



SURPLUS FEEDING REVERSES WORKER OVARIAN SUPPRESSION AND DISRUPTS SOCIAL COHESION IN QUEEN-RIGHT WASP COLONIES

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ABSTRACT

Nutrition is an important component of oogenesis and ovarian development in insects. In social insect colonies where a large proportion of females are sterile, suppression of reproduction caused by differential acquisition and retention of nutrition has been hypothesized to cause worker sterility. This study, using the primitively eusocial wasp *Ropalidia marginata* (Lepeletier) (Hymenoptera: Vespidae), show that worker ovarian development in naturally foraging colonies is more similar to experimentally food-limited colonies than to surplus-fed colonies, indicating nutritional castration in workers whose ovaries are inhibited by food availability. Further, experimental provisioning of surplus nutrition led to higher ovarian development in workers as compared to naturally foraging and food-limited workers. Surplus feeding also led to higher nest desertion by workers, leading to a breakdown of the colony's social structure, whereas food-limited colonies retained workers.

Key words: Nutrition, eusociality, oogenesis, worker sterility, caste differentiation, *Ropalidia marginata*, reproduction, suppression, ovarian development, nutritional castration, food availability, surplus feeding

Social insect colonies are characterized by reproductive division of labour. Reproduction is monopolised by one (or very few) females of the queen caste, and a large majority of females are sterile in the form of the worker caste such that queens' ovaries are well-developed and worker ovaries are arrested or atrophied at early stages of differentiation. Genetic as well as environmental factors are considered important in regulating ovarian development in social insect colonies. One important environmental factor is nutrition. The trophic castration hypothesis (Marchal, 1897) and the alimentary castration hypothesis (Roubaud, 1916) proposed that differential larval nutrition led to 'undernourished larvae' that developed into adults with 'underdeveloped ovaries'. The 'nutritional' castration hypothesis (Marchal, 1897) attributed worker sterility to energetically demanding worker roles, especially brood care by nursing workers (Hunt, 1991). Similarly, nutrient depletion due to foraging, and other worker behaviour may influence worker sterility and outcomes of caste differentiation (Chandrashekhara and Gadagkar, 1991; Krishnan et al., 2021). Natural inter-individual variation in nutrition created by unequal availability and distribution of food amongst nest-mates (Hunt, 1994) and differential disposition to consume food in adult stages (Gadagkar et al., 1991; Shukla et al., 2013) may further compound differential reproductive development in queens and

workers leading to the evolution and maintenance of sociality. Variation in levels of larval nutrition and maternal manipulation influence lipid reserves, ovarian development and reproductive caste differentiation in many wasp societies (Berens et al., 2015; Gadagkar et al., 1991; Gadagkar et al., 1990; Hunt and Dove 2002; Jandt et al., 2017; Judd et al., 2015; Suryanarayanan et al., 2011). Adult nutrition on the other hand can affect brood survival, nest growth, body size and fat deposition (Karsai and Hunt 2002; Mead et al., 1994; Toth et al., 2009). Social insect colonies have inherent mechanisms that can regulate the nutritional status of its members (viz., through dominance-subordinate behaviours, or through division of labour involving energetically costly behaviours such as foraging), and it is not entirely clear what levels of variation in ovarian development prevail in natural colonies and if natural colonies are more similar to starved or well-fed colonies in this respect? Also, if nutritional castration does indeed limit worker reproduction in social insect colonies, can surplus nutrition rescue worker ovarian development if excess food is made available?

This study uses the primitively eusocial wasp *Ropalidia marginata* to answer both the above questions. Importance of larval nutrition in worker sterility, egg laying and nest-founding has already been established in *R. marginata* (Gadagkar et al., 1990; 1991). Therefore,

this study describes ‘nutritional castration’ as an effect of adult nutrition on ovarian development, over and above the contribution of larval nutrition that is carried forward in wasps, without strictly adhering to the definition of ‘castration’ as provided by Marchal (1897) and Roubad (1916). Firstly, this study describes the ovarian development in natural free-foraging colonies and compares it with colonies that were limited by food and those that were fed surplus food. Secondly, this tests if surplus nutrition can rescue worker ovaries, by leading to higher ovarian development in queen-right workers. Additionally, this study predicts that surplus nutrition should lead to higher worker dispersal away from their natal colonies, either to explore opportunities of direct reproduction, or because surplus nutrition precludes conditions necessary for forming social groups. In contrast, starvation should suppress ovarian development in workers, reinforce worker status and curtail female dispersal.

Ropalidia marginata (Lepeletier) is a primitively eusocial, tropical, perennial nesting wasp species where queens are the sole egg layers of a colony. Queens and workers are morphologically identical. Reproductive castes are temporary, and though workers have poorly developed ovaries in the queen’s presence, they often replace queens to then develop ovaries and begin egg laying (Gadagkar et al., 1993). Wasps are capable of solitary nest initiation, and new nests can be founded singly or in groups (Shakarad and Gadagkar, 1995). Higher larval and adult nutrition significantly improve female ovarian development and egg laying when reared in isolated conditions, although with high variation (Gadagkar et al., 1991; Shukla et al., 2013). Queens are docile, and do not use aggression to regulate worker ovarian growth or colony labour, and worker ovaries may be inhibited through the queen’s pheromone (Bhadra et al., 2010), or through energetically demanding activities such as foraging (Chandrashekara and Gadagkar, 1991). Starvation increases dominance behaviours in workers, which use it to signal hunger to nestmates and foragers, which then leads to higher rates of foraging (Bruyndonckx et al., 2006; Lamba et al., 2008).

MATERIALS AND METHODS

Experimental setup: Ovarian development was studied in natural colonies and laboratory-reared experimental colonies. Natural colonies: Seven *R. marginata* colonies located at the Indian Institute of Science, Bangalore, India (13°00’ N, 77°32’ E) were

observed in a natural setting that allowed natural foraging activity. No nutritional supplementation of any form was provided experimentally. Experimental colonies: Eight *R. marginata* colonies were collected in Bangalore, India, brought to the laboratory and fixed in closed wooden cages (Gadagkar, 2001). Each cage housed a single wasp colony. Cages were closed to prevent wasps from foraging and bringing additional food to the colony but allowed for ventilation and ambient light to enter. The eight experimental close-caged colonies were further divided into two categories and randomly assigned to food-limited and food-surplus categories (four colonies each). From day one, when the cages housing the experimental colonies were closed, females were fed as per the following regimens for a total of ten days. Food-limited colonies: Maximum of two *Corcyra cephalonica* (Lepidoptera: Pyralidae) larvae was placed daily in a food tray inside each closed cage. None of the wasps were hand fed. Water, honey and soft wood were placed in all cages at all times. The number of *Corcyra* larvae consumed from the food tray was recorded. Food-surplus colonies: Each wasp present in the cage was individually hand-fed with as many *Corcyra* larvae as it accepted. This was performed thrice daily. Apart from hand feeding, ad libitum *Corcyra* larvae, honey, water and soft wood were kept in a food tray inside each closed cage at all times.

Behavioural observations: Wasps from natural and experimental colonies were marked with quick drying, non-toxic paints to assign unique colour codes for individual identification. Natural nests were observed for 20 hr in the natural setting and laboratory nests were observed for 10 hr (spread over four days and two days, respectively) as described before (Gadagkar, 2001). Behavioural observations were performed only after the wasps had finished eating the experimentally provided *Corcyra* larvae. All wasps from the experimental colonies were collected at the end of day 10. Food-surplus colonies consumed an average of 10.25 (s.d.± 4.63) *Corcyra* larvae/ day. Food-limited colonies consumed an average of 1.02 (s.d.± 0.25) *Corcyra* larvae/ day. Since foragers (or wasps in general) return to their colony after dusk, a night census was used to determine the proportion of nights that a wasp did not return to her colony, calculated as the number of nights spent outside the nest by the female (on the walls of the closed cage) divided by the total number of nights in the census.

Quantification of ovarian development: At the end of the experiment, all females were frozen at -20°C until

ovarian dissections. Number of mature oocytes, mean length of proximal oocytes, mean width of proximal oocytes, number of vitellogenic oocytes, and total number of oocytes was recorded for seven queens and 81 workers for natural colonies, and eight queens and 85 workers for the experimental colonies. Ovaries were measured in blind, i.e., the experimental treatment to which the wasp belonged to, was concealed during dissections. Ovarian measurements were subjected to principal component analysis (statistics XL, version 1.8) and principal component 1 scores (which explained 87.3% of the total variance) were used as an ovarian index for each female (Chandrashekhara and Gadagkar, 1991).

Statistical analysis: To test for differences between ovarian development between feeding regimens, ovarian indices were used as the response variable, and feeding category (natural, food-limited, and food-surplus) and female caste (queen and worker) were used as categorical factors in a generalized linear model (GLM), analysed in R (R Core Team 2015) using the *multcomp* package (Bretz et al., 2008). Post-hoc Tukey’s pairwise comparisons between feeding treatments were performed separately for workers and queens.

RESULTS AND DISCUSSION

There was a significant effect of feeding regimen on ovarian development in females of naturally foraging, food-limited, and food-surplus colonies (GLM, $F=4.27$, $p=0.015$; Table 1). Ovarian indices of workers belonging to naturally foraging and food-limited colonies were

similar to each other (GLM post-hoc test, $p=0.825$, Fig. 1; Table 2). Most workers from food-limited colonies as well as naturally foraging colonies had rudimentary ovaries, with no differentiating oocytes observed in them, arrested in early stages of ovarian development, and thus having low ovarian indices (Fig. 1). Workers from food-surplus colonies, however had significantly higher ovarian indices than naturally foraging workers and food-limited workers (GLM post-hoc tests, $p=0.009$ and $p=0.049$ respectively, Fig. 1) and thus suppression of worker ovaries was less effective in food-surplus colonies. The distribution of ovarian indices between food-surplus colonies and food-limited colonies differed significantly between workers (Fig. 2, Kolmogorov-Smirnov test, $D=0.30$, $p=0.039$) but not for queens (Fig. 2, Kolmogorov-Smirnov test, $D=0.75$, $p=0.228$). Workers from food-surplus colonies showed significant advances in ovarian development including higher number of differentiated oocytes, and higher number of vitellogenic oocytes that were not observed in workers from food-limited or natural colonies as reflected in their ovarian indices (Fig. 1, 2). There were no differences between ovarian indices of queens belonging to natural, food-limited, and food-surplus colonies (GLM post-hoc test, $p=0.228$, Fig.1, Table 1).

The similarity between ovarian indices of naturally foraging and food-limited workers in this study indicated that workers in natural colonies might already be in the lowest physiological level of ovarian development as starvation did not further reduce ovarian development in food-limited workers. Natural, free-foraging *R. marginata* worker ovaries

Table 1. Analysis of deviance table for generalized linear model (GLM) fit for the relationship between ovarian development (as estimated by the Ovarian Index), feeding regimens (naturally foraging, food-limited, and food-surplus colonies) and caste of the female (queen or worker)

	Df	Deviance	Residual degree of freedom	Residual deviance	F-statistic	p-value
NULL			180	710.23		
Feeding regimen	2	7.65	178	702.58	4.27	0.0153
Caste	1	544.37	177	158.20	609.05	<2e-16

Table 2. Results of post-hoc test of multiple comparison of means between each pair of feeding regimen, using Tukey’s contrasts performed for GLM analysis for worker ovarian indices. Non-adjusted p-values reported

Pair of comparisons	Estimate	Standard error	z value	p-value
Natural - food surplus	-0.43	0.16	-2.59	0.009
Food limited - food surplus	-0.39	0.19	-1.96	0.049
Food limited – natural	0.03	0.18	0.22	0.825

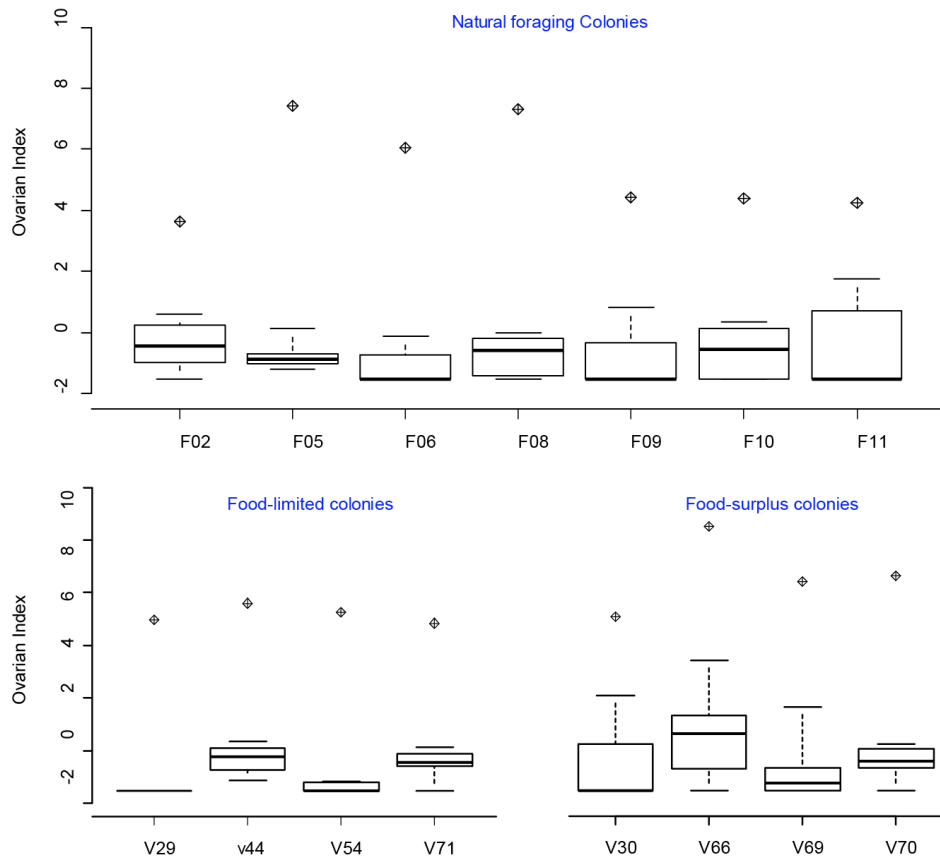


Fig. 1. Comparison of worker ovarian indices: workers from naturally foraging colonies and food-limited colonies did not differ in their ovarian indices. Workers from food-surplus colonies had higher ovarian indices than workers in food-limited and naturally foraging colonies. Queen ovaries did not differ between the natural, food-limited and food-surplus colonies (separate generalized linear models for queens and workers, post-hoc test, $\alpha = 0.05$). Worker ovarian indices are plotted as boxplots indicating median values inside the boxes. Queen ovarian index for each colony is plotted above the boxplot as a diamond plus. X-axis indicates individual colony IDs

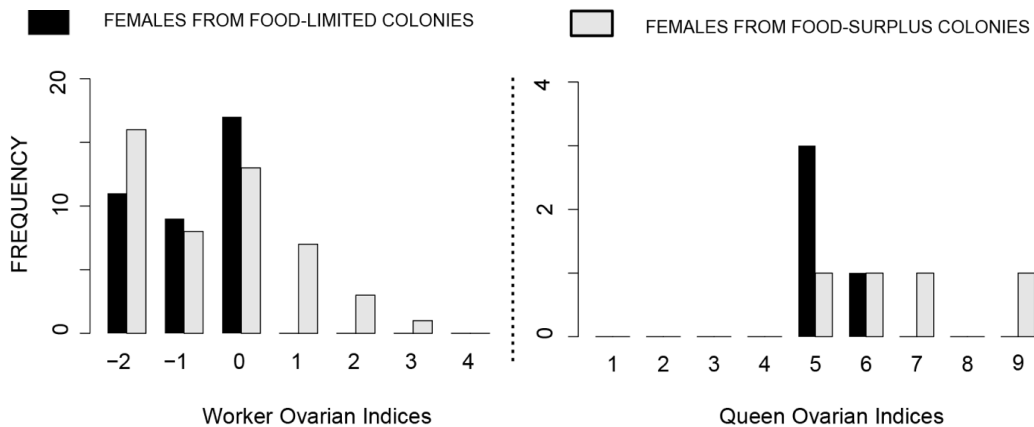


Fig. 2. Distribution of worker ovarian index (OI), a measure of the extent of ovarian development in individual females calculated using multiple ovariole parameters. OI values for food-limited colonies (black bars) were significantly different as compared to worker OI values for food-surplus (grey bars) colonies (Kolomogorov-Smirnov test, $p < 0.05$). Many surplus-fed workers belonged to higher OI classes that were absent in starved workers

were therefore limited by food availability. Surplus nutrition in adults reversed the effects of nutritional castration, leading to higher ovarian development in workers under queen-right conditions. Since food-surplus and food-limited colonies differed only in their availability of food, whereas energetic demands in tasks pertaining to brood care and colony maintenance were minimal and comparatively similar between the two treatments, surplus adult nutrition was the most likely cause leading to the observed differences in ovarian development. Since queen ovarian development was not different between food-surplus and food-limited colonies, *R. marginata* queens may already be at the highest physiological limit of oogenesis, selected for maximising their reproductive output as the sole egg layer of their colony (Table 3). Further, queens could have high-energy reserves that sustain oogenesis even during times of nutrient deprivation. It is possible that further starvation could reduce ovarian growth in queens, but *R. marginata* queens are docile, and generally do not participate in dominance behaviours, or energetically expensive behaviours such as foraging, and this could be another mechanism by which the queens conserve nutrient expenditure so as to maximise investment in oogenesis.

It was found that food-surplus colonies experienced higher nest desertion as compared to food-limited colonies. The proportion of nights that workers did not return to their natal nest was significantly higher in food-surplus colonies than in food-limited colonies (Fig. 3, Wilcoxon rank sum test, $W = 608.5$, $p = 0.002$). Thus, surplus-feeding increased nest desertion indicated by the number of wasps that did not return to their natal nests. There is evidence that excess fed *R. marginata* workers also show reduction in colony associated behaviours such as larval feeding, foraging, and dominance to nestmates (Bruyndonckx et al., 2006; Lamba et al., 2008). Such reduced worker activity may be a preliminary effect of surplus nutrition, and prolonged excess feeding (such as in this study) could eventually lead to nest desertion, and disintegration of the overall colony structure. Starvation on the other

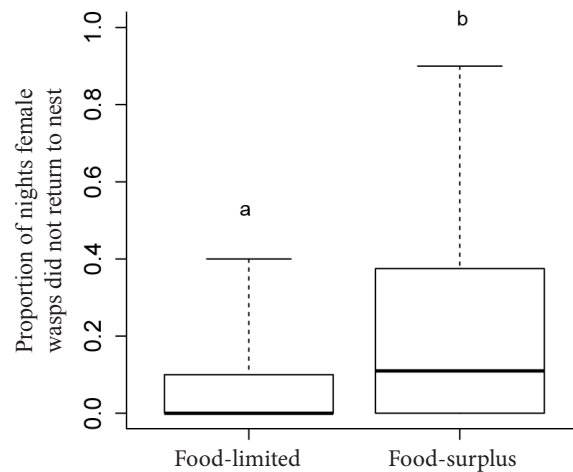


Fig. 3. Excess fed workers spent a significantly greater proportion of nights away from their natal nest as compared to starved workers (Wilcoxon rank sum test, $p < 0.05$). Workers that did not return to their nest were observed far from the nest, on the walls of the closed cage in which the nest was housed

hand ensured that more females remained back and continued on their nests as workers, consistent with earlier studies where starved *R. marginata* colonies show increased nest maintenance and care under starvation (Lamba et al., 2008). Surplus nutrition thus disintegrated colony structure, whereas starvation was associated with group cohesion. Surplus-feeding was accompanied by natural queen turnover in one colony, where one of the workers behaviourally dominated and drove away the existing queen, whereas separate nest founding by female(s) was observed in another surplus-fed colony. Taken together, these results suggest that surplus nutrition facilitates workers to overcome ovarian suppression and to desert natal nests. *Ropalidia marginata* wasps can desert colonies in groups, where multiple foundress colonies can be founded by two to 22 individuals (Shakarad and Gadagkar, 1995), which could explain why a large number of females in excess-fed colonies deserted their nests. Similar effects on colony demographics also occur in *Polistes* wasps, where honey-supplemented colonies produced higher proportion of reproductives and led to a lesser number of workers remaining on the nest (Hunt and Dove, 2002).

Table 3. Results of post-hoc test of multiple comparison of means between each pair of feeding regimens, using Tukey's contrasts performed for GLM analysis for queen ovarian indices. Non-adjusted p-values reported

Pair of comparisons	Estimate	Standard error	z value	p-value
Natural - food surplus	-1.30	0.82	-1.57	0.115
Food-limited - food surplus	-1.49	0.93	-1.59	0.110
Food limited - natural	-0.18	0.82	-0.22	0.819

Differential nutrition in social insects, especially in the larval stages can regulate gene expression and alter developmental trajectories committing or predisposing females to certain reproductive roles as adults (Berens et al., 2015; Gadagkar et al., 1991; Gadagkar et al., 1988; Jandt and Toth 2015; Judd et al., 2015; Karsai and Hunt, 2002; Kucharski et al., 2008). However, the quality and quantity of larval nutrition by itself is inadequate to explain the complex course of caste differentiation in reproductively totipotent primitively social wasps (Agrahari and Gadagkar, 2003; Gadagkar, 1991; 2001; Mead et al., 1994; Shukla et al., 2013; Shukla et al., 2014; Solis and Strassmann 1990; Suryanarayanan et al., 2011; Wheeler 1986). Asymmetries in adult nutrition arising through the disproportionate acquisition or retention of food, *viz.* through snatching of food by certain individuals from incoming foragers (Gadagkar, 2001), nutrient transfer through aggressive adult-adult trophallactic interactions (Pardi, 1948), self-feeding propensities either in isolation (Shukla et al., 2013; Tibbetts et al., 2011) or in the context of natal nests (Brahma et al., 2018; Judd et al., 2015; Markiewicz and O'Donnell, 2001) and the nutritional costs of worker behaviour (Hunt, 1991; Marchal, 1897; Markiewicz and O'Donnell, 2001; Richards, 2021) can influence worker ovarian development and probabilities of future nest founding. This current study therefore aimed at reversing potential nutritional deficiencies (and the arising nutritional castration) in queen-right workers and comparing ovarian development in workers of food-surplus colonies with food-limited and naturally foraging colonies. It was found that post-imaginal nutritional supplementation reversed ovarian suppression in queen-right workers and resulted in the loss of colony cohesion. Based on these results, it can be suggested that access to surplus nutrition and the resulting increase in ovarian development and nest desertion promotes colony fission and queen-turnover. Surplus-fed deserter workers should eventually explore reproductive options either in groups (as multiple foundresses) or individually (as single foundresses) at the expense of maintaining colony social cohesion (Krishnan et al., 2021). Patterns of worker- foundress transitions and of queen turnovers should be clearer when deserter wasps experience more optimal spatial conditions, as demonstrated in a followup study (Krishnan et al., 2021). Seen in the broader context of the effects of nutrition on body size, ovarian development, egg laying, and gyne production in social wasps (Gadagkar et al., 1991; Hunt and Dove, 2002; Karsai and Hunt, 2002; Shukla et al., 2013), this study further highlights the importance of nutrition

in regulating female ovarian development, as well as group cohesion in social insects. Nutritional control of female reproductive regulation may thus, not only affect mechanisms of caste differentiation in social insects but may also be a binding force for the maintenance of sociality.

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AUTHOR CONTRIBUTION

SPS and RG designed study and co-wrote the paper and SPS conducted the study. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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