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**Research article**

# Number of queens in founding associations of the ponerine ant *Pachycondyla villosa*

**P. D'Ettorre[1,2], K. Kellner[1], J.H.C. Delabie[3] and J. Heinze[1]**

[1] *Biologie I, Universität Regensburg, 93040 Regensburg, Germany*
[2] *present address: Institute of Biology, Dept. Population Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, e-mail: pdettorre@bi.ko.dk*
[3] *Laboratório de Mirmecologia, Convênio UESC/CEPLAC, CP 07, Itabuna, 45600-000 Bahia, Brazil*

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**Abstract.** In the ant *Pachycondyla villosa,* new colonies are usually started cooperatively by two or more young queens who establish a dominance order with a division of labour. Co-founding can lead to primary polygyny, where queens stay together after workers have emerged. Here we show that two queens associations are the most common (47 %) and also the most stable in the field. When offered additional nest sites in the laboratory, two-queen associations did not split, whereas larger associations did so. We also show that solitary foundresses always accepted experimentally introduced alien queens, while these were attacked and sometimes killed in queen associations. The removal of dominant alpha queens from three-queen associations resulted in beta queens obtaining the dominant role and sometimes the destruction of the existing eggs. It appears that two queens suffice for a successful association and that pleometrosis is favoured by ecological constrains, such as nest-site limitation.

*Keywords:* Pleometrosis, dominance hierarchy, primary polygyny, ponerine ants, *Pachycondyla*.

## Introduction

Social insects, and ants in particular, show a great variation in queen number and therefore in their social structure (Crozier and Pamilo, 1996). In many species of ants, unrelated, young queens co-operate during the foundation of new colonies (pleometrosis). Such founding associations appear to grow faster than colonies initiated by single queens (Hölldobler and Wilson, 1990). Co-founding thus shortens the risky phase during which the queens are without workers and either rely on body tissue to nourish their larvae or have to leave the nest to forage (Bartz and Hölldobler, 1982; Rissing and Pollock, 1988; Heinze, 1993; Keller, 1995). During the first weeks of co-founding, young queens show little aggression amongst each other. Once the first few workers have emerged, the queens of most species become intolerant of one another and finally all except one are eliminated by other queens or workers (reviewed by Bernasconi and Strassmann, 1999). Under some ecological conditions, the chances of a pleometrotic foundress becoming the only queen in a successfully established colony may be greater than those of haplometrotic foundresses (Herbers, 1993).

Co-founding is common in the Neotropical species complex *Pachycondyla villosa* (Heinze et al., 2001; Kolmer et al., 2002; Lucas et al., 2002), In *P. inversa* founding associations differ in two ways from those of other ants. First, young queens engage in aggressive interactions early during the founding phase and establish a rank order, which regulates the division of labour: the lowest ranking queens forage, whereas higher ranking queens care for the brood (Kolmer and Heinze, 2000). Rank also affects reproduction: though all queens lay similar numbers of eggs, the dominant queens may destroy and eat eggs laid by subordinates. The queens' contribution to the first workers therefore is considerably skewed (Kolmer et al., 2002). Second, cooperation in *P. inversa* does not end with the emergence of workers, as it does in most other ant species, but may lead to stable primary polygyny (Trunzer et al., 1998). Division of labour in founding associations has been reported only in a few species showing also primary polygyny (Rissing et al., 1989; Johnson, 2004), suggesting that these traits maybe correlated.

A stable division of labour between a forager and a nurse might increase the success of two-queen associations over that of solitary queens. However, founding colonies with three or more queens are characterised by strong aggression (Kolmer and Heinze, 2000), which raises the question about the optimal group size of queen associations (Reeve and Ratnieks, 1993) and the structure of a possible social contract (Reeve and Nonacs, 1992): a subordinate queen may have the alternative options of establishing an own colony or joining another solitary foundress over staying in a large multi-queen association (Nonacs, 2001). We therefore investigated in *P. villosa* how founding associations develop in the field, and in the laboratory when additional nest sites are available. We examined whether associations with more than two queens are less stable than those with two queens and whether founding queens may join solitary foundresses or associations. We also considered the hypothesis of subordinate queens adopting the strategy of hopeful reproductive (cf. Monnin and Ratnieks, 1999).

The *Pachycondyla villosa* complex was taxonomically clarified by Lucas et al. (2002), some of the previous studies referring to *P. villosa* (Fabricius 1804) were indeed performed on the sympatric species *P. inversa* (Smith, F. 1858) (e.g. Trunzer et al., 1998) and little is known about *P. villosa* itself.

## Materials and methods

The studied population of the ponerine ant Pachycondyla villosa is located in an experimental cocoa plantation at Centro de Pesquisas do Cacau (CEPLAC) near Ilhéus, Bahia, Brazil (14°45'S, 39°18'W). Founding queens of *P. villosa* usually nest in knotholes and rotting cocoa pods. We collected a total of 74 founding colonies with a total of 173 queens. Laboratory experiments were all carried out in the laboratory of myrmecology at CEPLAC. Ants were kept under natural temperature and light conditions and were fed honey and pieces of dead insects ad libitum.

### *Dynamics of founding associations in the field*

To investigate the stability of queen associations in the natural environment, we searched founding associations in the field by inspecting knotholes. Queens were gently removed with soft forceps, marked with a small dot of Edding paint markers on the thorax and immediately returned into their original nest site (Table 1). Previously inhabited nest sites and all additional knotholes up to approximately 1m from the original site were monitored and the location of queens was noted once per day over a period of two weeks. Because the knotholes were very exposed and covered only by a thin layer of moss it was possible to check nest sites without disturbing the queens.

### *Experimental manipulation of number of nest sites*

In the laboratory, additional nest sites were added to freshly collected, natural associations of two, three and four queens (Table 2). Queens were transferred into 20 cm × 30 cm × 5 cm plastic boxes, in which two to four pieces of rotten cocoa pods (concave squares of approximately 3 cm × 3 cm) provided with moistened cotton served as nesting sites. The walls of the plastic boxes were coated with fluon to prevent the ants from escaping. The location of queens was noted twice per day over a period of five days.

### *Introduction of alien queens*

Immediately after collection, additional founding associations and solitary queens were brought to the laboratory (total of 48 queens) and housed in plastic boxes provided with a darkened glass tube with moistened cotton serving as nesting site. To determine whether solitary queens and established founding associations accept new queens and how foundress associations are formed, we placed queens that were found solitarily in the field either together with another solitary queen (n = 6) or with a founding association of two (n = 4) or three queens (n = 6). Solitary founding queens were housed in circular plastic boxes of 10 cm in diameter and founding associations in plastic boxes of 12 cm diameter. All queens were individually marked with a small dot of Edding paint markers on the thorax. Right after the introduction of the alien queen, the behaviour of ants was observed continuously for 10 min. All interactions were noted and, in particular, the frequency of antennal contact, antennal boxing (ritualised aggressive interaction), mandibular opening (threat display), biting, biting with dragging, and stinging attempts was quantified. Experimental groups were checked again after one, two and 24 hours to determine whether the intruder was accepted or killed. These experiments were carried out one to two days after collection.

### *Removal of alpha queens*

Seven freshly collected three-queen associations with 2 to10 eggs were housed in circular plastic boxes of 12 cm diameter provided with a darkened glass tube with moistened cotton serving as nesting site and observed to determine the rank order among them (15 min continuous observation and then scans of 1min each until the rank order was clear). All interactions were noted and quantified as for the previous experiment. The alpha queen was then removed from each association and the remaining two queens were observed for 15 min continuously and then during five scans of 1min each every hour to determine the rank order and the aggression rate (Table 3).

## Results

The majority (47.3 %) of founding colonies collected in the field were formed by two queens. Solitary foundresses (17.6 %) and three-queens association (23 %) were less common, while four-and five-queen associations were relatively uncommon (respectively 8.1 and 4 %, Fig. 1).

### *Dynamics of founding associations in the field*

Monitoring eight founding associations in the field over a period of up to two weeks indicated a certain instability in the queen number. New queens were seen joining established founding associations and groups of queens were seen moving from one nest site to another close by (Table 1). In one particular case (nest G, Table 1), seven foundresses were observed in varying combinations in three closely adjacent knotholes in a cocoa tree over a period of 13 days. It was not possible to gather information about the rank order of queens in these field associations, consequently we do not know whether subordinates moved more often than dominant queens. Overall it appears that two-queen associations were the most stable in the field (B, D and E, Table 1). Several nest cavities inhabited were found deserted a few days later, inundated by rain water or inhabited by other ant species.

**Table 1.** Dynamics of founding queen associations observed in the field

| Nest (days of observation) | # of queens at day 0 | # of new queens (day) | # of leaving queens (day) | Final # of queens | Notes |
|---|---|---|---|---|---|
| A (13) | 1 | 2 (day 2)<br>1 (day 4) | 1 (day 7) | – | nest deserted (day 10) |
| B (11) | 3 | – | 1 (day 4) | 2 | stable 2-queen association from day 4 |
| C (13) | 2 | 1 (day 1) | 1 (day 4) | – | nest deserted (day 7) |
| D (7) | 2 | – | – | 2 | stable 2-queen association |
| E (11) | 2 | – | – | 2 | both moved to a new nest (day 4) and back to the old nest (day 8) |
| F (13) | 4 | 2 (day 2)<br>1 (day 4)<br>1 (day 7) | 2 (day 2) | 4 (2 old residents and 2 new queens) | some queens moved to a close nest site |
| G (13) | 4 | 4 (day 4) | 1 (day 3) | 3 (1 old resident and 2 new queens in the original hole, the other nest cavities deserted) | 7 queens observed in varying combinations in 3 close nest cavities |
| H (13) | 3 | – | – | – | nest deserted (day 1) |

**Table 2.** Number of nest sites occupied in the laboratory by associations of different queen number. Queens were observed in another nest site significantly less frequently in two-queen associations than in associations with more than two queens (Yates corrected $\chi^2 = 5.06$, $P = 0.02$).

| Association | # queens | # available nest sites | # occupied nest sites |
|---|---|---|---|
| A-1 | 2 | 2 | 1 |
| A-2 | 2 | 2 | 1 |
| A-3 | 2 | 2 | 1 |
| A-4 | 3 | 3 | 3 |
| A-5 | 3 | 3 | 2 |
| A-6 | 3 | 3 | 2 |
| A-7 | 3 | 3 | 2 |
| A-8 | 4 | 4 | 2 |
| A-9 | 4 | 4 | 3 |

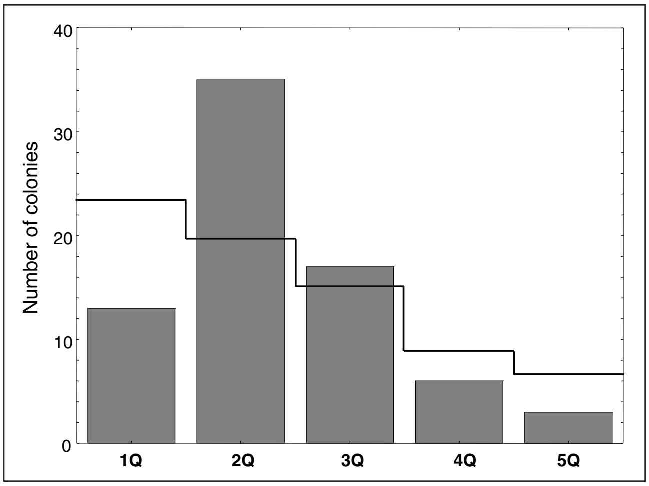

**Figure 1.** Distribution of queen number in incipient colonies collected in the field. The frequency distribution is significantly different from a Poisson distribution truncated at 1 ($\chi^2 = 20.26$, $P < 0.001$). Expected frequencies are represented by the solid line

### *Experimental manipulation of number of nest sites*

Results are summarized in Table 2. Associations with two queens were stable, queens explored the environment but were never found in a nest site different from the one they had inhabited during the previous scan. In contrast, in all 3- and 4-queen associations, several queens inspected the additional nesting sites and moved there either alone or with another queen and / or brood. Queens were observed in another nest site significantly less frequently in two-queen associations than in associations with more than two queens (0 of 3 versus 6 of 6; Yates corrected $\chi^2 = 5.06$, $P = 0.02$).

**Table 3.** Rank order in three-queen associations and experimental removal of dominant alpha queens. In all associations, queens were individually marked with a small dot of Enamel paint on the thorax or gaster, R = red, G = green, W = white, O = orange, B = blue, Y = yellow.

| **Association** | **Original rank order** (phase 1) | **Rank order after removal of alpha** (phase 2) | **Eating of existing eggs** |
|---|---|---|---|
| A-20 | R → G → W | G → W | yes |
| A-21 | O → R → G | R → G | no |
| A-22 | O → B → W | B → W | yes |
| A-23 | G → B → R | B → R | no |
| A-24 | O → R → G | R → G | no |
| A-25 | W → R → B | R → B | yes |
| A-26 | R → G → Y | G → Y | no |

Migration did not lead to the permanent split of associations during the observation time. Instead, queens continued to move between nesting sites inhabited by other queens until the experiment ended.

*Introduction of alien queens*

The alien queen was always accepted by solitary foundresses, but was always attacked and sometimes even killed by founding associations (three out of six trials in three-queen associations). Solitary foundresses and founding associations differed significantly in their behaviour towards intruders (Fig. 2). Queens in founding associations engaged in overt fighting with the intruder, but solitary foundresses showed more ritualised aggression and less violent attacks. Solitary foundresses antennated the intruder more, displayed more mandibular opening, but rarely stung or dragged. In contrast, queens in founding associations were more prone to bite, drag and sting the intruder (Fig. 2). In eight of ten trials (the four 2-queen and four of six 3-queen associations), overt aggression towards the intruder was almost exclusively shown by the most subordinate queen. Usually the intruder, when accepted, entered the hierarchy at the lowest rank, only in one case the intruder became the new dominant queen.

*Removal of alpha queens*

When alpha queens were removed from associations, aggression between the remaining two queens did not escalate but remained ritualised (Fig. 3). In all trials, the original beta queen became the new alpha of the two-queen association and in three out of seven colonies, the two queens ate all the eggs originally present in the association (Table 3). In one case, both remaining queens ate the eggs, in one case the new alpha-queen and in one case the new beta-queen ate the eggs.

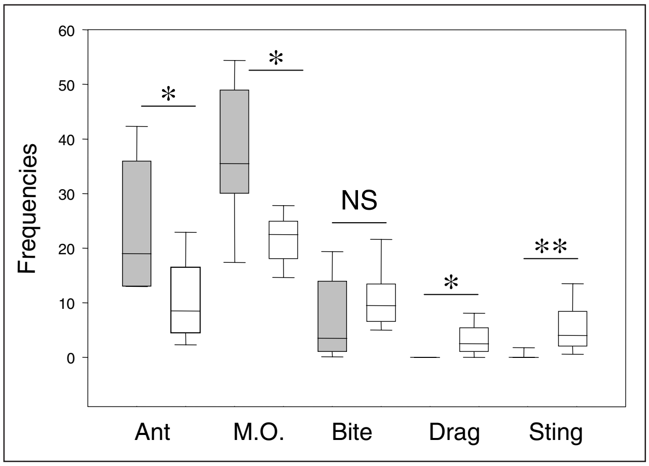

**Figure 2.** Behavioural interactions showed by solitary foundresses (grey boxes) and associations of two and three queens (white boxes) towards an intruder queen.
Ant = investigation by antennation, M.O. = mandibular opening, Bite = biting, Drag = biting with dragging, Sting = stinging. * = P < 0.05, ** = P < 0.01, Mann-Whitney U-test.

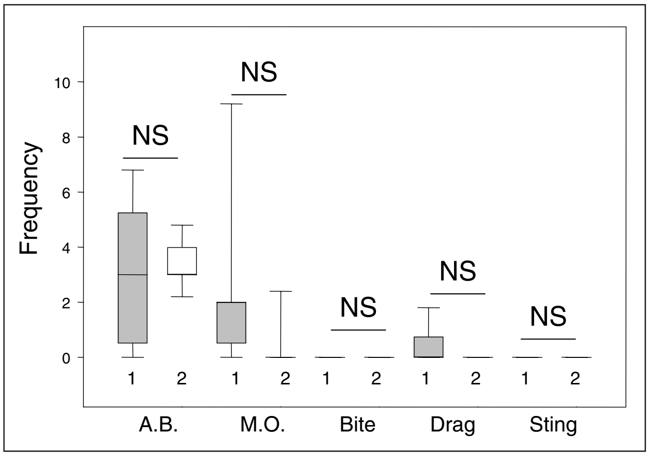

**Figure 3.** Internal aggression level showed by queen associations before the removal of the alpha queen (1) and after the removal of the alpha queen (2) (see also Table 3).
A.B. = antennal boxing, M.O. = mandibular opening, Bite = biting, , Drag = biting with dragging, Sting = stinging. There is no significant difference between phase 1 and 2 (Mann-Whitney U-test).

## Discussion

Our results show that in the ant Pachycondyla villosa founding associations of two queens are the most abundant in nature and that they are more stable, both in the field and in the lab, than larger associations. Founding associations of ant queens are considered to be stable over space and time as long as they do not yet contain workers. Queens are believed to form groups during the first days after mating and remain together in the same grouping until aggression in the late founding phase leads to the expulsion or elimination of all but one queen (Bernasconi and Strassmann, 1999). This appears to be strikingly different in founding associations of *P.*

*villosa*. Co-foundresses, that are typically unrelated (Kellner et al., in prep.), establish and maintain dominance hierarchies with a division of labour, similarly to *P. inversa* (Kolmer and Heinze, 2000). Whereas this division of labour appears to be well established in two-queen associations even when empty nest sites are available, the situation is more complicated in larger associations. In three- and four-queen associations, several queens left the nest with or without brood and settled in other nest sites provided in the lab. Similarly, in the field, individually marked queens were seen in different, adjacent nest cavities and with different companions on subsequent days. Even if these field data are merely descriptive and based on a small sample size, they are relevant because consistent with our laboratory data.

Such temporal fluidity of foundress associations, where subordinate have the opportunity to either switch groups or live solitarily, fits the definition of a social contract, as modelled in Polistine wasps (Nonacs, 2001). At present, we do not have enough data on reproductive skew to distinguish whether these groups are organized by conventions or transactions, even if the occurrence of aggressive interactions during the hierarchy formation works in favour of the transaction hypothesis (cf. Reeve et al., 1999).

As in other ant species, it appears that in *P. villosa* the optimal group size is around two (cf. Bartz and Hölldobler, 1982). Solitary foundresses readily accepted an alien queen to form a two-queen association, showing less overt aggression than two or three-queen associations towards an intruder. In these associations, the intruder was attacked mostly by the lowest ranking queen. Subordinate queens may be selected to prevent additional queens from joining the hierarchy (cf. Seger, 1993) or, if they do, last joiners must take the lowest rank and engage in the risky task of foraging. Established hierarchies may allow a smooth transition if the dominant queen should die. Our data suggest that a queen in a hierarchy of foundresses might be a hopeful reproductive who will increase her fitness if she becomes the alpha (Monnin and Ratnieks, 1999). Indeed, when alpha-queens were experimentally removed, beta-queens always became dominants without additional fighting. In some cases, beta-queens destroyed the eggs originally present in the association, but the interpretation of this finding is difficult since it was not consistent in all trials.

Pleometrosis and queen foraging have positive and interactive effects on survival and brood production in incipient colonies (Johnson, 2004). Given the occurrence of primary polygyny in *P. villosa* (P. D'Ettorre, K. Kellner and R. Rodrigues Da Hora, unpubl.), beta-queens might have higher chances of reproducing in a successful mature colony than in other ant species where queen number is reduced to one after the first workers have emerged. On the other hand, being in larger associations can be too costly if the chances of becoming dominant are low (Monnin and Ratnieks, 1999). In *P. inversa*, the rate of aggression per queen is considerably higher in colonies with three and more queens than in colonies with two queens (Kolmer and Heinze, 2000 and unpubl. data). Instead of staying in a single nest site with the same suite of nestmates, subordinates who do not have the possibility to place themselves at the top-positions of the hierarchy might simply escape the attacks by wandering from nest site to nest site and try to associate with different companions, at least during the first weeks of founding. Thus, larger foundress associations might eventually break into separate founding colonies. Alternatively, larger associations might develop into polygynous colonies inhabiting a network of neighbouring nest sites. Polydomy of mature nests has recently been documented in our study population (P. D'Ettorre, unpubl.) and might be an adaptation to nest site fragility. Polydomy is well known from ants nesting in decaying wood in temperate forests (Herbers, 1989) or carton nests, live or rotten plant material on trees in tropical habitats (Delabie et al., 1991). The nest sites of *P. villosa* are often quite ephemeral and not well concealed; they are easily destroyed by rain or taken over by other animals. It might therefore be advantageous for *P. villosa* queens to attempt to secure several nesting sites already during the founding phase.

Previously studied pleometrotic associations were all from ant species with seasonal production of sexuals, where all queens start new colonies synchronously (Hölldobler and Wilson 1990). In addition to spatial fluidity, the structure of founding associations of *P. villosa* might also vary over time. Similar to other tropical ants (e.g. Kaspari et al., 2001), young queens of the *P. villosa* complex appear to be produced during most of the year (Kolmer et al., 2002) without well-organized mating flights and young queens might eventually seek adoption into older founding associations.

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