# Genetic polyethism and nest building in the weaver ant *Oecophylla smaragdina* (Fabricius, 1775) (Hymenoptera: Formicidae)

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**Abstract**

The division of labour, i.e., the specialisation of workers on certain tasks, is a very prominent feature of eusocial insect colonies. Internal factors like physiological state and genetic makeup can influence the task an individual chooses to undertake. We examined nest construction in the weaver ant *Oecophylla smaragdina* (Fabricius, 1775) to explore the relationship between colony genetic diversity and division of labour. Single nests were collected from each of five colonies of this species in Townsville, Australia, and individually placed on small citrus trees. Nest reconstruction was elicited by displacing the walls of the transferred nest and the subsequent behaviour of workers classified into seven categories, three of which involved nest-construction activities. Workers were collected in approximately equal numbers from each behavioural group (equalling an average of 107 workers per colony) and identified to patriline using five microsatellite markers. All colonies contained only a single queen (monogynous). Three colonies were effectively monandrous while two colonies were polyandrous with the number of patrilines per colony ranging between 2 and 3. Genetic polyethism in nest-construction behaviour was detected only in the colony with three patrilines, with individual patrilines engaging in particular building activities more frequently than expected by chance alone. These results indicate that a genetic impact on the division of labour may influence the task choice of workers, though a larger study is necessary to determine the prevalence of this influence in *O. smaragdina*.

**Key words:** Division of labour, genetic polyethism, polyandry, nest building, weaver ant, *Oecophylla smaragdina*.

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## Introduction

Much of the ecological success of social insects is considered to be due to the phenomenon of division-of-labour, the process by which individuals specialise in particular behavioural tasks (Oster & Wilson 1978, Wilson 1979). Behavioural specialisation is typically associated with morphological castes (individuals with differing morphology) and / or temporal polyethism (a propensity to engage in different tasks as they age). A significant number of studies have examined how these and other mechanistic factors underlie division of labour and the emergence of enhanced colony-level efficiency (reviewed in Robinson 1992, Beshers & Fewell 2001, Seid & Traniello 2006).

The advent of molecular data has revealed a third determinant of division of labour, namely, genetic polyethism. First detected in honeybees (Calderone & Page 1988, Frumhoff & Baker 1988, Robinson & Page 1988), task specialisation based on genetic differences is now considered to potentially emerge in the presence of any genetic variation in individual response thresholds to particular tasks (Page & Mitchell 1998). Honeybee colonies containing greater genetic variability in task thresholds are more capable of maintaining homeostasis through behavioural means (Jones & al. 2004) and there is now strong support for a general relationship between colony genetic diversity, task specialisation and colony efficiency (Waibel & al. 2006).

It is plausible that genetic polyethism is generally present in eusocial Hymenoptera, at least in highly polyandrous species (Oldroyd & Fewell 2007), although the evidence for ants is generally indirect. Matrilines and patrilines within colonies of the ant *Formica selysi* Bondroit, 1918 differ in caste fate and task preferences (Schwander & al. 2005), as do different patrilines in *Acromyrmex echinatior* (Forel, 1899) (Hughes & al. 2003) and *Pogonomyrmex badius* (Latreille, 1802) (Rheindt & al. 2005). In contrast, in a study of the highly polyandrous *Cataglyphis cursor* (Fonscolombe, 1846), Fournier & al. (2008) did not find an effect of patriline on task preference. They hypothesize that in such cases polyandry may be favoured through other benefits of genetic diversity at the colony level (e.g., enhanced resistance to pathogens or reduced conflict over male production). Selection favouring genetic polyethism should be weaker in monandrous and monogynous species than in polyandrous ones (Page & al. 1989), and although genetic polyethism has been reported more frequently in highly polyandrous species, it is not clear whether this reflects a greater presence or greater ease of

| Primer | Primer sequence (5' – 3') | $T_A$(°C) | [MgCl$_2$] |
|---|---|---|---|
| MS6.7F MS6.7R | AGAGGGCACACATCCAACTAC CATCGTAAGGAGAATTTTCGT | 43 | 2.5 mM |
| MS7.4F MS7.4R | ATTGCCGAGTGAAAGAGGAAC AACTTCGCAGAATGACGAGTC | 56 | 2.5 mM |
| Osma37F Osma37R | GAATCCAGACCCGACGAACG CGAGAATCCGCCGCAATGAC | 58 | 2.5 mM |
| Osma101F Osma101R | ACCTTACGATCGTGGCAG AATACTCCGTGACAATCC | 50 | 3.0 mM |
| Ccon70F Ccon70R | GCATTAAAGTCGGGACGGAC CAGATGCGAAGAGCTCGC | 55 | 1.5 mM |

detecting relative to monandrous and / or monogynous colonies.

The weaver ant *Oecophylla smaragdina* (FABRICIUS, 1775) forms large arboreal colonies in tropical Asia and Australia (HÖLLDOBLER & WILSON 1990, reviewed in CROZIER & al. 2009). Workers construct arboreal nests in a process involving cooperating chains of ants pulling leaves together with other ants gluing the leaves together using silk from larvae (HÖLLDOBLER & WILSON 1977). A colony may have up to 500,000 workers and its territory may include several trees and scores of nests (HÖLLDOBLER & WILSON 1977). Worker size is strongly bimodal (COLE & JONES 1948): Smaller (minor) workers perform tasks within the nest while larger (major) workers carry out a range of tasks both within and outside the nest (HÖLLDOBLER & WILSON 1977).

*Oecophylla smaragdina* colonies are usually considered to be monogynous (HÖLLDOBLER & WILSON 1977), but there is some evidence for polygyny in restricted areas of their range (PENG & al. 1998, SCHLÜNS & al. 2009). In addition, a population in Queensland, Australia, has been found to be often monogynous and polyandrous (SCHLÜNS & al. 2009) opening up the possibility of studying phenomena such as genetic polyethism in this species. In this paper, we present the results of our study on genetic polyethism during nest building behaviour in *O. smaragdina*. We found task specialisation of different patrilines in a polyandrous colony, which may indicate the occurrence of genetically determined polyethism in this species.

## Methods

**Study species and behavioural analysis:** Five established colonies of *Oecophylla smaragdina* with several large nests and from well-separated trees were located on the James Cook University campus of Townsville, Australia. While the genetic colony structure was not known beforehand, most colonies were expected to be monogynous and polyandrous from a previous study (SCHLÜNS & al. 2009). The largest accessible nest from each colony was collected and placed individually in an indoor enclosure of ca. 2 m diameter made of a metal barrier on the floor, which was about 20 cm tall and coated at the top with general purpose motor grease to prevent ants escaping. A single potted citrus tree (approximately 1.5 m tall) was placed inside this enclosure. Nest construction was initiated by pulling apart the exist-

ing nest. Once construction of the new nest began, 12 to 16 major workers were sampled from 7 predetermined behavioural categories and genotyped. Individual ants were classified as being involved in 1 of 3 behaviours related to nest construction, CHAIN (forming chains to pull leaves together), WEAVE (holding larvae at the work site while they produced silk to glue leaves together), and HOLD (individually holding the edge of leaves), or 1 of 4 behaviours not involved in nest construction, BROOD (engaging in brood care in the old nest), STAY (moving on the surface of the old nest), WALK (moving on the tree away from both new and old nests), and MOVE (moving on the surface of the newly constructed nest but not assisting in nest construction). Minor workers did not participate in the construction of the new nest. The nests were maintained for the period of observation only and then returned to the tree from which they originated.

A randomisation approach (FISHER 1966) as implemented in the program MONTE CARLO RxC 2.2 (W.R. Engels, pers. comm.) was used to test for association between patriline and task performed within each colony. This approach gives an exact probability of obtaining the observed distribution by generating random new tables with the same marginal sums as the observed data and determining whether they deviate from the expected as much or more than the observed data. The resulting proportion of more extreme to less / equally extreme tables can then be used as a P-value. This procedure is appropriate when expected cell frequencies are low. We tested the data using all seven behavioural categories and by lumping these seven categories into building and non-building groups. We then implemented a False Discovery Rate correction using the "BY" method to correct for multiple (two) comparisons of the same hypothesis: an association between patriline and task (BENJAMINI & YEKUTIELI 2001).

**Microsatellite analysis:** All workers were genotyped at five microsatellite loci. DNA was extracted as in ELPHINSTONE & al.'s (2003) silica extraction method using a centrifugation rather than vacuum protocol. The locus Ccon70 was developed for *Camponotus consobrinus* (ERICHSON, 1842) (CROZIER & al. 1999), but the other four microsatellite loci we used were derived from *Oecophylla smaragdina*. For the population we studied, we used the same set of loci and conditions as described in SCHLÜNS & al. (2009). The primer sequences and PCR conditions are shown in Table 1. Forward primers were synthesized with a 5' 17-base tag devised by SHIMIZU & al. (2002) to enable the universal fluorescent labelling technique of SCHUELKE (2000). PCRs were performed with 0.1 µM of forward tagged primer, 0.4 µM of reverse primer and 0.4 µM of universal fluorescent tag (labelled with HEX, FAM or TET). PCR reactions were cleaned through Sephadex G50 before genotyping using an Amersham MegaBACE 1000 sequencer and the MegaBACE Fragment Profiler software.

**Identification of queen and patriline number per colony:** Since worker offspring was genotyped, the maternal and paternal alleles and a minimum number of queens and males per colony were inferred indirectly by the following method: Workers receive one allele from their mother (queen) and one allele from the father (male). When we found more than two alleles at a locus in the worker sample from one colony, the queen could be either homozygous or heterozygous at that locus. To decide which was the case

we applied a $\chi^2$ test to ascertain whether the queen's alleles segregated according to Mendelian expectations (for each locus separately) if heterozygosity was assumed. Once the queen genotypes were identified for all loci the male genotypes were determined as the combinations of non-queen alleles at all loci. In the case of the apparently monandrous colonies, we further checked against the possibility of the variation within them actually coming from two or more males mating with a homozygous queen by examining multilocus genotypes.

### Results

In all cases the distributions of colony worker genotypes were consistent with each colony having only one queen. In three cases these distributions also indicated monandry (single mating by the queen, Colony A, B & D) and in two polyandry (multiple mating, Colony C & E) (Tab. 2).

The relationship between a worker's patriline and the behaviours it engaged in varied between the two colonies in which multiple patrilines were detected. Colony C showed no significant association of patriline and behavioural tasks. The lack of association was consistent when tasks were either aggregated into two categories (building vs. non-building, P = 0.142), or analysed in the original 7 categories (P = 0.4371, Tab. 2). There was a significant relationship between patriline and the tasks performed in Colony E, the colony that contained the greatest number of patrilines. Individuals in Patriline E3 were more likely to engage in building behaviours than those of Patriline E2. Fifty of 101 individuals assigned to Patriline E3 were involved in nest building, while none of ten individuals of Patriline E2 engaged in these behaviours. The significant relationship between patriline and task in this colony was consistent when tasks were either analysed in the original 7 categories (P = 0.0055) or aggregated into two categories (building vs. non-building, P = 0.004). This association of patriline and task is not a function of how building behaviour was subdivided in the analysis, since it remained significant (for P < 0.05) after applying the False Discovery Rate correction for multiple comparisons.

### Discussion

The genetic structure of all five colonies examined here was consistent with the presence of only one mated queen per colony. Monogyny therefore appears to be the typical condition for *Oecophylla smaragdina* colonies in this region (see also SCHLÜNS & al. 2009).

Queens had mated with multiple males in at least two of the five colonies examined, with the number of multiple matings ranging from two to three, which fits the results of a previous larger study in the same area (SCHLÜNS & al. 2009). Variation in this trait is commonly found in multiply mating species, as for example in *Formica aquilonia* YARROW, 1955 (PAMILO 1993), and with the majority of ants species mating only once or twice the average mating frequencies of *Oecophylla smaragdina* are about average in ants (BOOMSMA & RATNIEKS 1996, STRASSMANN 2001). In the two polyandrous colonies, we found differences in the contributions of the males (paternity skew) with Colony E showing such a skew and Colony C with equal representations. This is again in accordance with previous studies (SCHLÜNS & al. 2009), which found about half of the polyandrous colonies in *O. smaragdina* had skewed par-

Tab. 2: Behavioural distribution of individual workers and patrilines during nest construction in five colonies of *Oecophylla smaragdina*. Patrilines are indicated by numbers.

| | Colony | A | B | C | | D | E | | |
|---|---|---|---|---|---|---|---|---|---|
| | Patriline | A1 | B1 | C1 | C2 | D1 | E1 | E2 | E3 |
| Building behaviours | CHAIN | 15 | 16 | 8 | 4 | 15 | 0 | 0 | 16 |
| Building behaviours | WEAVE | 14 | 15 | 11 | 4 | 16 | 1 | 0 | 15 |
| Building behaviours | HOLD | 15 | 16 | 8 | 4 | 16 | 0 | 0 | 16 |
| Non-building behaviours | BROOD | 16 | 16 | 9 | 5 | 16 | 0 | 4 | 12 |
| Non-building behaviours | STAY | 16 | 16 | 9 | 7 | 16 | 0 | 3 | 13 |
| Non-building behaviours | WALK | 13 | 16 | 5 | 10 | 15 | 0 | 0 | 16 |
| Non-building behaviours | MOVE | 12 | 16 | 9 | 6 | 16 | 0 | 3 | 13 |
| No. of workers per patriline | | | | 59 | 40 | | 1 | 10 | 101 |
| No. of workers per colony | | 101 | 111 | 99 | | 110 | 112 | | |

entages. These differences in skew could be due to varying amounts of sperm transferred during mating (KELLER & al. 1997) and / or sperm clumping in the spermathecae (e.g., SUNDSTRÖM & BOOMSMA 2000). But we would expect more consistently skewed male contributions across colonies if that were the case, because this effect would be the same in all multiply mated queens.

A potential genetic basis for behavioural specialization was detected in *Oecophylla smaragdina*, through a relationship between patriline membership and the probability of performing specific tasks associated with nest building behaviour in one of two polyandrous colonies (Tab. 2). These findings suggest that the hypothesis of a genetic impact on the division of labour in social insects may apply to *O. smaragdina* and further investigations of this phenomenon are needed. A variety of hypotheses have been proposed to explain the evolution, selection and maintenance of polyandry in social insects (reviewed by CROZIER & FJERDINGSTAD 2001). The expression of genetic polyethism within a colony may simply arise as a consequence of the presence of multiple patrilines, much as behavioural polyethism is considered to emerge as a consequence of increased colony sizes in insect societies (GAUTRAIS & al. 2002). Behavioural specialisation within a colony may be an unavoidable consequence of genetic variation. Genetic polyethism is therefore expected in *O. smaragdina* because of the large colony size and the moderate genetic variation within colonies.

The detection of genetic behavioural specialisation in *Oecophylla smaragdina* is even more significant, given that there are at least two reasons why it can be difficult to detect behavioural specialisation in the social system found in this species. First, when the total number of patrilines is low, the patrilines present in any one colony may not show differences in preferences for the tasks assayed, even if there is genetic polyethism present through the mother's genes. For example, SCHWANDER & al. (2005) found gen-

etic polyethism in *F. selysi* in only three of the four monogyne colonies they tested with twice-mated queens. Only two of the five *O. smaragdina* colonies tested in this study had multiple patrilines, and the number of patrilines in these two colonies were only two and three, respectively. Second, there is strong theoretical support showing that selection for genetical specialization in tasks will be weaker for monogynous and monandrous species than in species with significant incidences of multiple lineages per colony (PAGE & al. 1989). Given the low mate number and monogyny of *O. smaragdina*, at least in Queensland (this study and SCHLÜNS & al. 2009), it may be that the incidence of genetic polyethism is less for this species than in the highly polyandrous cases where it has been most studied. Further studies will clarify how widespread the example of a genetic behavioural specialisation detected in this study is in the *Oecophylla* population as a whole.

Furthermore, the behaviour of worker ants is also influenced by their age. Young workers typically perform tasks within the nest and progress to outdoor tasks with older age (BESHERS & FEWELL 2001). It is theoretically possible that sperm clumping in the queen's spermatheca and subsequent differential sperm use through time could produce age cohorts with different patriline composition and give the impression of genetic polyethism. Workers from Colony E that engaged in nest building might then simply represent the available group of individuals that can perform the task at that time. While we would generally expect temporal polyethism to play a role in task choice, there is only very limited evidence for sperm clumping in ants (CROZIER & BRÜCKNER 1981, FRANCK & al. 1999) and we therefore believe it unlikely that sperm clumping is the cause of the association between genotype and behaviour found in this study.

## Acknowledgements

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