

Effect of pleometrosis and brood transplantation on colony growth of the black garden ant, *Lasius niger*

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ABSTRACT. In response to intra- and interspecific competition, ant colonies have evolved mechanisms to maximize early colony growth. Through the analysis of photographic images, this study describes the individual effect of two different strategies, pleometrosis and pupae transplantation, on the growth of *Lasius niger* colonies. Results showed that both methods had an overall positive effect on colony production, albeit the magnitude at which pupae transplantation boosted colony growth was much larger than pleometrosis. After six weeks, colonies with two and three queens contained, respectively, 26% and 83% more brood than control colonies with only one queen. Queen association also led to overall decreased queen fecundity and death of all resident queens in over 60% of the colonies due to queen fights. After eight weeks, colonies that received 30 or 60 foreign pupae had produced on average 256% more brood than controls and nanitic workers that were 7% longer than those from colonies that did not receive transplanted pupae.

Keywords: pupae adoption, multiple queens, ant reproduction, colony founding, social insects, formicidae

INTRODUCTION

Most colonies of *Lasius niger* are started by a single claustral queen that uses her stored energy reserves to raise her first brood. However, the high numbers of *Lasius* queens occurring after mating flights, coupled with the fact that queens avoid areas frequented by workers of established colonies (Sommer & Hölldobler 1995), leads to a high density of new nest foundations in the field. In response to crowding and high inter-colony competition, incipient colonies may use two different strategies to gain competitive advantage. One strategy is pleometrosis, where queens enter facultative associations with non-related queens during colony founding (Bernasconi & Strassmann 1999). Such pleometrotic association allows claustral species like *L. niger*, in which

queens have a limited amount of resources to invest in reproduction, to produce more workers than single queens and to do so in shorter time (Waloff 1957, Bartz & Hölldobler 1982, Sommer & Hölldobler 1995). That may translate into (i) a higher foraging success, which will improve early colony growth and survival and (ii) an advantage when it comes to successfully defending the nest against brood raiding, usurpation (Bartz & Hölldobler 1982, Rissing & Pollock 1991, Tschinkel 1992b, Bales & Adams 1997) and predation (Jerome et al. 1998) by neighbouring colonies. That said, an alternative reason for pleometrosis can also be a strong pressure for the newly mated queens to leave the soil surface and use any available holes, including those excavated by other queens (Tschinkel 1998). Despite the potential benefits of pleometrosis, there is also an impor-

tant cost at the queen level, as the emergence of workers and the start of foraging activities generally trigger the onset of queen fights that continue until only one of the queens survives (Sommer & Hölldobler 1995, Bernasconi & Keller 1998, Aron et al. 2009).

A second strategy to improve competition by increasing early colony growth is the sequestration of foreign pupae and larvae through the raiding of brood from neighbouring colonies. This strategy provides ant colonies in the field with a competitive advantage derived from a rapid increase in the number of individuals in the nest, which leads to earlier colony maturation (Pollock & Rissing 1989, Rissing & Pollock 1991, Tschinkel 1992a, Gadau et al. 2003).

In addition to building up higher numbers of workers, it may also be advantageous for young colonies to produce larger individuals, as size influences the range of tasks a worker can perform (Wilson 1978, Miranda & Vinson 1981, Arnan et al. 2011). For example, mature colonies in the field have not only a higher number of workers, but also larger individuals compared to incipient colonies (Porter & Tschinkel 1985, Hasegawa & Imai 2011), and worker polymorphism and task repertoire have been found to correlate with colony size (Anderson & McShea 2001). Whether increased colony growth via pleometrosis or sequestration of foreign pupae leads to production of larger workers, and in this way further improves the competitive abilities of incipient colonies of *Lasius niger*, remains to be tested.

In order to elucidate the potential competitive advantage colonies can achieve through pleometrosis and brood raiding, the present study focused on analysing the effects of multiple founding queens and pupae adoption on early colony growth and worker size of *Lasius niger*.

METHODS

Experimental colonies

Queens of *Lasius niger* were collected immediately after their mating flight on 17th July, 2014, in Aarhus, Denmark (56°11'04.9"N 10°07'01.1"E), and were kept individually in plastic test tubes containing water behind a cotton plug. The indi-

viduals necessary for the multiple queen experiment (pleometrosis) were placed in groups of 1, 2 or 3 queens per test tube less than 60 minutes after collection to minimize a build-up of territorial behaviour over time. The day after collection, queens were cooled to 16.5°C for 60 minutes for easier manipulation before being transferred into artificial nests.

The artificial nests were created in Petri dishes (5.5cm Ø). The lids of the dishes were perforated (single hole, 4mm Ø) to allow the movement of workers in and out of the nest; the bases were partly filled (approximately 2/3 of their volume) with a substrate consisting of a combination of Plaster of Paris and charcoal (8:1). A small piece of tape on the substrate allowed the provision of a drop of water for the queens to drink until the first workers emerged, at which point workers provided the queen with water. The nests were then placed in a bigger Petri dish (14cm Ø) that contained a 10cm test tube filled with water and also served as an arena where the feedings would take place. *Ad libitum* sources of carbohydrate (20% sucrose solution in water) and protein were added when the first workers emerged. Protein consisted of house flies, pieces of shrimp and a 1% solution of peptone from bovine meat (Sigma-Aldrich, product number 70175), alternated weekly. All arenas and nests were kept inside opaque paper boxes in a dark 25°C chamber for the duration of the experiment. Feedings and population assessments were performed outside the chamber, in light conditions and at room temperature (August-September, 2014, mean daily temperature approximately 18°C).

Colony assessments

During the experiment, non-destructive assessments of colony sizes were based on photographic images taken every two weeks (camera: Canon EOS 1000D, lens: EF-S 18-55mm f/3.5-5.6 IS). The software *ImageJ* (Open Source: <http://imagej.nih.gov/ij/>) was used to analyse the images in order to perform counts of brood and workers and to measure colony areas and lengths of nanitic workers. After the last assessment on week eight after the nuptial flight, the ant colonies were exposed to gradually decreasing temperatures (5 days at 20°C followed by 5 more days at 15°C). Lower temperatures reduce the activity level of the colo-

nies, making it possible to perform a more exact manual count of the number of pupae and workers that were used to correct the estimations obtained through photographic analysis. The number of eggs and larvae, however, could not be counted manually in this way due to the delicate nature of these stages and our need for keeping the individuals alive for future experiments.

Multiple queen experiment

The multiple queen experiment started with 16 single queen colonies, 16 colonies with two queens and 13 colonies with three queens. During the experiment, a mite infection reduced the sample size to a total of 29 usable colonies: 11 control colonies with one queen, 8 colonies with two queens and 10 colonies with three queens. In addition, queen fights resulted in 12 colonies becoming orphan, as all the queens died from their injuries, which led to a stop in brood production. As a result, the set of data (images) analysed in this experiment belonged to week six of the experiment, where all queens were still present in all colonies and queen fights had not yet started.

Pupae transplantation experiment

In the pupae transplantation experiment, mite infections also reduced the sample sizes to 10 control colonies, 14 colonies receiving 30 pupae and 15 colonies receiving 60 pupae. All these colonies were founded by a single queen. Pupae used in the transplantation experiment were collected from mature colonies in the field (at coordinates 56°13'37.5"N 10°34'29.0"E) and were transplanted four days after the mating flight, at which point all queens but one had laid eggs. All queens readily accepted the transplanted pupae, albeit after one day all queens had divided the pupae into two groups: some were in close proximity to the queen and her eggs, and some were piled on the opposite side of the nest, as far away as possible, together with soil particles. The latter group was considered discarded material and was subsequently removed from the nest. Consequently, despite the two discrete transplantation treatments (30 and 60), the actual number of accepted pupae varied between colonies and treatments. However, the average number of pupae surviving in colonies in the 60 pupae treatment (mean \pm SE = 34.33 \pm 1.81) was significantly higher (GLM,

Poisson distribution and log-link function, Chi-square = 526.34, $p < 0.0001$) than that in the 30 pupae treatment (mean \pm SE = 19.93 \pm 1.31). The exact number of transplanted pupae hatching into imago workers was monitored and used for calculations of survival in relation to the number of pupae initially transplanted into the colony; i.e. discarded pupae were included as non-surviving. The number of surviving pupae in each colony was subtracted from the total number individuals to obtain intrinsic queen production.

Nanitic size

In the analysis of nanitic size, photos were used to measure length longitudinally from mandibles to the distal end of gaster using *ImageJ* software. Five to eight nanitic workers (much smaller and lighter in colour than adopted ones) were selected per colony, depending on availability, and their lengths averaged to provide a single value per experimental colony.

Statistical analysis

Seven response variables were analysed in the two experiments. Four of them were readily obtained through analysis of photographic images. These were (i) colony area (cm²) measured as the amount of substrate surface occupied by all brood, workers and queen(s), (ii) total number of imago workers (including transplanted individuals), (iii) total number of brood (i.e. eggs, larvae and pupae) and (iv) average length of intrinsically produced nanitic imago workers. For the brood transplantation experiment, total number of pupae and imago workers were corrected after a more accurate manual count was performed at the end of the experiment. These counts were not available for the multiple queen experiment, due to having to work with results from the analysis of images at week six, as explained above.

Per capita queen production was calculated in two different ways: (i) for the multiple queen experiment, the total number of eggs, larvae, pupae and imago workers at week six was divided by the number of queens in each colony, (ii) for the pupae transplantation experiment, as imago workers were a mixture of intrinsic adopted workers, queen production was calculated as the total number of egg, larvae, pupae and workers at week eight, minus the number of transplanted pupae that survived.

Because an unknown fraction of the eggs in the nest may be consumed by the colony, number of brood and per capita queen production were also analysed after excluding egg counts.

The software JMP 12.0 was used for statistical analysis. For data on nanitic length, ANOVA was used for overall analysis and Tukey-Kramer test for multiple comparisons between treatments within experiments. For data on colony areas, normality and variance homogeneity could not be achieved through data transformation and thus Kruskal-Wallis tests were used to analyse overall differences and paired Wilcoxon tests were used for multiple comparisons. Counts on brood and workers were analysed using generalized linear models with a Poisson log-linear model, adjusting p-values for pairwise comparisons with sequential Bonferroni correction (Holm 1979).

RESULTS

Figures 1 and 2 illustrate colony growth per week in the multiple queen and the brood transplantation experiments, respectively. It is clear that while both boosting methods had a positive effect on colony growth, pupae transplantation led to a more pronounced increase in numbers.

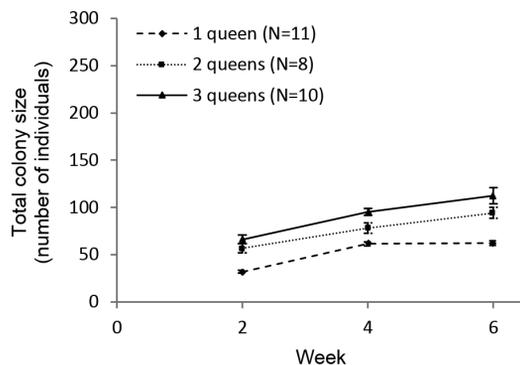


Fig. 1. Total colony size for the multiple queen experiment by week. Number of individuals included eggs, larvae, pupae and imago workers. Number of queens: dashed line = 1, dotted line = 2, solid line = 3. Vertical bars represent standard error.

Multiple queen experiment

Analysis of colony growth at week six revealed that queen associations had a significant positive effect on the number of workers and brood present in the nest as well as on the total nest area occupied by the colony, when compared to controls (Table 1).

The area occupied by colonies with two or three queens was on average 108% larger than that of colonies with a single queen. The number of workers in colonies with multiple queens was on average 142% higher. The only negative effect of multiple queen associations was a reduction in the average per capita queen production. When eggs were included in the analysis, queens that started a colony alone produced 60.8% more brood than queens in the pleometrotic colonies (calculated by dividing the total number of brood by the number of queens in each colony and pooling the two- and three-queen treatments). When the number of eggs was excluded from the calculations, a significant difference arose between treatments, with queens in groups of three producing less larvae and pupae than queens placed in pairs.

Despite the decrease in per capita production, nests with two and three queens still contained significantly more brood (including eggs, larvae and pupae) than control colonies started with a single queen (26.6% and 83.4%, respectively). However, when excluding the number of eggs from counts of brood, groups of two queens did not differ significantly from the experimental controls, while nests with three queens had significantly more larvae and pupae than the other two groups (Table 1).

Pupae transplantation experiment

The overall mean survival rate of pupae in the two transplantation treatments (number of pupae developing into imago workers/number of pupae added) was 61.8% (66.4% for 30 pupae and 57.2% for 60 pupae) and did not vary significantly between the two treatments (Binary logistic model, ChiSquare = 0.26, $p = 0.61$). Hence, colonies in the 60 pupae treatment received on average significantly more pupae than those in the 30 pupae treatment. The evaluation of the colonies at week eight of the experiment showed that pupae transplantation had a significant effect on all

Table 1. Mean (\pm SE) colony size by treatment after six weeks in the multiple queen experiment. Colony area was analysed with non-parametric Kruskal-Wallis and Wilcoxon tests. Count data was analysed with GLM using Poisson log-linear models. Multiple comparisons were corrected with the sequential Bonferroni method. Values in the same column with different uppercase letters are statistically different ($p < 0.05$). Total number of intrinsic brood included eggs, larvae and pupae. Per capita queen production included all developmental stages (eggs, larvae, pupae, imago workers).

Mean \pm (SE)	Colony area (cm ²)		Total number of workers		Total number of intrinsic brood		Total number of brood (eggs excluded)		Per capita queen production		Per capita queen production (eggs excluded)	
	Kruskal-Wallis	Mean \pm (SE)	Poisson	Mean \pm (SE)	Poisson	Mean \pm (SE)	Poisson	Mean \pm (SE)	Poisson	Mean \pm (SE)	Poisson	
Number of queens (sample size)	1 (11)	0.63 ^A (0.08)	7.2 ^A (1.84)	55.2 ^A (3.51)	39.4 ^A (4.07)	55.2 ^A (3.51)	39.4 ^A (4.07)	55.2 ^A (3.51)	46.5 ^A (5.11)	ChiSquare = 17.4 109.02 156.6 22.4 65.20 79.1 p < 0.0002 p < 0.0001 p < 0.0001 p < 0.0001	ChiSquare = 79.1 p < 0.0001	
	2 (8)	1.48 ^B (0.11)	23.4 ^B (1.15)	70.9 ^B (5.18)	40.4 ^A (4.47)	35.4 ^B (2.59)	40.4 ^A (4.47)	35.4 ^B (2.59)	31.9 ^B (2.45)			
	3 (10)	1.22 ^B (0.18)	19.8 ^B (3.04)	102.7 ^C (5.65)	52.2 ^B (6.48)	34.2 ^B (1.88)	52.2 ^B (6.48)	34.2 ^B (1.88)	24 ^C (3.03)			

Table 2. Mean (\pm SE) colony size by treatment at week eight in the pupae transplantation experiment. Colony area was analysed with non-parametric Kruskal-Wallis and Wilcoxon tests. Count data was analysed with GLM using Poisson log-linear models. Multiple comparisons were corrected with the sequential Bonferroni method. Values in the same column with different uppercase letters are statistically different ($p < 0.05$). Total number of intrinsic brood consisted of eggs, larvae and pupae and therefore not transplanted individuals, as they had developed into imagines at this point. Per capita queen production included all developmental stages (eggs, larvae, pupae, imago workers), but excluded transplanted workers.

<i>Mean \pm (SE)</i>	Colony area (cm ²)		Total number of workers		Total number of intrinsic brood		Total number of intrinsic brood (eggs excluded)		Per capita intrinsic queen production		Per capita intrinsic queen production (eggs excluded)	
	<i>Kruskal-Wallis</i>	<i>Mean \pm (SE)</i>	<i>Poisson</i>	<i>Mean \pm (SE)</i>	<i>Poisson</i>	<i>Mean \pm (SE)</i>	<i>Poisson</i>	<i>Mean \pm (SE)</i>	<i>Poisson</i>	<i>Mean \pm (SE)</i>	<i>Poisson</i>	
0 (10)	0.82 ^A (0.25)		15.7 ^A (3.05)		61.3 ^A (13.9)		58.5 ^A (4.1)		77 ^A (14.1)		74.2 ^A (4.75)	
Pupae transplanted (sample size)	4.28 ^B (0.21)	ChiSquare = 23.2	29.7 ^B (2.6)	ChiSquare = 121.7	220.2 ^B (11.7)	ChiSquare = 1276.7	182.4 ^B (12.4)	ChiSquare = 976.7	230 ^B (11.9)	ChiSquare = 1054.3	192.2 ^B (12.5)	ChiSquare = 776.2
	60 (15)	4.95 ^B (0.21)	39.3 ^C (2.5)	$p < 0.0001$	216.9 ^B (11.3)	$p < 0.0001$	191.9 ^B (13.2)	$p < 0.0001$	222 ^B (11.5)	$p < 0.0001$	196.9 ^B (13.4)	$p < 0.0001$

Table 3. Mean (\pm SE) average length of intrinsic nanitics by treatment in the two experiments. ANOVA tested for overall differences and Tukey-Kramer test was used for all pair comparisons. Values within the same experiment with different uppercase letters are statistically different ($p < 0.05$).

	Multiple queen experiment			Pupae transplantation experiment		
	1 (11)	2 (8)	3 (10)	0 (10)	30 (14)	60 (15)
Number of queens or transplanted pupae (<i>sample size</i>)						
Mean length of nanitics, mm (SE)	2.41 ^A (0.03)	2.42 ^A (0.02)	2.37 ^A (0.03)	2.42 ^A (0.03)	2.57 ^B (0.03)	2.61 ^B (0.04)
<i>ANOVA</i>	F = 0.69 P = 0.509			F = 7.03 p = 0.003		

parameters of growth, albeit only the total number of workers (which included imago workers developing from transplanted pupae) was significantly affected by the intensity of the transplantation (Table 2).

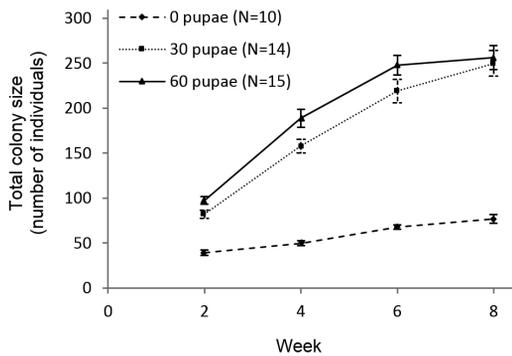


Fig. 2. Total colony size for the brood transplantation experiment by week. Number of individuals included eggs, larvae, pupae and imago workers. Number of transplanted pupae: dashed line = 0, dotted line = 30, solid line = 60. Vertical bars represent standard error.

For those variables where the number of pupae transplanted did not have a significant effect on the response, the average effect of the two treatments (30 and 60 pupae) was calculated. The results revealed that colonies that received pupae showed an average increase of 462% in colony area when compared to control colonies (Table 2). The number of total brood (eggs, larvae and pupae) in these colonies was also increased, in this case by 256%. This does not include transplanted individuals as they had already developed into

imago workers at week eight of the experiment. When excluding the number of eggs from total brood calculations, colonies that received transplanted pupae still contained an average of 220% more larvae and pupae than control colonies.

Lastly, pupae transplantation also boosted the intrinsic production rate of queens. Queens in colonies that received pupae produced on average 193% more brood than those in control colonies. When excluding eggs from the calculations and only looking at production of larvae and pupae, the average increase for the two treatment groups versus the control was of 162%.

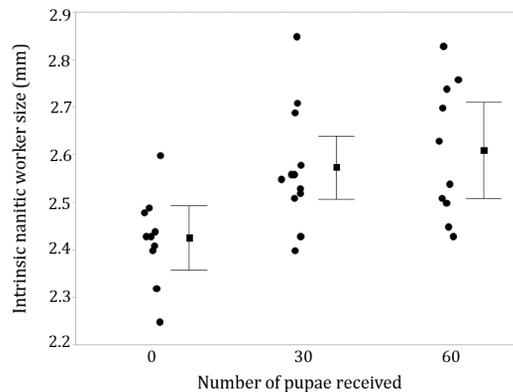


Fig. 3. Scatter plot showing length of intrinsic workers vs. number of pupae transplanted at the brood transplantation experiment. Black square shows category mean, vertical bars represent standard error.

Nanitic workers

Besides parameters associated with colony growth, the size of intrinsic imago workers was

also affected (Table 3). When comparing the different treatments within methodologies, we observed that the presence of more queens did not have an effect on imago size, whereas pupae transplantation led to queens producing nanitic imago workers that were significantly larger than the controls that did not receive pupae. Since there was no significant difference in nanitic size between the two transplantation intensities, a pooled average was calculated. Pupa transplantation then led to nanitic workers that were on average 7% larger than those in control colonies (Table 3, Fig. 3).

DISCUSSION

Both of the tested approaches to boosting colony growth, multiple queen foundresses and pupae transplantation, had a positive effect on colony development when compared to control colonies. However, queen associations had several drawbacks, e.g. reduced per capita production and risk of colonies becoming orphan after queen fights; added to the fact that the effect on colony growth was inferior to that obtained through brood transplantation.

Colony growth

As opposed to other studies in which colonies with multiple queens were successfully established (Johnson 2004, Offenberg et al. 2012b, Peng et al. 2013, Helms et al. 2013), our experiment resulted in 12 nests being left orphan and, therefore, unviable after the queen fight. These 12 nests represented 80% of the replicates in the three-queen treatment and 50% of the two-queen. *Lasius niger* is a monogynous species (Sommer & Hölldobler 1995, Buffin et al. 2010) and, therefore, queen fights are not surprising. However, similar experiments with this species reported all pleometrotic foundations reducing from two to one queen, with no nests being left orphan (Aron et al. 2009). This could point to the existence of geographic variation in colony founding strategies for *L. niger*, as described for some species of desert ants (Cahan et al. 1998, Rissing et al. 2000, Helms et al. 2013).

Compared to their corresponding controls, the increase in total colony brood derived from queen associations was 28% and 86% for two and three queens, respectively (Table 1). Beneficial effects of pleometrosis in *L. niger* were already reported in experiments where groups of two to six queens produced significantly more workers than single queens (Sommer & Hölldobler 1995). Therefore, starting colonies with more than one queen would benefit colony growth, assuming fatal queen fights in the colonies can be avoided. Nevertheless, the effect of pleometrosis is still inferior to that obtained by means of pupae transplantation, which was on average 256% higher than controls when both transplantation rates were pooled. In addition, the apparent benefits of pleometrotic colony founding on total number of brood were reduced when eggs were excluded (a fraction of which may be consumed by the colony). While it is true that the pupae transplantation experiment ran for a total of eight weeks and not six, as the multiple-queen experiment, it is unlikely that colonies in the latter experiment could have reached the aforementioned levels of brood production when given the extra time, even if queen mortality could somehow have been avoided.

The increment in brood numbers in colonies with more than one queen was exclusively due to having multiple producers at once, and not because the production of individual queens was somehow stimulated. On the contrary, we observed that per capita brood production decreased as the number of queens in the colony increased, especially when only looking at production of larvae and pupae (Table 1). The effect of pleometrosis on queen fertility varies, depending on the species involved. Reductions in per capita production have been previously reported in other ant species like *Pachycondyla villosa* (Trunzer et al. 1998), *Lasius flavus* (Waloff 1957) and *Solenopsis invicta* (Tschinkel 1993). Whether this is due to a lower investment in reproduction by the queens or to increased egg cannibalism remains to be studied. On the other hand, pleometrosis has been shown to boost queen fecundity in facultatively polygynous populations of *Oecophylla smaragdina*, assessed 68 days after their nuptial flight (Offenberg et al. 2012a). In other species like *Pogonomyrmex californicus*

and *Messor pergandei*, or in young *O. smaragdina* colonies, per capita queen production in single versus multiple queen associations has been similar (Rissing & Pollock 1991, Johnson 2004, Offenberg et al. 2012b).

Pupae adoption had a positive effect on all growth parameters studied, regardless of whether we included egg counts in the analyses or not. Unlike what we found in the multiple queen experiment, the increase in number of individuals is due to both the workers adopted in form of pupae and an increased intrinsic production of brood by the queens, triggered by the presence of such pupae. These results are consistent with what has been found in analogous experiments with *O. smaragdina* and *O. longinoda*, where pupae transplantation also led to an increased per capita queen production and larger colony size in nests where brood were adopted (Krag et al. 2010, Offenberg et al. 2012b, Ouagoussounon et al. 2013, 2015). This pupae transplantation strategy is equivalent to brood raiding behaviours observed in laboratory experiments with *L. niger* (Sommer & Hölldobler 1995), *Messor pergandei* (Rissing & Pollock 1987) and *Myrmecocystus mimicus* (Bartz & Hölldobler 1982). Also, a number of studies report high levels of competition and brood raiding among incipient colonies of fire ant *S. invicta* as a means to boost early colony growth in both natural and laboratory conditions (Tschinkel 1992b, Adams & Tschinkel 1995). Thus, raiding and acceptance of non-nestmate brood may be a widespread method of improving colony growth among ant species.

Nanitic workers

Pupae transplantation led to an increment in the size of intrinsic nanitics, which has also been found in experiments with the weaver ant *O. longinoda* (Ouagoussounon et al. 2015). Pleometrotic associations in the current experiment, however, had no detectable effect on the size of the intrinsic nanitic workers, although a reduction in worker size in multiple-queen colonies compared to single-queen colonies has been reported for mature colonies of *Formica selysi* (Schwander et al. 2005). It stands to reason that a single queen that receives pupae, and therefore is expecting to have foragers soon, will invest more resources in producing more and/or larger individuals than a

queen that has to raise her first batch of brood exclusively using her body reserves. One might also hypothesize that pleometrotic queens may limit their reproductive effort (and, thus, investment in larger workers) in anticipation of a fight when finding themselves in pleometrosis, as seen for *S. invicta* (Bernasconi & Keller 1996, 1998). While *L. niger* showed no adjustment of investment strategy to relative fighting ability (Aron et al. 2009), there is evidence that, when the colony is given sufficient brood, cohabiting queens conserve resources by reducing productivity (Holman et al. 2010). Whether this reduction is a selfish action towards saving energy for the elimination stage remains unclear, as inhibition of oviposition in *L. niger* with juvenile hormone did not increase the chances of queens surviving in foundress groups (Sommer & Hölldobler 1995).

An increase in the size of intrinsic nanitics adds an extra benefit to the adoption of foreign brood, with colony performance being potentially improved due to a wider range of tasks that can be efficiently handled by the colony. Similar experiments with *O. longinoda* reported an increase of 12% in nanitic length in nests where 100 pupae had been transplanted versus controls that did not receive any pupae (Ouagoussounon et al. 2015). That opens the possibility that a higher transplantation rate could result in higher increases than the 7% observed in our experiment, although the issue of pupae discard by the queen and possible transplantation limits still needs to be further investigated for *L. niger*.

In conclusion, pupae adoption seems to be a powerful strategy to achieve increased growth and competitiveness among incipient *L. niger* colonies, at least under the pupae adoption rates used in this study. Not only will it boost the colony's workforce, but queen production will also increase, resulting in more and even larger workers. Hence, strong selection for brood raiding may be expected. Selection for pleometrosis may also be present, albeit less intense, as the effect is much smaller in terms of worker number (with no significant effect on worker size) and due to the risks associated with queen fights. On the other hand, pleometrosis may provide an advantage for claustral colonies in two ways: (i) it may result in earlier emergence of the first workers (Waloff 1957, Rissing & Pollock 1987, Offenberg et al.

2012a), thus allowing pleometrotic colonies to raid single queen colonies before their nanitic workers are present to defend the nest and (ii) the lead in worker numbers at the time of worker emergence can make pleometrotic colonies more successful in brood raiding (Sommer & Hölldobler 1995). In other words, pleometrosis could be a prerequisite for effective brood raiding and the advantages associated with this latter strategy.

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REFERENCES

- Adams ES, Tschinkel WR, 1995. Density-dependent competition in fire ants: effects on colony survivorship and size variation. *Journal of Animal Ecology* 64: 315 – 324.
- Anderson C, McShea DW, 2001. Individual versus social complexity, with particular reference to ant colonies. *Biological Reviews of the Cambridge Philosophical Society* 76(2): 211 – 237.
- Arnan X, Ferrandiz-Rovira M, Pladevall C, Rodrigo A, 2011. Worker size-related task partitioning in the foraging strategy of a seed-harvesting ant species. *Behavioral Ecology and Sociobiology* 65(10): 1881 – 1890.
- Aron S, Steinhauer N, Fournier D, 2009. Influence of queen phenotype, investment and maternity apportionment on the outcome of fights in cooperative foundations of the ant *Lasius niger*. *Animal Behaviour* 77(5): 1067 – 1074.
- Bales MT, Adams ES, 1997. Intraspecific usurpation of incipient fire ant colonies. *Behavioral Ecology* 8(1): 99 – 103.
- Bartz SH, Hölldobler B, 1982. Colony founding in *Myrmecocystus mimicus* wheeler (Hymenoptera: Formicidae) and the evolution of foundress associations. *Behavioral Ecology and Sociobiology* 10(2): 137 – 147.
- Bernasconi G, Keller L, 1996. Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). *Proceedings of the Royal Society of London B: Biological Sciences* 263(1369): 509 – 513.
- Bernasconi G, Keller L, 1998. Phenotype and individual investment in cooperative foundress associations of the fire ant, *Solenopsis invicta*. *Behavioral Ecology* 9(5): 478 – 485.
- Bernasconi G, Strassmann JE, 1999. Cooperation among unrelated individuals: the ant foundress case. *Trends in Ecology & Evolution* 14(12): 477 – 482.
- Buffin A, Maillieux A-C, Detrain C, Deneubourg JL, 2010. Trophallaxis in *Lasius niger*: a variable frequency and constant duration for three food types. *Insectes Sociaux* 58(2): 177 – 183.
- Cahan S, Helms KR, Rissing SW, 1998. An abrupt transition in colony founding behaviour in the ant *Messor pergandei*. *Animal Behaviour* 55(6): 1583 – 1594.
- Gadau J, Strehl C-P, Oettler J, Hölldobler B, 2003. Determinants of intracolony relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): mating frequency and brood raids. *Molecular Ecology* 12(7): 1931 – 1938.
- Hasegawa E, Imai S, 2011. A trade-off between number and size within the first workers of the ant *Camponotus japonicus*. *Journal of Ethology* 30(1): 201 – 204.
- Helms KR, Newman NJ, Helms Cahan S, 2013. Regional variation in queen and worker aggression in incipient colonies of the desert ant *Messor pergandei*. *Behavioral Ecology and Sociobiology* 67(10): 1563 – 1573.
- Holm S, 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* 6(2): 65 – 70.
- Holman L, Dreier S, D’Ettorre P, 2010. Selfish strategies and honest signalling: reproductive conflicts in ant queen associations. *Proceedings of the Royal Society of London B: Biological Sciences* 277(1690): 2007 – 2015.
- Jerome CA, McInnes DA, Adams ES, 1998. Group defense by colony-founding queens in the fire ant *Solenopsis invicta*. *Behavioral Ecology* 9(3): 301 – 308.

- Johnson RA, 2004. Colony founding by pleometrosis in the semiclaustral seed-harvester ant *Pogonomyrmex californicus* (Hymenoptera: Formicidae). *Animal Behaviour* 68(5): 1189 – 1200.
- Krag K, Lundegaard R, Offenberg J, Nielsen MG, Wiwatwittaya D, 2010. Intercolony transplantation of *Oecophylla smaragdina* (Hymenoptera: Formicidae) larvae. *Journal of Asia-Pacific Entomology* 13(2): 97 – 100.
- Mirenda JT, Vinson SB, 1981. Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta* buren. *Animal Behaviour* 29(2): 410 – 420.
- Offenberg J, Peng R, Nielsen MG, 2012a. Development rate and brood production in haplo- and pleometrotic colonies of *Oecophylla smaragdina*. *Insectes Sociaux* 59(3): 307 – 311.
- Offenberg J, Peng R, Nielsen MG, Birkmose D, 2012b. The effect of queen and worker adoption on weaver ant (*Oecophylla smaragdina* F.) queen fecundity. *Journal of Insect Behavior* 25(5): 478 – 485.
- Ouagoussounon I, Offenberg J, Sinzogan A, Adandonon A, Kossou D, Vayssières J-F, 2015. Founding weaver ant queens (*Oecophylla longinoda*) increase production and nanitic worker size when adopting non-nestmate pupae. *SpringerPlus* 4(6).
- Ouagoussounon I, Sinzogan A, Offenberg J, Adandonon A, Vayssières J-F, Kossou D, 2013. Pupa transplantation to boost early colony growth in the weaver ant *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae). *Sociobiology* 60(4): 374 – 379.
- Peng R, Nielsen MG, Offenberg J, 2013. Utilisation of multiple queens and pupae transplantation to boost early colony growth of weaver ants *Oecophylla smaragdina*. *Asian Myrmecology* 5: 177 – 184.
- Pollock GB, Rissing SW, 1989. Intraspecific brood raiding, territoriality, and slavery in ants. *The American Naturalist* 133(1): 61 – 70.
- Porter SD, Tschinkel WR, 1985. Fire ant polymorphism (Hymenoptera: Formicidae): factors affecting worker size. *Annals of the Entomological Society of America* 78(3): 381–386.
- Rissing SW, Johnson RA, Martin JW, 2000. Colony founding behavior of some desert ants: geographic variation in metrosis. *Psyche: A Journal of Entomology* 103(1-2): 95 – 101.
- Rissing SW, Pollock GB, 1987. Queen aggression, pleometrotic advantage and brood raiding in the ant *Veromessor pergandei* (Hymenoptera: Formicidae). *Animal Behaviour* 35(4): 975 – 981.
- Rissing SW, Pollock GB, 1991. An experimental analysis of pleometrotic advantage in the desert seed-harvester ant *Messor pergandei* (Hymenoptera; Formicidae). *Insectes Sociaux* 38(2): 205 – 211.
- Schwander T, Rosset H, Chapuisat M, 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behavioral Ecology and Sociobiology* 59(2): 215 – 221.
- Sommer K, Hölldobler B, 1995. Colony founding by queen association and determinants of reduction in queen number in the ant *Lasius niger*. *Anima* 50(2): 287 – 294.
- Trunzer B, Heinze J, Hölldobler B, 1998. Cooperative colony founding and experimental primary polygyny in the ponerine ant *Pachycondyla villosa*. *Insectes Sociaux* 45(3): 267 – 276.
- Tschinkel WR, 1992a. Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. *Ecological Entomology* 17(2): 179 – 188.
- Tschinkel WR, 1992b. Brood raiding in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): laboratory and field observations. *Annals of the Entomological Society of America* 85(5): 638 – 646.
- Tschinkel WR, 1993. Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology* 33(4): 209–223.
- Tschinkel WR, 1998. An experimental study of pleometrotic colony founding in the fire ant, *Solenopsis invicta*: what is the basis for association? *Behavioral Ecology and Sociobiology* 43(4-5): 247 – 257.
- Waloff N, 1957. The effect of the number of queens of the ant *Lasius flavus* (Fab.) (Hym., Formicidae) on their survival and on the rate of development of the first brood. *Insectes Sociaux* 4(4): 391 – 408.
- Wilson EO, 1978. Division of labor in fire ants based on physical castes (Hymenoptera: Formicidae: *Solenopsis*). *Journal of the Kansas Entomological Society* 51(4): 615 – 636.

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