one-tailed}} = 0.033$, calculated by the Wilcoxon two-sample rank-sum test for data combined from several experiments).

Experiment 3: queens in closed vials

Aim

To measure the productivity and survival of founding groups of experimentally manipulated sizes.

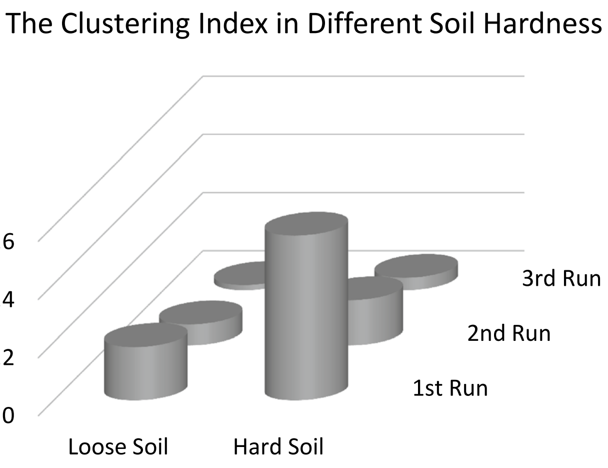

Fig. 1 The soil hardness experiment: The clustering index (see text) in loose and hard soil for the three runs of the experiment

Table 2 Clustering in the soil hardness experiment

Description	Loose soil	Hard soil
1st Run: 6 queens in each of 6, 36 × 30 × 15 cm boxes (1991)	0.067, 0.200, 0.200, 0.267	0.133, 1.000
2nd Run: 8 queens in each of 5, 60 × 40 × 15 cm boxes (1991)	0.036, 0.107	0.107, 0.143, 0.214
3rd Run: 8 queens in each of 20, 36 × 30 × 15 cm boxes (1993)	0.000, 0.000, 0.000, 0.000, 0.000, 0.036, 0.036, 0.036, 0.036, 0.036	0.000, 0.000, 0.000, 0.036, 0.036, 0.036, 0.036, 0.071, 0.107, 0.107

The entries are the clustering index (see text) for each box, that is, the probability of finding any two, randomly chosen queens from the box dug in together in the same burrow

Methods

Queens were collected after landing from their nuptial flight and placed in glass vials (2.4 cm in diameter and 10.0 cm long) that were closed by cotton plugs. The closed vials were put in the same box, which was covered by a transparent red plastic filter, and the cotton was moistened every day.

In the autumn of 1989, 21 such vials were arranged: 13 vials with a single queen, and eight vials with three queens. In 1990, 20 vials were arranged: seven vials with a single queen, seven vials with two queens, and six vials with three queens. The queens were observed daily for 5 months. Their behavior (aggression, brood tending), as well as the progeny in each vial (i.e., eggs, larvae, and pupae), were recorded. Hierarchy was determined by observing the different postures in behavioral interactions—one queen (the dominant) was standing higher, while the other (the subordinate) was in passive crouching postures. At the end of the observation period, the productivity of each vial was ranked among all the vials of that year, and a Wilcoxon two-sample rank-sum test was performed.

A similar experiment, comprising 60 vials, 30 with a single queen and 30 with a pair of queens, was also arranged in the autumn of 1992, but observations in each vial were carried out only until eggs were deposited (ca. 3 months). In vials containing several queens, we could not attribute egg-laying date or productivity to specific queens.

Results

Total productivity per vial was somewhat greater in both 1989 and 1990, but not significantly so, in vials which initially contained a single queen when compared to vials which initially contained a group (two or three queens). When the per-queen output was considered, the advantage of the single queens was much more pronounced, with a pooled p value of 0.018. This is not surprising, as in a claustral species such as *M. semirufus* queens probably eat some eggs. Although consumption was not directly observed, the fluctuating number of eggs during time in some vials serves as an indicator of egg consumption.

The first year's experiment was continued until the appearance of the first workers. While most of the single queens (9 out of 13) succeeded in producing workers, only 2 (out of 8) triplets achieved this stage ($p = 0.127$ by Fisher's exact test).

Survival rate in the third year's experiment (until the first egg was laid in the experiment's setup) was 97% among the single queens, but only 60% among the queens placed in pairs, mainly as a consequence of aggressive interactions. This agonistic behavior starts with chases and bites, and quite often terminates after hours or days with the death of one of the queens. Thus, survival rate is much higher for single queens than for queens in pairs ($p < 0.002$, by a t-test that takes into account that data for pairs were obtained by cluster sampling).

In each of the three years, single queens laid their first egg early than groups of queens. While the overall level of significance is 0.106 (calculated by the Wilcoxon two-sample rank-sum test for data combined from several experiments), this non-significant result is contrary to what one should expect if queens lay their eggs independently of the presence of other queens. (In such a situation, the minimum of several observations is expected to be smaller than a single observation taken from the same distribution).

Experiment 4: digging rates in vials

Aim

In addition to measuring productivity and survival of founding groups of different sizes, the rate of digging of the different groups was also taken into consideration. Since we could not find any advantage of groups over single queens with respect to productivity, we looked for a possible advantage in the rate of digging. Are groups (i.e., pairs or triplets) quicker than single queens in reaching a safe depth?

Methods

Queens were collected after landing from their nuptial flight in the autumn of 1990, and placed in soil-filled digging vessels. These small vessels were constructed of vertical glass vials (2.4 cm in diameter and 10.0 cm long), each inserted into a larger plastic jar (through a hole in its bottom), and filled with soil. Altogether, 44 such vessels were arranged: 12 with a single queen, 11 with two queens, 11 with three queens—all these queens were placed simultaneously, on the same day. In the remaining 10 vessels, three queens were placed consecutively, that is, one queen was put on the first day, a second queen on the third day, and a third queen on the fourth day. The queens' behavior (digging, aggression, brood tending), as well as the progeny in each vial (i.e., eggs, larvae, and pupae), were recorded daily during 5 months. Hierarchy was determined in the same way as in the closed vials experiment. At the end of the experiment, the productivity of each vessel was ranked, and Wilcoxon two-sample rank-sum tests were performed.

Results

Also here, the productivity per vial was greater (though not significantly) for single queens than for groups, and more so when the per-queen success is considered ($p = 0.066$).

Table 3 The digging rates in vials experiment

	Single queens		Pairs/triplets		Remarks
	+	−	+	−	
After 1 day (depth of 1.0 cm)	7	15	3	19	More singles than groups ($p=0.281$)
After 2 days (depth of 1.5 cm)	11	11	4	18	More singles than groups ($p=0.055$)
After 3 days (depth of 2.0 cm)	7	5	3	19	More singles than groups ($p=0.020$)

The number of single queens and the number of groups that succeeded (+) or did not succeed (−) in reaching a safe depth. The two-tailed *p* values were obtained by Fisher's exact test

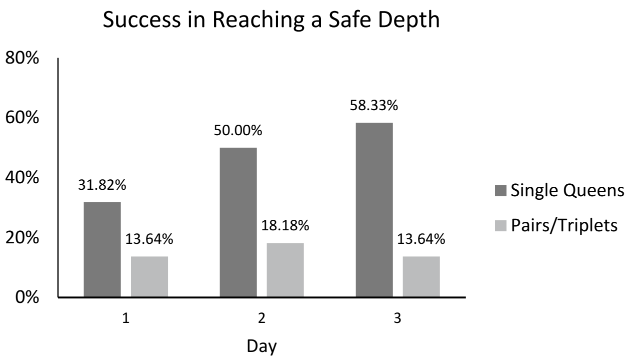

Fig. 2 The digging rates in vials experiment: Percentage of burrows dug by a single queen and by pairs/triplets that have reached a safe depth after 1, 2, and 3 days

Again, although not significant, the per-vial result is contrary to what one should expect if a queen's production is independent of the presence of other queens. (In such a situation, the more producers there are, the greater is the total expected production). Productivity for simultaneously placed triplets was significantly greater than for consecutively placed triplets ($p=0.038$). The observations (see Table 3; Fig. 2) show that groups are slower than single queens in their rate of digging.

Survival (until the end of the experiment) was 92% for single queens, but only 64% for queens that were placed in pairs, 64% for queens that were simultaneously placed in triplets, and only 47% in consecutively introduced triplets. Thus, survival rate is higher for single queens than for queens in a group ($p<0.010$, by a *t*-test that takes into account that data for pairs and triplets were obtained by cluster sampling).

Experiment 5: the effect of prior-residence

Aim

The aim was to assess the possibility of prior-residence advantage in multiple founder associations. Our hypothesis is that in owner–intruder interactions, the prior resident is more likely to be the dominant queen.

Table 4 Hierarchy in the effect of prior-residence experiment

Day	'Owner' dominant/ winner	'Intruder' dominant/ winner	Undetermined pairs	*p* Value
1	33 (52.4%)	15 (23.8%)	15 (23.8%)	0.007
3	27 (42.9%)	17 (27.0%)	19 (30.2%)	0.087
7	28 (44.4%)	15 (23.8%)	20 (31.7%)	0.033
30	23 (36.5%)	19 (30.2%)	21 (33.3%)	0.322

The first column gives the number of days after the introduction of the second queen; the next three columns give the number of pairs in each category (with their percentages in parentheses); the fifth column presents the one-tailed *p* values of the prior-residence advantage test

Methods

Sixty-three queens were collected after landing from their nuptial flight in November 1992, weighed and placed in 63 soil-filled digging vessels (such as those used in the digging vials experiment), with one queen in each vessel. On the following day, another nuptial flight took place, and 63 more queens were collected, weighed, and added to the already occupied digging vessels, one to each vessel. Thus, each vessel contained two queens, placed consecutively, with a one-day interval between the first (the 'owner') and the second (the 'intruder'). On the average, the 'intruders' were slightly (but not significantly) heavier than the 'owners': 48.03 ± 3.24 versus 47.38 ± 3.45 mg (mean ± SD), respectively. For distinction between the 'owner' and the 'intruder' in each vessel, one of the queens (either the 'owner' or the 'intruder', to avoid any confounding effects) was marked with a silver dot on her thorax (made by an "Edding 750 metallic" marker), and its identity ('owner' or 'intruder') was registered. The queens' behavior (e.g., digging activity and aggressive interactions), as well as the dominance hierarchy (see Methods in the closed vials experiment), were observed and recorded. Cases where no clear hierarchy was established, or where the structure of the burrow concealed one or both queens, were termed as undetermined.

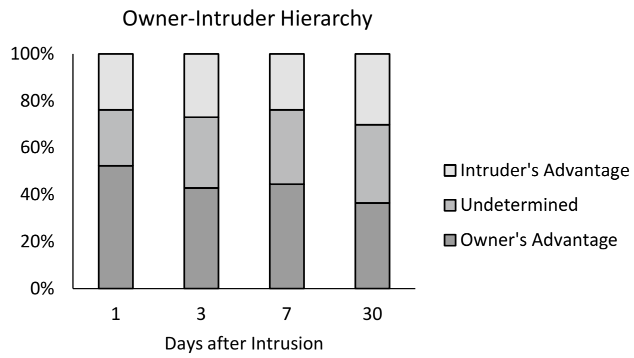

Fig. 3 The effect of prior-residence experiment: dynamics of prior-resident advantage along 30 days since introduction of the intruder

Results

Within the 63 pairs, 11 of the intruders and five of the owners died during the first month. In pairs where the hierarchy could be determined, it was more often the 'owner' that was the dominant queen (Table 4; Fig. 3). In general, interactions between a resident and an intruder are more commonly won by the resident (e.g., Maynard Smith and Parker 1977); thus, the prior-residence advantage was statistically tested in our experiment by a one-tailed binomial test, and the significance levels are presented in the rightmost column of Table 4. Within some pairs, aggressive interactions caused the death of one of the queens already during the first days, and the survivor was considered the winner thereafter. The relevance of initial 'ownership' fades with time, as can be seen by the decreasing advantage of the original 'owner'.

A slightly different measure of dominance within a pair, which takes into account the fading effect of initial ownership, is as follows: on each of the first 7 days, the pair was assigned either $+1$ (if the 'owner' was the dominant queen on that day), -1 (if the 'intruder' was the dominant), or 0 (if the situation was undetermined). These numbers were weighted such that each day received only half the weight of the previous day (to take into account the fading effect of initial ownership), and summed over these seven days. The measure thus obtained can range from -1 (i.e., consistent dominance of the 'intruder') to $+1$ (i.e., consistent dominancy of the 'owner'). The mean dominance measure of the 63 pairs was $+0.327$ (with a standard error of 0.068), indicating a significant ($t_{62} = 4.809\ p < 0.001$) prior-residence advantage.

Was the prior-residence effect stronger if the 'owner' had already invested in digging a burrow? We divided the 'owners' into two groups: those which had already dug a burrow (of at least 1.0 cm deep) during the day before the introduction of the 'intruder', and those which had not, and compare the frequency of dominant queens (on the day after the introduction of the 'intruder') in each group. If we leave out the undetermined pairs, then among the 17 'owners' which had a burrow, 13 (i.e., 76%) were dominant over the 'intruder', while among 31 'owners' which did not have a burrow, only 20 (65%) were dominant, and these two proportions are not statistically different ($p = 0.604$, using the Fisher's exact test).

Conclusions

In *M. semirufus*, queen associations occur more frequently under less favorable nest founding circumstances—high queen density and hard digging conditions. These associations do not confer any advantage neither in survival or productivity nor in facilitating digging rate. On the contrary—single queens survive longer and exhibit greater per-queen productivity than queens in groups, and digging of single queens is faster than of pairs/triplets. These results are even stronger given that aggression within groups happened quite quickly, and mortality affected a fairly large proportion of the groups so that the nests became the property of a single queen. Our results indicate that these seemingly pleometric interactions are probably forced associations, where the intruder tries to invade a digging site, although the prior resident has (at least temporarily) an advantage in these interactions.

Discussion

In most ant species in which pleometrosis takes place, the new colonies eventually end up as monogynous, having a single queen. Although only one of the foundresses will be the successful queen, a sufficiently large *a priori* probability of being the survivor motivates each queen to join other queens in pleometrotic nest founding. This implies an advantage of pleometrotic over single foundress nests, at least at some crucial stages during the establishment of the new colony, an advantage that compensates for the sharing of chances of success among the founding queens. Bartz and Hölldobler (1982) report that when young queens of the honeypot ant *M. mimicus* were grouped together, no aggressive behavior was observed. Groups had greater productivity, as well as a much lower mortality rate, than single queens. Only after the first workers had emerged, the queens in the groups started to space themselves out in the test tubes, and some kind of hierarchy among the queens was observed, with the α-queen closer to the brood. Workers fed and groomed the α-queen, and eventually either expelled or killed the rest of the queens. The workers also raided neighboring incipient colonies, and at the end of this process all the brood of an area ended up in the winning nest. Nonacs (1992) reports that in *L. pallitarsis*, pairs of queens raise more workers in the first brood than single queens. Mintzer (1979) reports that when he formed groups of young queens of *Camponotus vicinus*, some combinations of ants led to fighting, while others were compatible for the entire period of colony founding. The compatible pairs produced more workers than single females.

Our experiments regarding queen associations in *M. semirufus* indicate that groups do not exhibit any advantage over single queens. Generally, single queens had greater productivity than queens in groups, and single queens were not lagging after grouped queens in laying their first egg (their laying was even earlier, albeit not significantly so). Individual survival was higher among single than among queens in groups. Moreover, the rate of nest digging was faster for single queens than for groups. Since digging is costly—Johnson (2000) demonstrates some aspects of its cost to the digging queens—we can expect a slower rate of digging *per capita* in groups than for single queens. This sub-additive outcome is quite common in various situations, for example, in the familiar parental investment conflict (Maynard Smith 1977). Still, the finding that the *total* rate is slower for a group is less expected, and points to the intensity of conflict in the founding group. Note that Clark and Fewell (2014) report similar findings in *P. californicus*: escalated aggression in pairs of normally solitary queens, leading to a lower productivity, compared to pairs from the pleometrotic population.

Within groups in our experiments, aggressive interactions between the individual queens were quite common, starting immediately after the group was formed. These interactions either affected a stable dominance hierarchy or continued undecidedly. In most of the cases involving prior-residence asymmetry, it was the prior resident that came off as the dominant queen or the winner. In cases where withdrawal was prevented by the conditions of the experiment, the aggressive interactions were often fatal. Dead queens, however, were never devoured, although in *S. invicta*, Tschinkel (1993) observed cannibalism, and found this behavior as having a reproductive advantage.

The importance of digging conditions on queens association is exhibited by the density and by the soil hardness experiments. Indeed, as density of queens increases and digging becomes more straining (arising from increased soil hardness), the queens' tendency to cluster increases. We did not test the relative importance of these two factors in our study, but for *S. invicta*, Tschinkel (1998) has shown the greater importance of digging conditions (as demonstrated by the attraction to partially formed holes) as a cause of pleometrosis.

The features of nest co-founding that we found in *M. semirufus* are not typical of pleometrotic nest foundation. Possibly, queen associations of these species are not even short-term cooperation, but rather the attempts to invade nesting sites by wandering queens. One of the most crucial stages in the process of the claustral colony founding in *M. semirufus* is the interval between landing from nuptial flight, through the search for a suitable nesting site, until relative safety is reached in the depths of the new burrow. During the search, the queens wander around, exposed to high risks of predation and desiccation, while frequently testing the ground for digging conditions and finding out about the density of other queens in the area (Motro et al. 2016). After an appropriate place is found, the task of digging the burrow, which is also risky and energy consuming, takes place. Upon encountering an already digging queen, there can be a temptation to a wandering queen to avoid most of this toil, and to try to invade the burrow. The owner has then to decide whether to fight and chase the intruder away, or stay in the burrow and let the intruder in, postponing the costly struggle to a later stage (there is always the possibility that in the meanwhile, the intruder will anyhow pass away), or give in and leave the burrow to the intruder. Our field observations, as well as the results of the prior-residence experiments, indicate that usually (but not necessarily always) the prior resident is the dominant queen, determined to keep her investment and to chase away the intruder.

Our findings are quite similar to those of Pfenning (1995) for the facultatively pleometrotic ant *V. pergandei*. In his experimental setup, Pfenning found that pairs of queens did not last longer than single queens, and did not have any advantage regarding survival rate and productivity over single foundresses. Instead, he attributes the multiple foundress associations found in this species to selection on females to join any nest, in order to decrease their risk of being preyed upon or of desiccating aboveground. Nest owners appear to tolerate such joiners, presumably because fighting is costly and most intruders die naturally. Nevertheless, Pfenning's conclusions are different from those of Rissing and Pollock (1987), who found for the same species that colonies started by multiple queens opened 9 days earlier than those started by single queens, and were more successful at brood raiding.

Perhaps, such forced associations as observed in *M. semirufus* open the way for the subsequent evolution of facultative association and of cooperative nest founding, as suggested by Schwarz et al. (2011).

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