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RESEARCH ARTICLE

Lipid content influences division of labour in a clonal ant

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ABSTRACT

The fat body, a major metabolic hub in insects, is involved in many functions, e.g. energy storage, nutrient sensing and immune response. In social insects, fat appears to play an additional role in division of labour between egg layers and workers, which specialize in non-reproductive tasks inside and outside their nest. For instance, reproductives are more resistant to starvation, and changes in fat content have been associated with the transition from inside to outside work or reproductive activities. However, most studies have been correlative and we still need to unravel the causal interrelationships between fat content and division of both reproductive and non-reproductive labour. Clonal ants, e.g. *Platythyrea punctata*, are ideal models for studying task partitioning without confounding variation in genotype and morphology. In this study, we examined the range of variation and flexibility of fat content throughout the lifespan of workers, the threshold of corpulence associated with foraging or reproduction and whether low fat content is a cause rather than a consequence of the transition to foraging. We found that lipid stores change with division of labour from corpulent to lean and, in reverted nurses, back to corpulent. In addition, our data show the presence of fat content thresholds that trigger the onset of foraging or egg-laying behaviour. Our study supports the view that mechanisms that regulate reproduction and foraging in solitary insects, in particular the nutritional status of individuals, have been co-opted to regulate division of labour in colonies of social insects.

KEY WORDS: Eusociality, Reproduction, Nutrition, Fat content, Clonality, Formicidae

INTRODUCTION

Insect societies are typically characterized by a well-ordered division of labour among their nestmates. While egg layers ensure colony reproduction, workers specialize in non-reproductive tasks inside and outside their nest (Wilson, 1971). There has been increasing interest in understanding how this division of labour has emerged and which factors proximately control the specialization of individuals. Variation in genotype, morphology and age clearly play a role in the propensity of individuals to undertake particular tasks in colonies of honeybees or ants (Stuart and Page, 1991; Robinson, 1992; Smith et al., 2008). However, how individuals specialize for a task may be shaped by many other factors (Jeanson and Weidenmüller, 2014). Indeed, several studies have highlighted the importance of environmental influences – in particular, nutrition – on the development of individual traits and caste determination (e.g. Hunt, 2007; Berens et al., 2015). For example, in bees, the quantity and quality of food fed to larvae trigger changes in gene expression, which result in different caste phenotypes (Maleszka, 2008; Smith et al., 2008; Berens et al., 2015).

Recent genomic studies suggest that nutrition and fat content also act as key players in task specialization in other social insects, and this is supported by measuring fat content in different castes. Nest workers are often fatter than foragers and the depletion of fat content in workers correlates with the transition from inside to outside tasks (‘lean forager–corpulent nest worker’; e.g. Blanchard et al., 2000; Toth and Robinson, 2005; Toth et al., 2005, 2009; Daugherty et al., 2011; Tibbetts et al., 2011; Smith et al., 2011; Robinson et al., 2012). Conversely, in the ant *Lasius niger*, foragers that reverted to nest activities showed an increase in fat content as well as an increased longevity relative to normal foragers under conditions of starvation (Dussutour et al., 2016).

Variation in fat content may also be associated with division of reproductive labour. Queens are consistently more corpulent than nest workers and the last to die in a starving colony (Rüppell and Kirkman, 2005; Smith and Tschinkel, 2006). A minimum fat level is probably a physiological prerequisite to support the synthesis of yolk and for laying eggs (Wheeler, 1996; Canavoso et al., 2001; Markiewicz and O’Donnell, 2001; Toth et al., 2009; Arrese and Soulages, 2010; Daugherty et al., 2011; Roy et al., 2018).

Taken together, these results suggest a link between lipid metabolism and division of labour, most likely via several signalling cascades, including the nutrient-sensing IIS and TOR pathways (Hansen et al., 2013; Bustos and Partridge, 2017; Roy et al., 2018). Though suggestive, most studies have remained correlative, and the link has rarely been substantiated by direct physiological and behavioural studies (e.g. Toth et al., 2009; Daugherty et al., 2011; Berens et al., 2015; Okada et al., 2017). A causal effect of fat on non-reproductive tasks in individuals of standardized age has so far been documented only in honeybee workers, which after treatment with an inhibitor of fatty acid synthesis began to forage precociously (Toth et al., 2005). Whether similar interrelationships underlie the transition from indoor tasks to foraging in other insects, in which eusociality evolved convergently to honeybees, remains unclear. More manipulative studies are therefore needed to clearly unravel the interrelationships between fat content, division of both reproductive and non-reproductive labour, and chronological age.

Platythyrea punctata is one of a dozen or so ant species in which workers can produce female offspring from unfertilized eggs via thelytokous parthenogenesis (Heinze and Hölldobler, 1995; Kellner and Heinze, 2011). Colonies are clonal, and all workers are genetically identical. Nevertheless, colonies show a well-ordered reproductive division of labour based on social status: young workers establish dominance hierarchy and rank order by antennal boxing (Heinze and Hölldobler, 1995). In each colony, one, occasionally several, socially dominant workers reproduce while the majority of workers are subordinate, do not lay eggs and instead engage in non-reproductive tasks. Dominant, egg-laying workers outlive most of their coeval nestmates (Hartmann and Heinze, 2003).

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In previous studies, it has been shown that the transition from inside to outside nest activities is a key determinant of worker lifespan: the later that workers started foraging, the longer they lived (C. Hartmann and A.B., unpublished data; for honeybees, see Rüppell et al., 2007). We also found a link between division of labour and nutritional status of workers: foragers were leaner than intranidal workers, i.e. workers engaged in duties in the nest (Bernadou et al., 2015). These results suggested that the nutritional status of *P. punctata* workers might play a role in regulating the transition from inside to outside nest activities and also their lifespan (Bernadou et al., 2015). *Platythyrea punctata* is therefore an ideal model for investigations on the emergence of division of labour under controlled environmental conditions without any confounding variation in genotype, morphology or ontogeny (Hartmann and Heinze, 2003; Bernadou et al., 2018b).

Here, we built upon our previous work to provide a more comprehensive view on the interrelationships between fat content and division of labour. In particular, we examined the range of variation and flexibility of fat content throughout the lifespan of workers, the thresholds of corpulence associated with foraging or reproduction and whether low fat content is a cause rather than a consequence of the transition to foraging. Finally, we tested whether fat workers may mobilize their fat reserves and serve as ‘repletes’ to feed nestmates and larvae in times of food scarcity (Børgesen, 2000).

We document how lipid stores change with division of labour from corpulent to lean and, in reverted nurses, back to corpulent, and identify the minimal threshold of fat content needed for reproduction and onset of foraging. Food restriction affected colony productivity, and it is unlikely that fat workers have a nutritional function as repletes. Manipulation of fat content by providing clonemates with different quantities of food affected their probability of foraging, showing that fat content has a causal effect on the onset of foraging. Based on results from this and our earlier studies (Bernadou et al., 2015, 2018a,b), we propose a model that links physiological and behavioural components of division of labour.

MATERIALS AND METHODS

Study species and rearing conditions

Colonies of *P. punctata* (F. Smith 1858) were collected in 2012 at El Verde Field Station, El Yunque National Forest, Puerto Rico. Stock colonies have been kept since then in plastic boxes (20 cm×20 cm×9 cm) with a plaster floor in climate chambers under near-natural conditions (22–26°C with a 12 h light:12 h dark cycle, 75% humidity). Cavities dug in the plaster of the plastic box and covered by a glass plate and red plastic film served as nesting site. Colonies were provided with water *ad libitum* through a plastic tube plugged by cotton wool. The plaster floor was regularly moistened to control humidity. The colonies were fed with diluted honey, cockroaches and *Drosophila* 3 times per week (Bernadou et al., 2015). Because of colony fusion and the adoption of alien individuals, natural colonies may contain workers from different clones (Kellner et al., 2010). However, the social structure of colonies with mostly only a single reproductive and the limited lifespan of the latter (Hartmann and Heinze, 2003) make it likely that after several years in the laboratory, colonies essentially consist of single clones (see also Bernadou et al., 2018b, for a genetic confirmation of clonality).

Fat content measurement

Fat content was measured as described in Bernadou et al. (2015). Workers were placed individually in labelled Eppendorf cups, killed and stored by freezing at −20°C. Ants were dried at 60°C for 3 days and weighed to the nearest 0.0001 mg with a Sartorius SC2 ultra-microbalance (dry mass). Subsequently, we extracted the fat by soaking each worker for 4 days in 2 ml petroleum ether (boiling range 40–60°C; Merck, Darmstadt, Germany) at room temperature. After 2 days, the workers were transferred into new Eppendorf cups, and the petroleum ether was changed. The ants were weighed again after drying in a fresh tube at 60°C for 3 days (lean mass). The percentage fat content was calculated as the proportion of dry mass for each worker as: [(dry mass−lean mass)/dry mass]×100.

As it was not possible to determine the fat content of abdomens that had been dissected to determine ovarian status, we investigated whether abdominal fat content is reflected in the fat content of the head and thorax. Using the same procedure as for complete workers, we determined the fat content of individual body parts (head, thorax including the epinotum, abdomen including the petiole) of 10 young intranidal workers, 10 old intranidal workers and 10 foragers (see below for the characterization of these three worker groups) each from two stock colonies.

Ovary development

To determine reproductive status, we dissected the ovaries of workers following standard methods (Hartmann and Heinze, 2003). The ovaries were placed on a microscope slide, covered by a coverslip, and photographed under a microscope (Zeiss Primo Star) at 40× magnification. For each dissected worker, we measured the length of the largest oocyte (if no oocytes were observed, workers received a score of 0). Images were analysed using the free software ImageJ v1.48.

Experiments

Experiment 1: does fat content differ among workers?

To test whether fat content differs among workers, we measured fat content in five worker groups: foragers, old and young intranidal workers, newly eclosed workers (callows) and workers that had recently died (i.e. most likely old foragers; Bernadou et al., 2015). We distinguished foragers, which were found in the foraging area outside the nest and responded aggressively when provoked with forceps, from intranidal workers. Intranidal workers were selected from workers still present in the nest 1 h after all the foragers had been removed from the foraging area. Among the intranidal workers, we differentiated between callows, young and old intranidal workers based on the colour of the cuticula, which in *P. punctata* changes from yellowish to blackish within 3–4 weeks (Bernadou et al., 2015). Before starting the experiment, we marked all young individuals present in the nest with a colour spot (Edding® 751 paint marker) and cleaned the colonies by removing all worker corpses. Thereafter, colonies were checked 3 times per day at 09:30 h, 14:00 h and 17:00 h, and all newly hatched callows and workers that had died were collected. Three stock colonies were used with 10 workers in each group (i.e. 150 workers in total).

Experiment 2: is foraging effort associated with fat content and/or ovary development?

In this experiment, we examined whether foraging effort was related to fat content and/or ovary development. In order to cover the full range of fat content present in workers in stock colonies (see experiment 1), we marked young intranidal workers, old intranidal workers and foragers from three mother colonies with a colour code (10 workers in each group, i.e. 90 workers in total). We used the scan sampling method (i.e. proportional time) to observe as many workers as possible to disentangle the interrelationship between fat content, ovary development and foraging intensity. Scan sampling began 1 day after marking to allow ants to recover from handling and marking. We conducted 10–12 scans per day for 5 days per week (from Monday to Friday) between 09:30 h and 18:00 h (minimum time interval of 30 min between two consecutive scans of the same worker). During each scan, we recorded the location of all marked individuals (inside or outside the nest). Observations stopped once we had recorded 80 scans per worker. At the end of the observation period, ants were placed individually in labelled Eppendorf cups and stored by freezing at −20°C. We separated the abdomen from the head and thorax for subsequent analyses. The thoraces were used to quantify fat content and the abdomens were dissected to determine the reproductive status of workers (10 workers had to be discarded because of poor dissection quality).

Experiment 3: does fat content differ between reproductive and non-reproductive workers?

In unmanipulated colonies, age, tasks performed and also food access may vary widely among clonemates. In addition, one or occasionally several individuals are reproductive, while the majority of workers are non-reproductive. Hence, getting enough replicates to determine how the different factors affect fat content in colonies would have been extremely laborious. Instead, we set up dyads of similar-aged workers to examine whether fat content differs with reproductive status, i.e. ovary development. The experimental isolation of two young intranidal clonemates in a new nest box quickly leads to the establishment of a stable division of labour between a dominant egg layer, which remains in the nest and lays eggs, and a subordinate forager (Bernadou et al., 2018b). Young intranidal nestmates with a similarly brownish cuticula colour were transferred to a Petri dish (diameter 13.5 cm, height 3 cm) consisting of a plaster floor and a cavity covered by a microscope slide and darkened by a piece of red plastic film that served as a nest (10 stock colonies were used to set up 16 pairs of workers). Ants were fed 3 times per week with diluted honey and *Drosophila*. After 10–14 weeks, ants were placed individually in labelled Eppendorf cups and stored at −20°C. We separated the abdomen from the head and thorax for subsequent analyses of fat content and ovarian status as above. Workers were categorized in each dyad as reproductive dominants (individual with the most developed oocytes) and subordinate foragers (individual with the least developed oocytes) based on their oocyte development (*N*=15 pairs, oocyte length: 0.984±0.095 mm and 0.145±0.105 mm for dominants and subordinates, respectively; one pair was excluded because of a similar oocyte development, 0.777 versus 0.790 mm).

Experiment 4: does fat content have a causal effect on foraging behaviour?

To test whether fat content has a causal effect on foraging behaviour independent of worker age, we manipulated the nutritional status of same-aged workers by reducing their access to food. Stock colonies of *P. punctata* were checked daily for newly hatched workers. All callows were marked with a colour code on the day of eclosion and returned to their mother colony to recover. After 3 days, clonemates that had eclosed on the same day (or within the same 24 h) were placed individually into a plastic tube (diameter 1 cm, length 6.5 cm) covered by red plastic film for a period of 10 days. Tubes were half-filled with water and plugged by moist cotton to maintain humidity. The entrances were similarly plugged with dry cotton to prevent the ants from escaping. Nutritional status of workers was manipulated by providing callows with different amounts of food (3 *Drosophila* per day during 10 days or 3 *Drosophila* only on day 5). During the isolation treatment, five poorly fed and one well-fed worker died. After 10 days, same-aged but differently treated callows were either frozen for fat content measurement (to confirm treatment efficacy – fat content measured in the abdomen, *N*=16 and 17 for poorly fed and well-fed workers, respectively) or placed in pairs in miniature nests (as in experiment 3). Ants were fed every 2 days with diluted honey and *Drosophila*. We monitored the behaviour of individuals twice daily for 10 min (20 min per day per nest) over six consecutive days and in particular documented time spent outside the nest (*N*=25 pairs of workers). Data of the first and last 3 days were pooled in time blocks to facilitate their visualization and analysis. Worker behaviour in dyads is in general episodic and their probability of being recorded by scan sampling would have been low. Eleven stock colonies were used for this experiment to set up all the different groups of workers.

Experiment 5: does fat content increase in reverted nurses?

We have previously shown that *P. punctata* foragers, in the absence of nurses, can revert to intranidal tasks, including egg laying and brood care behaviour (Bernadou et al., 2015, 2018a). To test whether this reversal also involves an increase in fat content among workers, we set up 12 experimental colonies consisting of 10 foragers each. Each group of foragers was transferred without brood into a 20 cm×10 cm×6 cm plastic box with a plaster floor and a nest chamber as above. On the same day, six additional foragers (from the same mother colonies) were collected, placed individually in labelled Eppendorf cups and stored by freezing at −20°C to serve as control. Colonies were kept under standard conditions. After 30 days, all workers still alive were collected and stored at −20°C in Eppendorf cups until fat extraction. Nests were inspected after 30 days for the presence of eggs. As we did not know the quantities of fat gained by reverted nurses after 1 month, we decided to use the whole body to get the most accurate fat measurements.

Experiment 6: does food restriction affect colony productivity?

To test whether workers, which may contain a large amount of fat (see Results), might serve as ‘repletes’ and use their fat to feed nestmates and larvae in times of food scarcity, we determined how food availability affects colony productivity of *P. punctata*. Six mother colonies were split each into two sub-colonies of equal size. Treatment began 2 months after splitting to allow the colonies to regrow and develop a new stable hierarchy among workers (colonies were fed normally during this period). Six sub-colonies were then fed with diluted honey (2.5 ml, 15% w/v) and 80–100 *Drosophila* 3 times per week (high food quantity) while the other six sub-colonies received the same amount of honey but 80–100 *Drosophila* only once a week (low food quantity). During 1 month, we counted every week the number of pupae and workers present in each colony.

Statistical analyses

All analyses were performed and graphs generated with the statistical software R 3.3.2 (https://www.R-project.org/). For experiment 1, we tested whether the percentage of fat content differed among the five worker groups by a linear mixed-effect model (LME, lmer function, lme4 package; Bates et al., 2015). Because of heterogeneity of variance among groups, we ran the analysis by allowing a different variance structure per worker group (varIdent function, nlme package, https://CRAN.R-project.org/package=nlme; Zuur et al., 2009). In addition, we conducted pairwise comparisons between groups using Tukey contrasts (glht function, multcomp package; Hothorn et al., 2008). The association between fat content in the head, thorax and abdomen was investigated using Spearman rank correlation tests.

The number of times workers were found outside the nest in experiment 2 was analysed by a generalized linear mixed model (GLMM) with a binomial distribution (glmer function, lme4 package; Bates et al., 2015). We ran the model with the proportion of time a worker was outside as a response variable and fat content, ovary development and their interaction as explanatory variables.

For experiment 3, we tested whether the percentage of fat content differed among dominant and subordinate workers with an LME model. We ran the model with fat content as a response variable and the time individuals spent together in the miniature colonies, social status (two levels: dominant, subordinate) and their interaction as explanatory variables.

To test whether receiving different quantities of food for 10 days resulted in a difference in fat content between workers (experiment 4), we used an LME model. We ran the model with fat content as a response variable and treatment (two levels: well-fed workers, poorly fed workers) as an explanatory variable. To study whether fat content has a causal effect on division of labour, we ran another LME model with time spent outside as a response variable and treatment (two levels), blocks of observation and their interaction as explanatory variables.

In experiment 5, to examine whether reversion involves an increase in fat content among workers, we ran an LME model with fat content as a response variable and eggs (two levels: presence, absence), days when workers were collected (0 for controls or 30 for reverted nurses) and their interaction as explanatory variables.

To determine how food availability affects colony productivity (experiment 6), we used a GLMM with a Poisson distribution. We ran the model with number of workers or pupae (count data) as response variables and treatment (two levels: high food quantity, low food quantity), weeks and their interaction as explanatory variables.

We obtained minimal models by successively removing the least non-significant fixed factors and by comparing the nested models by likelihood ratio tests. The *P*-values for the final models were obtained by the function Anova from the ‘car’ package (Fox and Weisberg, 2011). To account for pseudo-replication, colony, miniature colony or subject nested within colony were introduced in the models as a random effect factor. Residuals of LME models were checked for assumptions of normality (Shapiro–Wilk test of normality) and homoscedasticity (Levene’s test for homogeneity of variance). Data were log or log(x+1) transformed when necessary. Poisson and binomial models were checked using the DHARMa package (http://florianhartig.github.io/DHARMa). If overdispersion was detected, we added an observation-level random factor (Harrison, 2014).

RESULTS

Experiment 1: fat content differs among and within workers

The percentage of fat in the whole body of workers decreased significantly from callows to freshly dead workers (LMM: $\chi^2_4 = 1469.1$, $P<0.001$; Fig. 1A). Each group differed significantly from each other except for callows and young intranidal workers (GLHT contrast: $Z=-1.429$, $P=0.153$; Fig. 1A).

In each worker group, the highest absolute fat content was found in the abdomen followed by the thorax and head (mean over all groups: 0.120±0.024, 0.033±0.004 and 0.027±0.002 mg, respectively; *N*=60). Using the same dataset, we checked whether the percentage of fat in the abdomen correlated with the fat content of the head and thorax (Fig. 1B,C). In both cases we found a strong positive

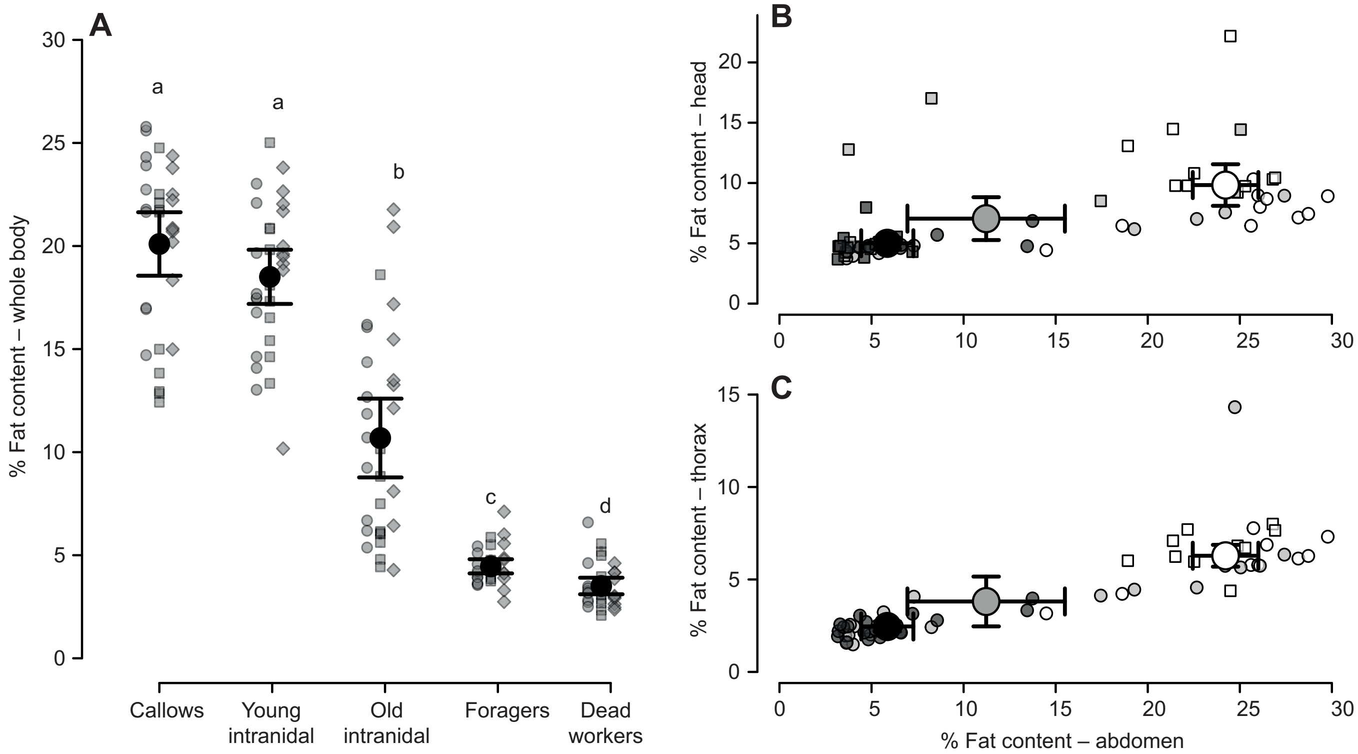

Fig. 1. Percentage fat content in workers of the clonal ant *Platythyrea punctata*. (A) Percentage whole-body fat content in five categories of workers: callows, young and old intranidal workers, foragers, and workers that died recently (*N*=30 for each group, the three symbols in each group represent the three different colonies used; each small symbol represents a worker). Different letters on the plot indicate datasets that are statistically different from each other. The large black circles indicate the mean±95% confidence interval ($\text{CI}_{0.95}$) of each group. (B,C) Percentage of fat in the head (B) or thorax (C) against fat content in the abdomen (*N*=60). The larger circles indicate the mean±$\text{CI}_{0.95}$ of each group (young and old intranidal workers, and foragers in white, grey and black, respectively; the two symbols in each group indicate the two different colonies used; each small symbol represents a worker).

correlation (Spearman rank correlation: head versus abdomen, r_s=0.71, N=60, P<0.001; Fig. 1B; thorax versus abdomen, r_s=0.87, N=60, P<0.001; Fig. 1C). We therefore used fat content of the thorax as a proxy for the fat content of the abdomen (because of its higher Spearman correlation coefficient) in the experiments in which the abdomen was dissected to determine ovarian status.

Experiments 2 and 3: fat content is associated with foraging effort and reproductive status

The frequency of a worker being observed outside the nest was significantly influenced by both fat content (GLMM: $\chi^2_1 = 52.359$, P<0.001; Fig. 2) and ovary development (GLMM: $\chi^2_1 = 14.145$, P<0.001; Fig. 2). A decrease in fat content level or ovary development was associated with an increase in foraging effort. There was also a significant interaction between fat content and ovary development on foraging effort (GLMM: $\chi^2_1 = 12.788$, P<0.001; Fig. 2). That is, ants with low fat content tended to forage more if they had undeveloped ovaries. When individuals were fatter, foraging effort remained low whatever their ovary development (Fig. 2). To illustrate the interaction between oocyte length and fat content (Fig. 2), we discretized the variable 'oocyte length' and plotted the probabilities according to three oocyte categories: undeveloped (0.0 mm, N=49), developed(−) (>0.0–≤0.2 mm, N=16) and developed(+) (>0.2 mm, N=15). In addition, oocyte length and fat contents were positively and significantly correlated (Spearman rank correlation: r_s=0.56, N=80, P<0.001).

When placed together in pairs, same-aged clonemates quickly established rank order and stable division of labour by aggressive antennation. In the 15 nests with a clear division of reproductive labour, reproductive status was significantly associated with fat content: dominant reproductives had a higher fat content than their subordinate clonemates (LMM: $\chi^2_1 = 9.865$, P=0.002; Fig. 3). Neither the time individuals spent together in the miniature colonies (10–14 weeks) nor the interaction between social status and time spent together had an effect on fat content (LMM: $\chi^2_1 = 0.112$, P=0.737 and $\chi^2_1 = 0.441$, P=0.506, respectively).

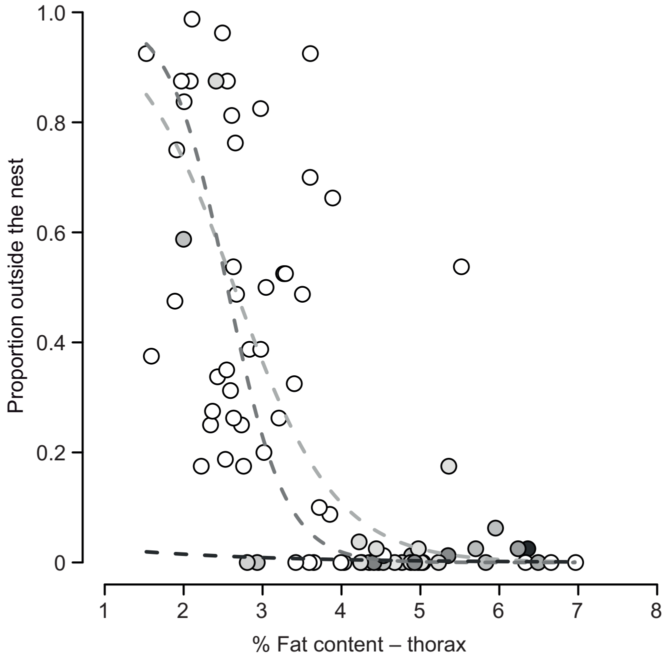

Fig. 2. Percentage fat content (thorax) in *P. punctata* workers with different foraging effort. Circles represent workers (N=80) and different tones of grey (dark, 1.148 mm maximum; light, 0.0 mm minimum) reflect oocyte development. To illustrate the interaction between oocyte development and fat content on foraging effort, we discretized the variable 'oocyte length' and plotted the probabilities according to three oocyte categories: undeveloped (0.0 mm, N=49, light grey curve), developed(−) (>0.0–≤0.2 mm, N=16, grey curve) and developed(+) (>0.2 mm, N=15, dark grey curve).

Experiment 4: fat content affects foraging behaviour

Receiving different quantities of food for 10 days resulted in a significant difference in fat content (well-fed workers: 19.04±4.54%, N=17; poorly fed workers: 5.84±3.78%, N=16; LMM: $\chi^2_1 = 24.478$, P<0.001; Fig. 4A). Poorly fed workers had similar fat levels to those of foragers (5.84% versus 5.85%, respectively, see the values of fat content of the abdomens of foragers in Fig. 1C). In contrast, well-fed workers (19.04% fat) had a fat content more or less intermediate between that of young (24.22%) and old (11.22%) intranidal workers (see fat content in the abdomens of young and old intranidal workers in Fig. 1C).

Building on this result, we grouped same-aged, but differently treated callows in miniature nests to test whether fat content has a causal effect on division of labour. The nutritional manipulation showed a non-significant trend concerning the time the workers spent outside the nest: poorly fed workers spent more time outside than well-fed workers (LMM: $\chi^2_1 = 3.435$, P=0.064; Fig. 4B). The time spent outside the nest was also influenced by the blocks of observation: it was significantly longer in the first block (1–3 days) than in the second block (4–6 days) (LMM: $\chi^2_1 = 29.788$, P<0.001). The interaction between observation block and nutritional manipulation was not significant (LMM: $\chi^2_1 = 1.415$, P=0.234; Fig. 4B).

Experiment 5: fat content increases in reverted nurses that lay eggs

After 1 month, eggs had been laid in five of 12 colonies of foragers. Therefore, we distinguished between colonies with and without eggs. Concerning fat content, we found a significant interaction

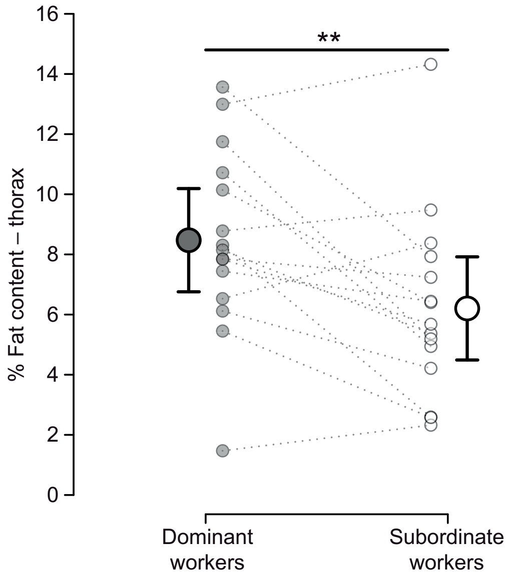

Fig. 3. Percentage fat content (thorax) in pairs of similar-aged dominant and subordinate *P. punctata* workers maintained for 10–14 weeks in miniature colonies. Each small circle represents a worker (N=15 for each group), the larger circles indicate the mean±CI$_{0.95}$, and the dotted lines represent dyads. **P<0.01.

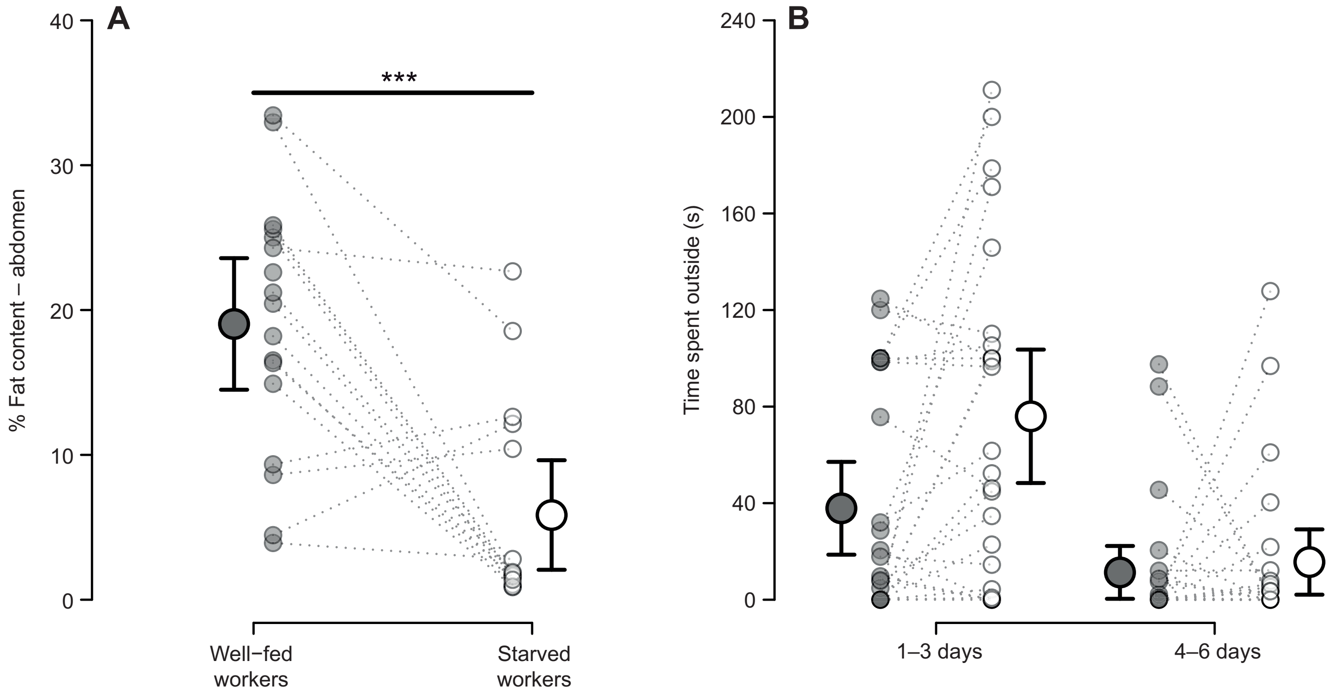

Fig. 4. Fat content and foraging behaviour. (A) Percentage fat content (abdomen) in well-fed (*N*=17) and poorly fed (*N*=16) *P. punctata* workers after 10 days of receiving different quantities of food (****P*<0.001). (B) Mean time poorly fed (white) and well-fed (black) *P. punctata* workers maintained in miniature nests spent outside the nest during the first 3 days and the last 3 days of observation (*N*=25 for each group). Each small circle represents a worker, the larger circles indicate the mean±CI$_{0.95}$, and the dotted lines represent dyads.

between the factors egg presence and time (LMM: $\chi^2_1 = 9.102$, *P*=0.002; Fig. 5). That is, fat content did not differ between control foragers (which had been frozen before the start of the experiment) from colonies in which 1 month later their nestmates had or had not laid eggs (day 0 – control; Fig. 5). However, after 30 days, foragers from colonies with eggs had a higher fat content than foragers from colonies without eggs (Fig. 5). This result implied that, after 30 days, foragers that had not regained fat also failed to reproduce. The factors eggs presence and time were not significant (LMM: $\chi^2_1 = 0.871$, *P*=0.351 and $\chi^2_1 = 0.000$, *P*=0.992, respectively).

Experiment 6: food restriction affects colony productivity

Food restriction influenced colony productivity, i.e. the number of newly produced workers and pupae, after the first 2 weeks of treatments (Fig. S1). The number of workers was influenced by time since the start of food treatment (GLMM: $\chi^2_1 = 14.783$, *P*=0.001) but not by food treatment (GLMM: $\chi^2_1 = 1.073$, *P*=0.300; Fig. S1A). The interaction between food treatment and time was also significant (GLMM: $\chi^2_1 = 10.186$, *P*=0.001; Fig. S1A): the number of workers increased steadily in high food quantity colonies while it remained almost the same for the low food quantity colonies.

The number of pupae was influenced by both food treatment (GLMM: $\chi^2_1 = 9.580$, *P*=0.002) and time since the start of food treatment (GLMM: $\chi^2_1 = 12.570$, *P*<0.001). The interaction between food treatment and time was also significant (GLMM: $\chi^2_1 = 39.763$, *P*<0.001; Fig. S1B): the number of pupae decreased quicker in low food quantity colonies than in high food quantity colonies.

DISCUSSION

The fat body is a major metabolic hub in insects, i.e. a centre of important metabolic processes (Arrese and Soulages, 2010; Musselman and Kühnlein, 2018). It is involved in many functions, e.g. energy storage, nutrient sensing and immune response (Zheng et al., 2016; Mirth et al., 2019). Here, we demonstrate in the ant *Platythyrea punctata* that the fat body is a dynamic tissue that responds to environmental and social conditions. While fat levels decreased steadily in most workers from hatching to death, a high lipid content was maintained in reproductives and it even increased on average in reverted nurses. By following the behaviour of individual workers and quantifying their ovary development and fat content, we revealed the existence of a threshold in fat content that controls the onset of foraging (Fig. 2). Lastly, manipulating fat content among same-aged workers, though not significant, suggested a causal role of fat depletion. Although a causal role of fat in foraging was hypothesized from studies in honeybees, it had not been confirmed in ants (Toth et al., 2005).

Fat tissue was present throughout the workers' body (for queens, see Jensen and Børgesen, 2000) and the highest lipid stores were located in the abdomen, presumably around the digestive tracts and the ovaries (Roma et al., 2010). Ants collected in the nest had a higher fat content than workers collected outside the nest, which matches the hypothesis of 'lean forager – corpulent nest worker', i.e. the depletion of body reserves triggers foraging behaviour (e.g. Toth and Robinson, 2005; Toth et al., 2005; Tibbetts et al., 2011; Smith et al., 2011; Robinson et al., 2012; Bernadou et al., 2015). However, the full variation in body stores of workers from hatching to death has, to our knowledge, never been reported. We showed that ants outside the nest and freshly dead workers had a similarly low fat content (Fig. 1A). Moreover, foragers had 75% less fat than callows (4.5% fat and 20.1% fat for foragers and callows, respectively; Fig. 1A). Many old intranidal workers had similarly low fat content to that of foragers, suggesting that the main losses in body reserves occur before the onset of foraging. The low variation of fat content in foragers suggests that foraging activities had only a slight effect on fat depletion (Toth and Robinson, 2005). Fat-depleted workers might not rely on lipid content to sustain their foraging activity and

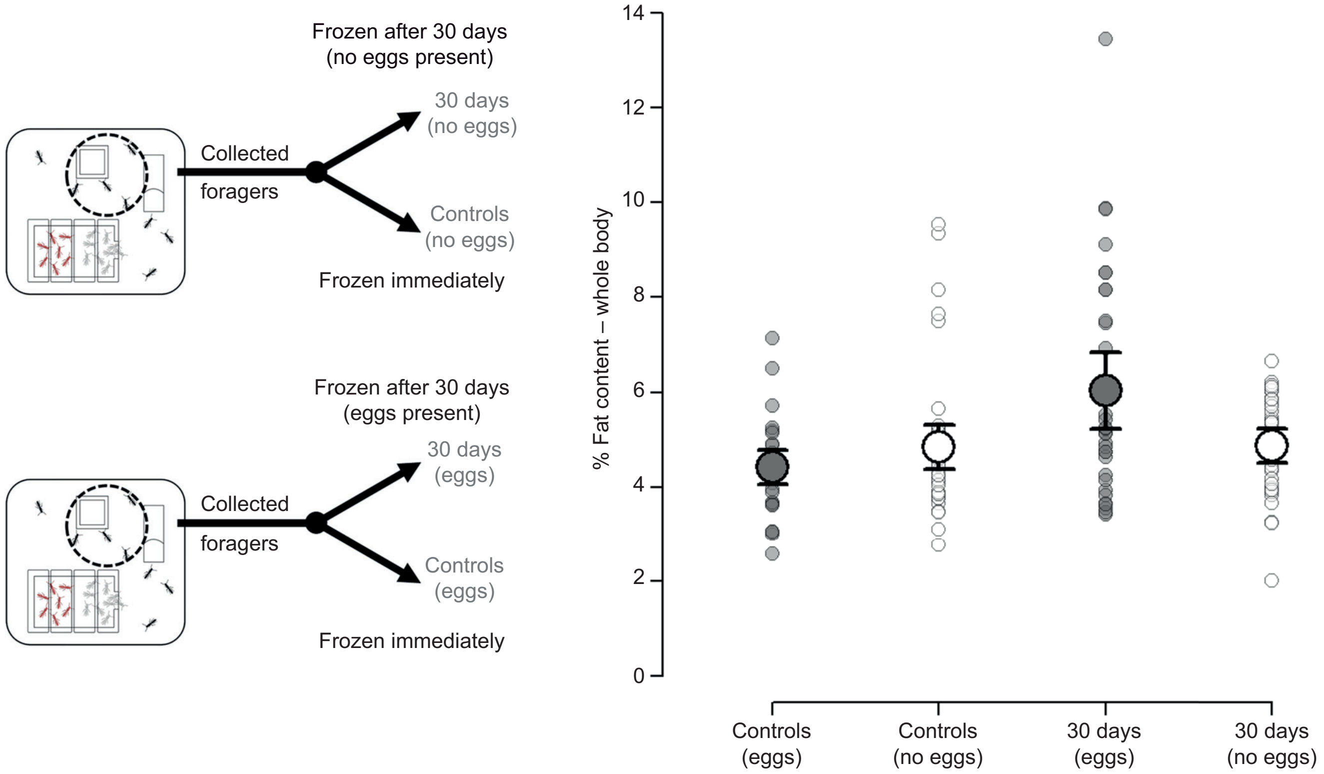

Fig. 5. Fat content in reverted nurses. Percentage fat content (whole body) in *P. punctata* foragers collected in stock colonies (controls; *N*=30 eggs, *N*=42 no eggs) or after they had been maintained for 30 days in colonies consisting of foragers only (30 days; *N*=34 eggs, *N*=35 no eggs). Colonies of foragers that had laid eggs after 30 days were distinguished from colonies that did not lay eggs (eggs had been laid in five of 12 colonies of foragers). After 30 days, foragers from colonies with eggs had a higher fat content than foragers from colonies without eggs (LMM, interaction between egg presence and time: $\chi^2_1 = 9.102$, *P*=0.002). Each small circle represents a worker; the larger circles indicate the mean±$\mathrm{CI}_{0.95}$.

must use another energy resource, most likely carbohydrates (Toth and Robinson, 2005).

Behavioural observations from experiment 2 revealed that the likelihood of workers beginning foraging increases strongly when their fat content falls below 3–4% (in the thorax, reflecting approximately 8–12% in the abdomen; see Fig. 1C). More corpulent individuals, whatever their ovary development, usually stayed in the nest (Fig. 2). This result matches previous assumptions that division of labour, including the transition from nest work to foraging, is triggered by self-organization through fixed or self-reinforcing variation in 'stimulus–response thresholds' (e.g. Camazine et al., 2001; Theraulaz et al., 1998). Although other mechanisms, e.g. individual experience (Ravary et al., 2007; Robinson et al., 2012), life expectancy (Moroń et al., 2008) or age (Wilson, 1971), may also affect the onset of foraging activities, worker corpulence provides a simple physiological mechanism to monitor food availability in the nest and to organize the colony's foraging effort (Schulz et al., 1998). From an evolutionary perspective, having lean foragers also keeps fat as a valuable resource in the relative safety of the nest and minimizes resource loss through the death of corpulent foragers (foragers as a 'disposable caste'; Porter and Jorgensen, 1981; O'Donnell and Jeanne, 1995; Toth and Robinson, 2005).

Recently hatched workers had the highest quantities of fat but also showed considerable inter-individual variation in this trait (Fig. 1A). As callows were collected 3 times per day, it seems unlikely that this variation in body reserves is associated with different food intake following emergence. Instead, different fat content of callows might have already been determined at the larval stage (Hunt, 2007; Judd et al., 2010, 2015). In social insects, early nutritional bias may have long-lasting consequences for division of labour (e.g. Judd et al., 2015; Lawson et al., 2017; Bernadou et al., 2018b). Workers hatching with high fat content may be more prone to initiate dominance interactions than leaner callows and may have a head start concerning reproduction (Shukla et al., 2013; Judd et al., 2015; Bernadou et al., 2018b). Different initial fat content might underlie the variation in reproductive performance among callows when placed into a new nest site together with older nestmates (A.B. and J.H., unpublished results; see also Shukla et al., 2013).

Interestingly, manipulation of the nutritional status of workers and thus their fat content tended to induce a non-significant behavioural bias: well-fed callows stayed inside the nest while poorly fed callows spent more time being active outside (Fig. 4B; see also Bernadou et al., 2018b). These results have two implications: (1) they confirm that the onset of foraging is associated with fat content and (2) it seems that there is a causal connection between fat and division of labour (Toth and Robinson, 2005; Toth et al., 2005). Poor nutritional conditions may have increased the likelihood of foraging in young workers indirectly via an effect on fat content (Fig. 4). In many organisms, poor nutritional conditions, e.g. food deprivation or access to imbalanced food, result in hyperactivity and increased foraging activity in order to locate new food sources (e.g. Dussutour and Simpson, 2012; Yang et al., 2015; Scharf, 2016; Landayan et al., 2018; Poissonnier et al., 2018). The decrease in time spent outside the nest between the two observation periods is probably explained by poorly fed workers retrieving prey and bringing them back to the nest, which reduced their foraging activity during the second observation period.

Do lipid stores invariably become depleted in all workers? Our results confirm that the fat body is a dynamic tissue that responds to environmental and social conditions (Fig. 3; Arrese and Soulages, 2010). Although we generally expect a decrease in fat content over time, there may also be differences among individuals (i.e. for reproductive workers and reverted nurses). In experiment 3, ovary development and social status appeared to be associated with a high fat content, probably triggered by the need to sustain metabolic demands for egg production (Wheeler, 1996; Arrese and Soulages, 2010). However, in this experiment we could not substantiate whether differences in fat content between dominants and subordinates were a consequence or a cause of reproductive division of labour. But interestingly, neither the time individuals spent grouped nor the interaction between social status and time had an effect on fat content. This indicates that both workers maintained their fat level over time. While reproductive individuals may need fat to maintain efficient egg-laying activity, we would have assumed subordinates would lose fat over time. The discrepancy is probably resolved by the fact that food was provided *ad libitum* and was not a limiting factor, explaining why individuals in dyads had higher thorax fat levels than those in normal colonies (see Figs 2 and 3).

Isolated groups of *P. punctata* foragers can reverse their behavioural development and switch back to intranidal tasks (reverted nurses; Bernadou et al., 2015, 2018a). This reversal is associated with a broad range of behavioural changes, including waste management and worker localization, but also an increase in residual lifespan (Bernadou et al., 2015, 2018a; but see Herb et al., 2012; Dussutour et al., 2016). Our results suggest that reversion is correlated with physiological modifications, i.e. an increase of fat content, but only in groups that have laid eggs (Fig. 5; but see Dussutour et al., 2016). We previously compared egg laying among worker groups and showed that reverted nurses, if they started laying eggs, did it later than young or old intranidal workers (Bernadou et al., 2015). Either ovaries take longer to re-activate in such worker groups or foragers needed to rebuild fat reserves and improve their nutritional status before they can start laying eggs, or both. Indeed, egg laying depends on the internal condition of individuals (Wheeler, 1996). These results are also supported by experiment 2, which suggests that workers with developed ovaries and low fat content do not occur.

Based on our results, we propose a model to explain how fat content and reproduction are associated and how they regulate the onset of foraging in *P. punctata* (Fig. 6). The decision to forage is probably mediated by a worker's nutritional status and the presence of food in the colony. Workers will not start foraging until they have lost most of their lipid stores. Below a threshold of 3–4% fat content in the thorax and in the absence of food, the probability of a worker beginning to forage increases steeply. Leanness is not a consequence of foraging activity but has a causal effect on the transition from duties inside the nest to outside tasks. Workers that start foraging are expected to switch from lipid to carbohydrate metabolism to fuel their daily activity. In the absence of reversion (and an increase in fat content), the ovaries of foragers will degenerate irreparably (Fig. 5). Contrary to most workers, reproductive individuals maintain their fat level. A well-developed fat body is probably needed to support the synthesis of the lipoprotein vitellogenin (Wheeler, 1996; Toth and Robinson, 2005). Moreover, changes in fat content might affect hormone titres and biogenic amine levels (Page et al., 2012; Okada et al., 2015), both of which have been shown to affect division of labour. For example, the fat body may be a target of dopamine, which may cause reproductive differentiation (Okada et al., 2015).

Two questions remain and deserve further attention: (1) why do nurses lose their fat prior to foraging?; and (2) why cannot all foragers regain fat and reproduce? In ponerine ants, workers do not perform trophallaxis (but see Hashimoto et al., 1995; Liebig et al., 1997). It is, therefore, unlikely that nurses are used as 'fat-body repletes' to feed nestmates (Wheeler and Martinez, 1995; Børgesen, 2000). Competition among workers for food resources might be another explanation. Dominant individuals may monopolize prey

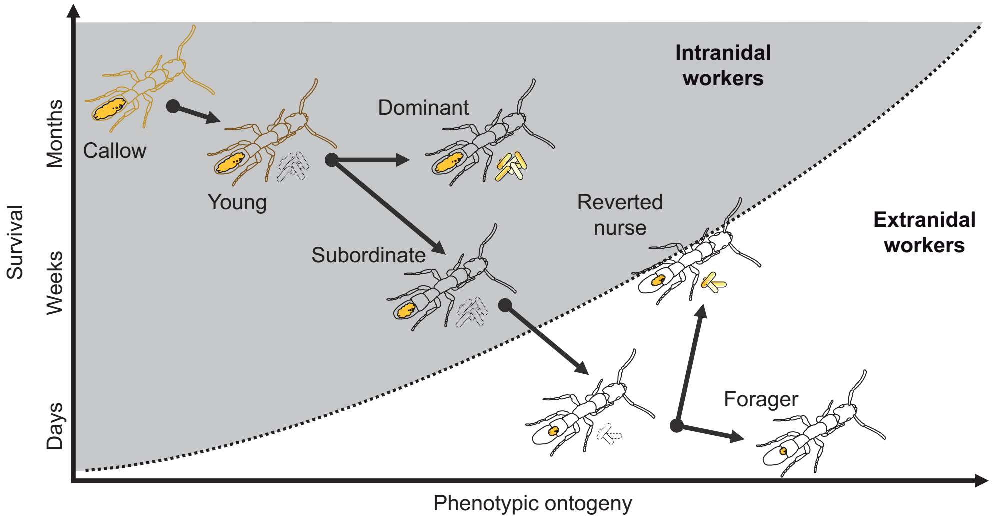

Fig. 6. Model for the association among fat body, reproduction and foraging behaviour in the ant *P. punctata*. The decision to forage is mediated by a worker's nutritional status (fat body in yellow) and presence of food in the colony. Workers will not start foraging until they have substantially lost lipids. Below a given fat threshold and in the absence of food, the probability of an individual starting to forage increases steeply. In the absence of reversion (reverted nurse), the ovaries of foragers will degenerate. Contrary to most workers, reproductive individuals (dominant, with pile of eggs) maintain their fat level, probably to support the synthesis of vitellogenin. Workers with a dotted brood pile may still have the capacity to lay eggs but will not oviposit in normal colonies. Foragers will become reverted nurses, gain fat and start laying eggs only when they are isolated from the rest of the colony. The design of this figure was inspired by fig. 1 in Münch and Amdam (2010).

and/or feed on high-quality nutrients and limit food access to submissive workers, which eventually lose their fat (O'Donnell, 1998; Salomon et al., 2008). Workers would therefore shift their diet away from high-quality proteins to more carbohydrates, e.g. in honey bees (Paoli et al., 2014). The inability of several foragers to revert to egg laying might be caused by the degeneration of nurse cells and oocytes in late age (Okada et al., 2010). As a consequence, such foragers, with no ovarian feedback from their nurse cells and oocytes on their fat body, would remain fat depleted. A decrease in lipid reserves associated with a loss of reproductive performance has been reported in several species of solitary Hymenoptera (e.g. O'Neill et al., 2015). Whether the same pattern observed in our study, i.e. the link between fat content and reproductive performance in *P. punctata* workers, results from ancestral physiological constraints underlying division of reproductive labour remains to be tested ('ovarian ground plan'; West-Eberhard, 1996; Okada et al., 2010).

In conclusion, our study confirms the role and importance of nutrition in division of labour in social insects. In bees, Toth et al. (2005) noted that 'mechanisms linking internal nutritional physiology to foraging in solitary insects have been co-opted to regulate altruistic foraging in a social context'. Our study supports this view and is consistent with the idea that similar processes shaped the convergent evolution of eusociality. Whether the genomic and physiological pathways controlling fat dynamics and its link with reproduction and foraging behaviour are ant specific or share similar genes and regulatory networks with other social insects remains to be elucidated (e.g. Daugherty et al., 2011; Berens et al., 2015; Okada et al., 2017).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.B., J.H.; Methodology: A.B.; Validation: A.B., E.H., J.P., J.H.; Formal analysis: A.B.; Investigation: A.B., E.H., J.P.; Data curation: E.H., J.P.; Writing - original draft: A.B.; Writing - review & editing: A.B., J.H.; Visualization: A.B.; Supervision: A.B.; Project administration: J.H.; Funding acquisition: J.H.

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Data availability

Data are available from the Dryad Digital Repository (Bernadou et al., 2020): dryad.2kh498h

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.219238.supplemental

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SUPPLEMENTARY FIGURES

Figure S1. (A) Number of workers and (B) pupae present in sub-colonies of *P. punctata* fed three times per week (black dots) or only once a week (white dots). Six stock colonies were split into equal sub-colonies 8 weeks before starting the treatment. Each small dot represents a sub-colony, the bigger dots indicate the mean.