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Utilisation of multiple queens and pupae transplantation to boost early colony growth of weaver ants *Oecophylla smaragdina*

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ABSTRACT. Weaver ants (*Oecophylla smaragdina* (Fabricius)) have been increasingly used as biocontrol agents of insect pests and as insect protein for human food and animals. For either of these purposes, mature ant colonies are essential. However, for a newly established colony to develop to a suitable mature size takes three years, which is too long for most users to spend rearing them. Multiple queens and non-nestmate pupae transplantation may be ways to boost early colony growth. An experiment on newly-founded *O. smaragdina* colonies with two, three and four founding queens, together with transplantation of 0, 30 and 60 non-nestmate pupae from a mature donor colony, was conducted in 2010 at Darwin, Australia. The survival rates of the workers from transplanted pupae ranged between 73 and 97%, suggesting that queens in incipient colonies accepted foreign pupae. At the end of the experiment, colony size was positively related to the number of founding queens. Compared with the colonies without pupae transplantation, colonies with 30 and 60 transplanted pupae produced 110% and 200% more brood, respectively. Production of 476% more brood was achieved by four-queen colonies with 60-pupae transplantation than by two-queen colonies without pupae transplantation. These results suggest that selecting incipient colonies with multiple queens, and increasing worker numbers by transplanting pupae from other colonies, will promote early colony growth of weaver ants.

Keywords: *Oecophylla smaragdina*, *Oecophylla* ant farming, incipient colony boost, biological control, edible ant production.

INTRODUCTION

The weaver ant *Oecophylla smaragdina* (Fabricius) has been utilised for biological control of insect pests in a number of tropical crop trees (Peng & Christian 2005, 2010; Peng *et al*. 2004, 2010; Van Mele *et al*. 2007; Lim & Kirton 2003), for production of edible insect protein as human health food (Offenberg 2011; Offenberg & Wiwatwitaya 2010; FAO 2008), for traditional medicine (Rastogi 2011) and for feeding animals (César 2004). For either of these purposes, mature colonies of the ant are essential. *Oecophylla* ant-colony rearing under ambient tropical conditions showed that it took 18 to 24 months for a young colony with founding queens to become mature (emergence of sexual forms) (Vanderplank 1960; Peng *et al*. 1998). A newly-mature colony normally occupies only a couple of trees, with a couple of dozen leaf nests

(Peng *et al*. 2004). For the purpose of biocontrol, human food production or animal feeding, one more year is needed for a newly-mature colony to develop to a suitable size. Thus, a total of three years are needed, which is too long for most users to spend rearing a colony. However, this long period could be shortened, if early colony growth can be boosted.

Multiple queens and non-nestmate pupae transplantation may be a way to boost early colony growth. Bernasconi & Strassmann (1999) reported that some ant species can found new colonies with multiple queens (pleometrosis), which results in an increase in the probability of survival during the initial phase of colony development through the faster production of more workers. *Oecophylla smaragdina* is also known to found new colonies with multiple queens (Fig. 1) (Peng *et al*. 1998; Peeters & Andersen 1989; Binh Thanh Nguyen, pers. comm.), but it is not known whether pleometrosis in this species promotes initial colony growth. Based on the findings of other ant species (Bernasconi & Strassmann 1999), we hypothesised that incipient colonies with a higher number of founding queens in *O. smaragdina* would produce bigger colonies.

The adoption of non-nestmate brood from other colonies, too, may increase colony growth. Several ant species that are able to rob intraspecific brood from neighbour colonies accelerate colony growth by adding the robbed individuals to their worker force (Bartz & Hölldobler 1982; Rissing & Pollock 1987). It is not known whether *O. smaragdina* actively rob intraspecific brood, but it is known that transplanted non-nestmate larvae of *O. smaragdina* are accepted by young colonies (R.L. Nielsen unpublished data), and queenless worker colonies accept and culture larvae from other conspecific colonies (Krag *et al*. 2010). Pupae may be even valuable for colony growth because a receiving colony does not need to fuel the growth of the pupae. However, given that *O. smaragdina* is antagonistic between colonies (Hölldobler & Wilson 1990; Peng *et al*. 2004; Crozier *et al*. 2009), it is not recorded in the literature whether a colony accepts foreign pupae. Lenoir *et al*. (2001) showed that individual chemical-recognition cues in several species

Fig. 1. New queens of *Oecophylla smaragdina* use empty cocoons of the saturniid moth, *Syntherata janetta* (White), on African mahogany *Khaya senegalensis* (Desv.) A.Juss. trees to found colonies (this cocoon contains at least 6 queens).

of ants were developed shortly after worker eclosure from the pupa. Therefore, we expected that transplanted non-nestmate pupae would be accepted by *O. smaragdina* colonies.

In this study, we therefore evaluated: (1) whether queens in young colonies of *O. smaragdina* accept foreign pupae; and (2) whether multiple queens and transplanted foreign pupae promote the early growth of the colony.

MATERIALS AND METHODS

The study was conducted in February 2010 at Darwin, Australia. Newly founded *O. smaragdina* colonies with queens and their eggs were collected from trees on Casuarina Campus of Charles Darwin University. To help new queens establish their colonies on trees, 104 artificial nests were made on 13 trees by rolling a single leaf together, fixing it with a plastic ring (1.3 cm in diameter) in the middle part and sealing the tip end with a paper clip (Fig. 2). A total of 64 nests were utilised by queens after their nuptial flight in the early morning on 7 February 2010. These nests were collected in later afternoon on 7 and 8 February. Of 64 nests, 73% had multiple queens. After collection, each colony (nest) was immediately transferred into an open cylindrical transparent plastic container (diameter = 4.5 cm; height = 10.5 cm) with two fresh citrus leaves for maintaining humidity, and then, a piece of nylon mesh material was used to cover the open end of the container and secured with a rubber band.

A total of 27 colonies were used for this experiment, and they were divided into three groups: nine 2-queen colonies (each colony with two queens), nine 3-queen colonies and nine 4-queen colonies. Each group was divided into three treatments (0, 30 or 60 transplanted non-nestmate pupae), and each treatment had three colonies.

Non-nestmate pupae were all obtained from a single mature *O. smaragdina* colony, which was located more than 500 m away from the location where new colonies were collected. The pupae transplantation took place seven days after the nuptial flight. At this time, each colony contained queens, eggs and some early-instar

Fig. 2. An artificial nest used for trapping new queens of *Oecophylla smaragdina* (the opening of the nest has been sealed with a thin layer of thread, showing that a new colony is already founded claustrally).

larvae. For pupae transplantation, each colony was first transferred to a petri dish (diameter = 9.5 cm) with several fresh citrus leaves. After counting the right number of non-nestmate pupae, these were quickly put into the colony. Then, the petri dish was covered by a big transparent plastic container (top diameter = 7.0 cm fixed with nylon mesh, bottom diameter = 8.5 cm attached to the petri dish, and height = 12.5 cm). The 27 colonies were placed on a table in partial open shade under ambient climatic conditions. During the experiment, all colonies were provided with a few drops of pure water every day to allow the queens to drink. When the first worker from the transplanted pupae emerged, two drops of 20% of sugar water were provided for each colony every day. The transparent plastic containers allowed daily external inspection of brood development. The experiment was terminated twelve days after the pupae transplantation because the colony´s intrinsic brood (brood produced by the queens of the colonies) reached the pupal stage. At this stage, there was no overlap between intrinsic and transplanted pupae cohorts because all transplanted pupae had either emerged as workers or died. On the experiment termination day, the numbers of brood (eggs, larvae and pupae), workers and queens in each of the 27 colonies were counted.

All the data of brood, except workers, were normally distributed and showed variance homogeneity. Two-way ANOVA was used to compare means with queen number (2, 3 or 4) and treatment (0, 30 or 60 transplanted pupae) as the main effects and with an interaction term, using JMP 9.0.0 statistical software. A whole model was calculated to test if all regression parameters were zero (Table 1). The whole model test should be significant before the significance of the interaction term and the main effects can be evaluated (JMP 9.0.0). Because the data of workers were not normally distributed, the numbers of workers were analysed with a Kruskal-Wallis test (nonparametric statistics) using the same software.

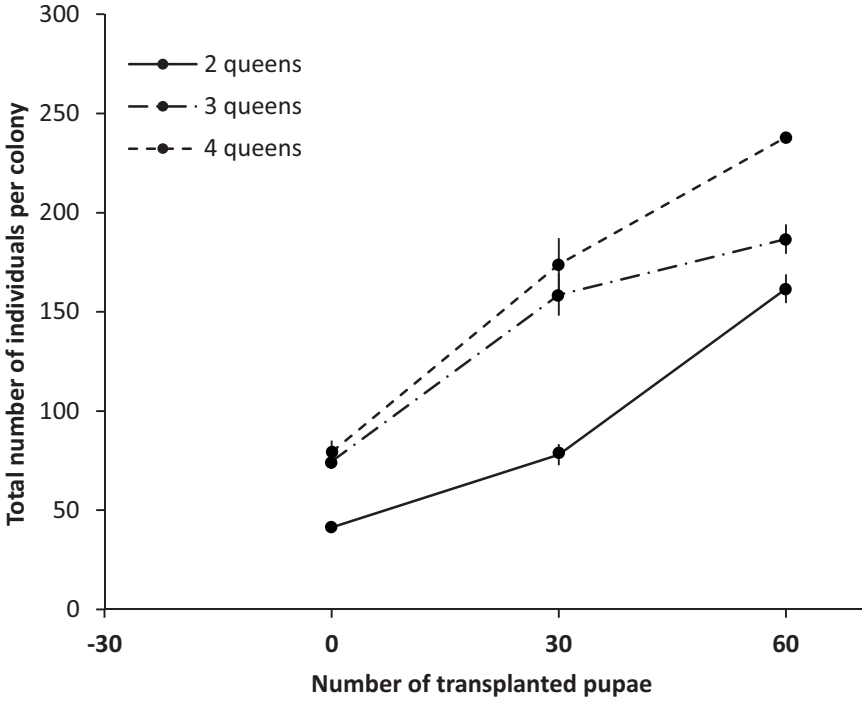

Fig. 3. The mean (± SE) number of individuals (including egg, larvae, pupae and workers) per colony at the end of the experiment.

RESULTS

Pupae acceptance

At the end of the experiment, all queens in each of the 27 colonies survived, and workers from transplanted pupae behaved normally. Survival rates (No. of emerged workers / No. of transplanted pupae x 100) ranged between 73 and 97% (mean % ± SE = 84 ± 1.5), and the survival rates were not affected by transplantation intensity (mean ± SE, 30-pupae transplantation = 85 ± 1.4; 60-pupae transplantation = 81 ± 2.6; $F_{1.12} = 2.12$; $P = 0.17$) or by queen number (mean ± SE, 2-queen colonies = 86 ± 3.2; 3-queen colonies = 81 ± 2.7; 4-queen colonies = 83 ± 1.9; $F_{2.12} = 1.18$; $P = 0.34$). The mean (± SE) number of workers that emerged from non-nestmate pupae was 25.4 (± 0.41) and 48.8 (± 1.57) in the colonies that received 30 and 60 pupae, respectively (one-way Kruskal-Wallis; Chi square = 24.22, df = 2, $P < 0.0001$).

Colony production

Colony production was positively related to the number of founding queens and transplanted pupae. The mean number of individuals per colony followed the following pattern: 4-queen colonies > 3-queen colonies > 2-queen colonies. This result applied to eggs, larvae and pupae (Table 1). Compared with the total population size of colonies without transplanted pupae, colonies with 30 and 60 transplanted pupae produced populations 110% and 200% higher, respectively (Table 1). It is noted that after 12 days there was no significant difference in number of pupae per colony according to the number of transplanted pupae ($F_{2.18} = 0.62$, $P = 0.55$; Table 1). Four-queen colonies with a 60-pupae transplantation produced an average of 238.0 (± 1.73 SE) individuals, which was 476% more brood than 2-queen colonies without pupae transplantation (41.3 (± 2.60 SE) individuals). There was a significant interaction between queen number and transplantation intensity on the egg and the total production (Table 1).

Table 1: Mean (± SE) number of individuals (egg, larvae, pupae and workers) per colony 12 days after the transplantation of pupae in relation to queen numbers and transplantation intensity.

		Eggs per colony		Larvae per colony		Pupae per colony		Brood + workers per colony	
		Mean ± SE	Two-way ANOVA	Mean ± SE	Two-way ANOVA	Mean ± SE	Two-way ANOVA	Mean ± SE	Two-way ANOVA
Queens	2	39.0 (8.17)		26.4 (3.99)		2.2 (0.92)		93.7 (18.01)	
	3	64.7 (9.84)	$F_{2.18} = 13.69$; $P = 0.0002$	47.0 (3.47)	$F_{2.18} = 33.39$; $P < 0.0001$	4.6 (1.25)	$F_{2.18} = 5.97$; $P = 0.010$	140.0 (17.27)	$F_{2.18} = 71.86$; $P < 0.0001$
	4	72.1 (11.02)		58.3 (6.06)		8.8 (1.81)		163.8 (23.42)	
Transplantation	0	27.6 (4.89)		32.0 (4.14)		5.6 (1.81)		65.1 (1.03)	
	30	64.7 (10.25)	$F_{2.18} = 36.83$; $P < 0.0001$	42.8 (5.71)	$F_{2.18} = 20.09$; $P < 0.0001$	4.0 (0.94)	$F_{2.18} = 0.62$; $P = 0.55$	136.9 (3.01)	$F_{2.18} = 240.87$; $P < 0.0001$
	60	83.6 (5.35)		57.0 (6.40)		6.1 (2.02)		195.4 (2.07)	
Queens * transplantation interaction			$F_{4.18} = 2.96$; $P = 0.048$		$F_{4.18} = 1.97$; $P = 0.14$		$F_{4.18} = 1.11$; $P = 0.38$		$F_{4.18} = 7.44$; $P = 0.0010$
Whole model			$F_{8.18} = 11.9$; $P < 0.0001$		$F_{8.18} = 14.36$; $P < 0.0001$		$F_{8.18} = 2.20$; $P = 0.078$		$F_{8.18} = 81.90$; $P < 0.0001$

Colonies with 3 and 4 queens had a high production gain from 0 to 30 transplanted pupae (an average of 73% of the overall increase in the total brood production) and a smaller gain from 30 to 60 pupae (27%; Fig. 3). Populations in colonies with 2 queens showed a gain of 18% from 0 to 30 pupae, but of 82% from 30 to 60 pupae (Fig. 3).

DISCUSSION

Foreign pupae acceptance by young colonies

Queens in young *O. smaragdina* colonies adopted foreign pupae. An average of 84% of transplanted pupae developed to the adult stage and the workers displayed normal behaviour, suggesting that non-nestmate pupae were readily accepted by the residential queens. Krag *et al*. (2010) showed that *O. smaragdina* non-nestmate larvae were adopted by queenless colonies containing only mature workers. Having considered antagonistic behaviour of *O. smaragdina*, we suggest that the nestmate recognition cues are developed after the pupal stage in *O. smaragdina*. Working with a few other ant species, Lenoir *et al*. (2001) showed that individual chemical cues were developed shortly after worker eclosure.

Influence of multiple queens on early colony growth

More founding queens increased colony production. The presence of more queens in young colonies led to significant gains in the number of intrinsic brood (Table 1 and Fig. 3). Pleometrosis has evolved in several other ant species as a way to boost early colony growth (Nonacs 1993; Sommer & Hölldobler 1995). Offenberg *et al*. (2012) showed that pleometrotic *O. smaragdina* colonies were competitively superior to haplometrotic (single-queen-founded) colonies, as they produce more workers faster and shorten the claustral phase (see below), leading to increased queen fecundity.

Influence of transplantation of foreign pupae on early colony growth

The transplantation of foreign pupae directly increased colony size in proportion to the number of pupae added, and also indirectly promoted colony growth. Four-queen colonies with 60 pupae transplantation produced almost six times as much brood as 2-queen colonies without pupae transplantation (Fig. 3). Colonies with transplanted pupae resulted in a great increase in per-capita production of eggs and larvae (Table 1), suggesting that transplanted pupae can stimulate queens to continue laying eggs.

In species where queens are not helped by nestmate workers (independent foundation), the survival of the first generation of workers depends entirely on the food given by the queen (Peeters & Molet 2010). Independent foundation in *Oecophylla* is 'claustral', meaning that the queen must have sufficient metabolic reserves because she does not forage outside her nest as indicated in Fig. 2. At the initial stage of colony establishment, young brood accounts for all energy expenditure of the queens in terms of nursing. Eclosed workers from transplanted pupae quickly replaced the queens' role of nursing brood, and they also looked after the queens. As a result, the nutrients and energy saved by the queens enable them to continue laying eggs. *Oecophylla smaragdina* colonies less than 1.5 years old produce smaller and slimmer workers (nanictics), with an associated narrower range of tasks compared with older and larger colonies (Peng *et al*. 2004). Eclosed workers emerging from non-nestmate pupae of a mature colony are normal size (big), and they should conduct much wider range of tasks than nanictics in young colonies. Thus, it would be interesting in future studies to test how quickly the incipient colonies with transplanted pupae can develop to the mature stage.

The survival rates of workers derived from transplanted pupae were not affected by the number of queens and transplanted pupae, suggesting that pupae required only a minimal amount of nursing if any. This implies that colonies can be boosted with even higher numbers of foreign pupae. However, this is not the case with larvae as they need to be fed and groomed

by queens. Thus, pupae are better than larvae for promoting early colony growth.

As expected there was no significant difference in the number of intrinsic pupae produced by colonies with and without transplanted pupae (Table 1). When non-nestmate pupae were transplanted, all the colonies were seven days old, and each colony had already produced its first batch of eggs with some early-instar larvae. The experiment lasted 12 days, and this period was about the time needed for early-instar larvae to develop to pupae (Peng *et al*. 1998). Also, the transplanted pupae should not affect the development of the intrinsic larvae.

Oecophylla smaragdina has a sister species in tropical Africa, *O. longinoda* (Latreille), which is also an efficient biocontrol agent of the insect pests of tree crops. For sustainable agriculture, the use of *O. longinoda* has been promoted in a number of African countries (e.g. Ayenor *et al*. 2007; Van Mele *et al*. 2007; Dwomoh *et al*. 2009). Therefore, the culture of *O. longinoda* colonies will be important for meeting farmers' requirements for larger-scale biological control in the near future. The bio-ecology of *O. longinoda* is often regarded to be similar to *O. smaragdina* (Way & Khoo 1992). Whether pleometrosis is involved in early colony establishment is not known in *O. longinoda*, but the results from this study are likely to be relevant for boosting the early colony growth of this species.

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