THE UNIVERSITY of ADELAIDE

# NUTRIONAL ECOLOGY IN SOCIAL INSECTS

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*“If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos.”*

E.O.Wilson

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# Abstract

*Most living organisms must regulate their nutrient intake to survive and reproduce. This regulation is challenging because animals must manage the fluctuating demands of their own metabolism within the context of nutritionally heterogeneous environments. For social insects, the survival of the group relies on the efforts of only a small number of individual foragers. These foragers do not possess a direct knowledge of the colony's nutritional state, yet they are able to accurately regulate their intake to meet the varying needs of their nestmates. To further our knowledge of the nutritional ecology of social insects, we need to understand the rules that foragers follow in order to maintain the collective nutrition of the group. Most advances in the field of collective nutrition come from the development of the Nutritional Geometric Framework (NGF). The NGF is a modelling platform that allows the integration of: the animal's nutritional state, the optimal state it could reach, the foods available and the consequences of eating those foods. The present study combines the use of modelling and experiments implementing the NGF to explore how social insects utilise collective nutrition to fight pathogens and how specialist feeders meet their nutritional needs.*

*Solitary species have been shown to alter their intake of nutrients to fight infections, but how would such a response be achievable on a collective scale? We adapted an existing individual based model of nutrition to investigate the impact of collective nutrient balancing on pathogen spread in a social insect colony. In our model, foragers not only altered their food collection according to their own infection status but also to the status of nestmates, and this social immunity strategy was highly beneficial to the colony when immune responses were short lived. Impaired foraging in infected workers favoured colony resilience when pathogen transmission rate was low (by reducing contact between colony members), or triggered colony collapse when transmission rates were fast (by depleting the pool of foragers). Our findings therefore suggest a new mechanism by which colonies could defend*

*themselves against pathogens and provide a conceptual framework for experimental investigations of the nutritional immunology of social animals.*

*For the rest of my PhD, we investigated the regulation of nutrition in groups of specialist feeders. We developed artificial diets and experimental setups to run the first NGF study on termite macronutrient regulation. We confined termite groups to single diets with varying macronutrient compositions. Diet composition did not affect food intake, but impacted lifespan and foraging. This finding is in direct contrast observation of generalist insects studied thus far. The amount of carbohydrate eaten had a strong effect on lifespan, and foraging activity increased with global intake. We subsequently offered termites various food pairing with highly different protein:carbohydrate ratios. Foragers collected the same amount of food, regardless of protein type or group caste composition. These results validate a nutritional ecology theory predicting that animals specialised on an invariant food type would lose the ability to regulate nutrient composition and would instead only regulate the amount of food collected.*

# Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Laure-Anne Poissonnier Date 16/07/2018

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And to conclude, a short citation by one of the greatest women in science, Marie Curie, underlying the importance of gaining and sharing knowledge.

"*Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.*" — Marie Curie

# Statement of Authorship

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ii. permission is granted for the candidate to include the publication in the thesis; and

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# Chapter 1

# General introduction

*"People who think they know everything are a great annoyance to those of us who do."*

Isaac Asimov

# Chapter 1 - General introduction

Nutrition is defined as *"a process in animals and plants involving the intake of nutrient materials and their subsequent assimilation into the tissues"* in the Collins dictionary. From this simple and minimalist definition it is already clear that nutrition influences all the aspects of animal lives.

## *1. Nutrition is a complex process that influences and links all living organisms*

Nutritional science encompasses a wide range of fields and techniques. The uptake and effects of nutrients on metabolisms span molecular and cellular biology as well as immunology, while the feeding decisions and nutritional interactions between individuals and groups are considered in fields such as ecology, evolution and behaviour. Animals' interactions with their nutritional environment are highly complex, and necessitate an approach that has the power to combine all their disparate aspects (Douglas 2009, Raubenheimer et al. 2009).

Most research conducted in nutrition is application-orientated, and historically there has been a gap between targeted nutrition studies, which investigate the effect of a specific nutrient on a specific parameter, and studies from the field of ecology, which attempt to understand broader phenomena and usually considers foods as an energy value. As Ponton et al. (2011) pointed out, *"Many studies consider foods as uniform commodities and manipulate the amount available without considering the food's nutritional composition or having a*

*quantitative understanding of the animal's nutrient requirements"*. Studying nutrition is decisive to improve our understanding of the biological world, from individual behaviour to group interactions and evolution. To gain better recognition as a discipline of its own, nutritional science has to develop general laws and principles (Döring and Ströhle, 2015), which is a challenging task. Nutritional science is uncontestably a 'multi-disciplinary' field, but it is however not considered as an 'inter-disciplinary' one. The difference between the two is that an 'inter-disciplinary' field has principles that link and unify the sub-disciplines with one another, a state that nutritional science has not fully reached yet (Döring and Ströhle, 2015). Nutritional ecology has however recently emerged as a field which aims at bridging the gaps between the various fields of nutritional science.

## 2. Towards an integrative approach to study nutrition, the Nutritional Geometric Framework

Nutritional ecology aims to combine the diverse fields that compose nutrition and took an integrative approach, encompassing ecology, nutrition, behaviour, physiology, life history, and evolutionary biology. One of the most fruitful and innovative approach in nutritional ecology is called the Nutritional Geometric Framework (NGF), and was conceptualised in 1992 by Simpson and Raubenheimer (and is reviewed and detailed in the book by Simpson and Raubenheimer, 2012).

The NGF established that by looking only at the global energetic values of food, researchers were likely to misinterpret some observations. Instead, the NGF considers foods as ratios of nutrients, and led to the discovery that macronutrient composition is one of the key factors influencing food consumption. Remarkably, considering the protein to carbohydrate ratio of foods instead of their caloric value has put an end to years of debate

regarding the effect of caloric restriction on longevity (Simpson et al., 2015), in both flies and mice (Lee et al., 2008; Piper et al., 2011; Solon-Biet et al., 2014). It was established that the balance of protein to non-protein energy ingested by the organisms is responsible for lifespan extension and not caloric restriction per se. This is just one example of the many achievements of the NGF, which has been successfully applied in fields as varied as neurobiology (Wahl et al., 2016), human health (Raubenheimer et al., 2015; Simpson and Raubenheimer, 2012), conservation (Rothman, 2015), collective behaviour (Lihoreau et al., 2015; Simpson et al., 2006), pet food development (Hewson-Hughes et al., 2011), and genomics (Leulier et al., 2017; Simpson et al., 2017).

## *2.a. Nutrient regulation*

The NGF follows a state-space modelling approach. A state-space model is a mathematical model which describes the dependence between state variables in a dynamic system. The state of the system or the measurement can be represented in an orthogonal space where variables are the axis, as either continuous or discrete. In the case of the NGF, the axis are usually nutrients of interest. If we consider 2 nutrients, for instance protein (P) on the x-axis and carbohydrate (C) on the y-axis, we will have a 2 dimensional space where food can be represented as lines (called **"nutritional rails"**). The slope of the rail corresponds to the ratio of C/P for that food, and the position on the line corresponds to the quantity of food eaten by an organism (Figure 1). The **nutritional states** of individuals can be added as points, and their evolution through time quantified by recording the individuals' consumption of different foods.

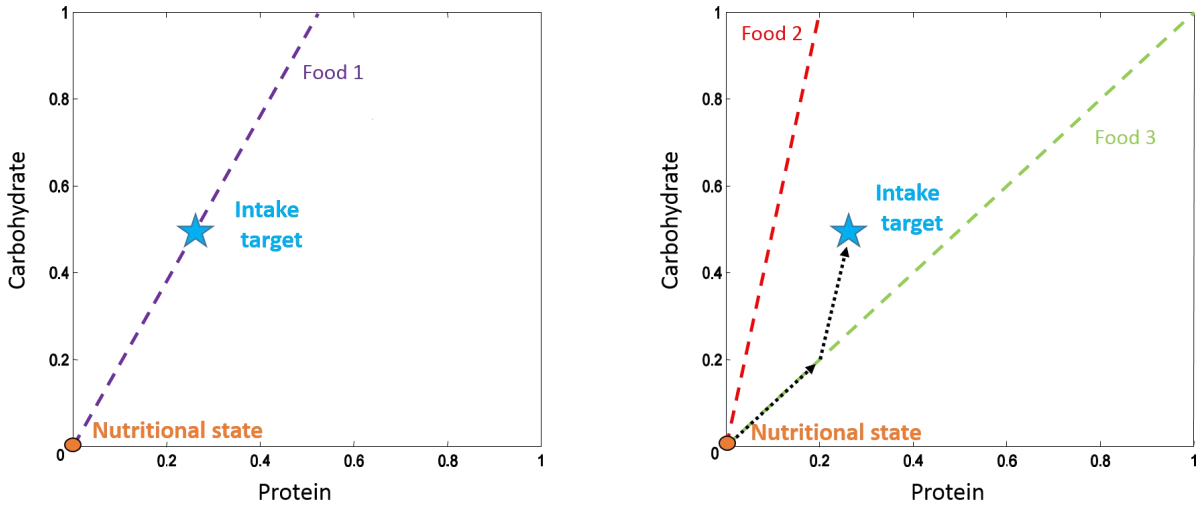


Fig 1: Illustration of the Nutritional Geometric Framework in a hypothetical case where the nutritional state of the animal is zero at the beginning of the experiment, and has an Intake Target of 0.5 units of carbohydrate, and 0.25 units of protein. In this case, Food 1 represented by the dotted line on the left panel is a balanced food, as its composition is made of two units of carbohydrate for one unit of protein. By eating the right amount of Food 1 which is considered as balanced, the hypothetical animal would get to its Intake Target. On the right panel, Food 2 and 3 are imbalanced and do not allow the animal to reach its intake target. However by eating a certain amount of each food, the animal can reach the Intake Target (represented by the black doted lines), as the two foods are complementary (*i.e.* they fall on opposite sides of the Intake Target).

A key parameter of the NGF is the **intake target (IT)**. The intake target is the optimal amount of nutrient that should be eaten by the animal to optimize its fitness. Every animals studied so far actively regulate their nutritional intake in order to reach their IT (but see chapter 4 and 5) provided they have a choice between different complementary foods. Foods are considered complementary if they fall on each side of the IT, allowing the animal to reach the IT by eating the appropriate amount of each food (Figure 1). ITs can therefore be determined experimentally in two ways. The obvious method is to give the individuals access to a single food, and measure the resulting fitness effects through time. This is highly time consuming, and require a high number of replicates to investigate the effects of different ratio and concentrations of nutrients. Since animals naturally regulate their intake to reach their IT, another method consist in recording what they eat when given a choice between a range of complementary foods. Synthetic diets have to be designed to manipulate the chemical composition of foods, in order to cover the largest area possible in the nutrient space, and to have standardized diets which composition is controlled. Classically at least two pairs of ratio

are used, to make sure that the IT is actively defended, *i.e.* the measured intake does not result from a random choice. If the intake is the same for both pairs of diets, one can be confident that it is indeed a true IT. This second method is non-destructive and highly beneficial in endangered animals, where the first technique cannot be used, as the individuals' health would be negatively impacted if constrained to a sub-optimal diet. It is also highly advantageous in the measurements of fitness traits in long-lived animals, which imply in some cases years of study, on top of a high number of individuals.

Finally, it is important to keep in mind that ITs are not fixed but "dynamic over physiological, developmental, and evolutionary timescales" (Simpson and Raubenheimer, 2012). For instance the IT of an individual changes with reproductive activity (such as in Drosophila (Bowman and Tartar, 2016; Camus et al., 2018; Lee et al., 2008, Lee et al., 2013; ), infection by pathogen (see Chapter 3), and age (such as in honeybee workers (Paoli et al., 2014)). It is also noteworthy that the regulation of nutrition can take place after foods have been ingested. Two ways of post-ingestive regulation have been described: the efficiency by which nutrients are absorbed can be modulated, and/or the efficiency by which absorbed nutrients are retain or excreted can be modified. For the first option, three possibilities exist: 1) the secretion of enzyme can be regulated, 2) the speed of passage through the gut can be tuned, and 3) over longer time scales the anatomy of the gut can be adjusted in response to diet (Simpson and Raubenheimer, 2012). The second option relies on modifying the efficiency with which nutrients are retained rather than cleared from the body (reviewed in Simpson and Raubenheimer 2012).

## 2.b. Nutrient effects on life history traits and feeding rules

In the same space as represented in Figure 1, parameters related to fitness can be represented and linked to the consumption of nutrients, such as lifespan, fertility or resistance to pathogens (Figure 2).

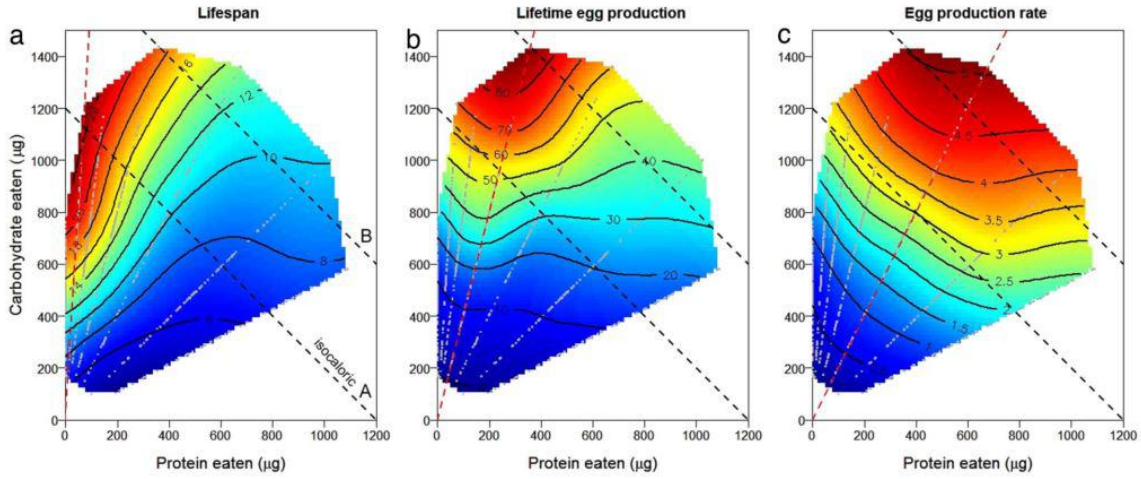


Fig. 2: Effects of protein (P) and carbohydrate (C) intake on lifespan (a), lifetime egg production (b), and egg production rate (c), recorded for individual flies confined to 1 of 28 diets varying in both the ratio and the total amount of P and C. Gray dots are actual intakes over the first 6 days of individual flies. Brown dashed lines represent the nutritional ratio at which each fitness component was maximized. Black dashed lines show isocaloric intakes (Lee et al., 2008).

Apart from elucidating nutrient regulation, the NGF has proven very useful in studying the consequences of nutrient intake on life history traits. By constraining individuals to a single diet with a known nutrient content, the effects of different nutrients and their interactions can be measured. The costs of over and under-eating can vary according to nutrients, and for the same nutrient the effects can also differ according to whether they are in deficits or excesses (Figure 3). For instance, eating an extra gram of carbohydrate might not be as harmful as undereating 1g of carbohydrate, and/or eating an extra gram of protein might be more detrimental than overeating 1g of carbohydrate. Diet can affect virtually all aspects of an organism's life, from immune and reproductive systems to lifespan.

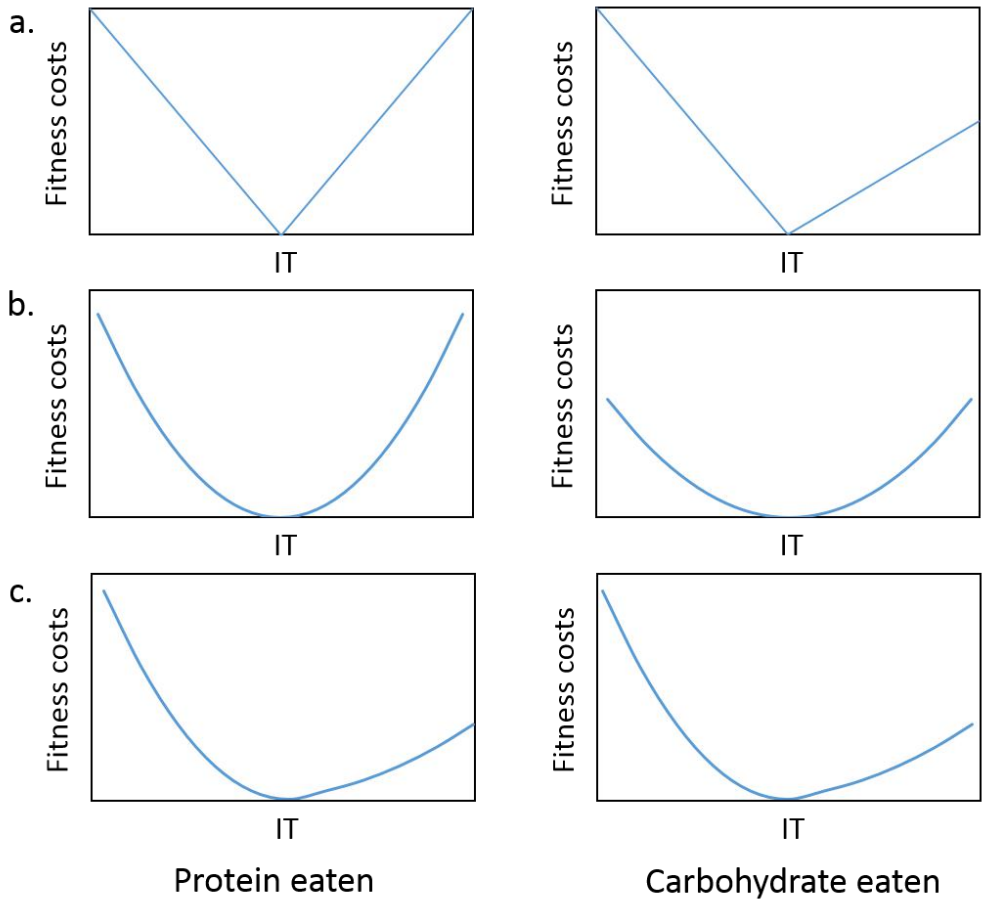


Fig. 3: Fitness costs of protein (left) and carbohydrate (right) deficits and excesses. Panel a. shows linear fitness costs, where the costs increase linearly with distance from the IT. For the protein equal costs of deficit and excesses are represented, while for the carbohydrate the costs of deficits are represented as stronger than the excesses. Panel b. shows quadratic costs, symmetrical for deficits and excesses but stronger for protein than for carbohydrate. Panel c. shows asymmetrical quadratic costs, *i.e.* the costs of deficits are stronger than the excesses. Adapted from (Simpson and Raubenheimer, 2012).

When faced with imbalanced foods preventing them from reaching their intake targets, individuals have to decide which nutrients they might under or over ingest (the so-called "**rules of compromise**", see Figure 4). The rules of compromise vary depending on the species. In some species the priority is given to a nutrient, to the detriment of others, that are readily over or under-eaten. For instance, the protein content of foods dictates the daily intake of spider-monkey (Felton et al., 2009) and humans (Simpson and Raubenheimer, 2012) (the "protein leverage hypothesis"). Ants on the contrary prioritise carbohydrates (Arganda et al., 2014). Other organisms such as locusts eat as to minimize the distance between their IT and their nutritional state (*e.g Locusta migratoria,* Simpson and Raubenheimer, 2012). This is

known as the "Closest Distance rule". Another alternative is to balance the excesses of one nutrient with the shortages of the other one (*e.g. Schistocerca gregaria*, Simpson and Raubenheimer, 2012). This rule is known as the "Equal Distance rule". Finally animals can display intermediate rules between those described above, such as prioritising a nutrient, but not under highly imbalanced diets, to avoid too much excesses. For instance, honey bee workers use an "asymmetrical quadratic" rule of compromise when balancing carbohydrates and essential amino acids (Paoli et al., 2014).

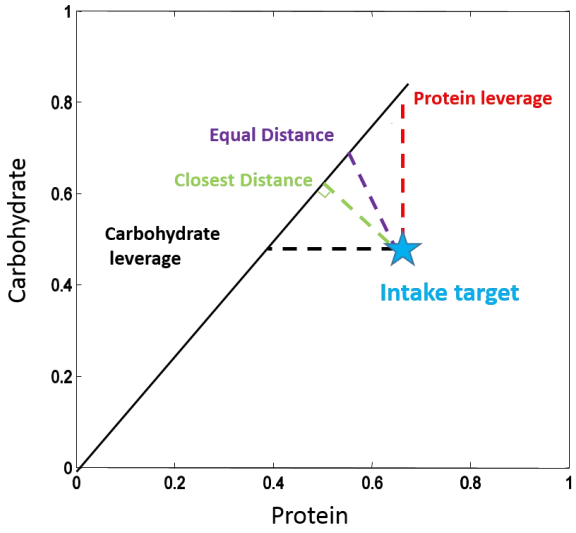


Fig. 4: Rules of compromise adopted by animals when restricted to a diet that do not allow them the reach their intake target. The values of carbohydrate and protein are arbitrarily chosen.

## 3. Nutrition and immunity in social insects

So far, most of the research in nutritional ecology has focused on individual animals. But individuals interact with one another - in family groups, aggregations, swarms and societies - and most of these interactions involve nutrition. Living in groups provides many advantages, such as a reduced predation risk (Beauchamp, 2014; Ward and Webster, 2016) through the increased chance of detecting predators ('many-eyes effect'), as well as common defence, confusion of the predator, and the 'dilution effect' (the risk for one individual to get killed is spread among the group). Preys can then allocate more time to other behaviours such

as foraging. Groups can also operate as information gathering and processing systems, allowing individuals to make faster and more accurate decisions collectively than how they would independently (Couzin, 2009). For instance by interacting with each other ants and cockroaches can rapidly focus their activity on the most optimal food source (Beckers et al., 1990; Lihoreau et al., 2010, respectively).

However, living in groups is not trivial as it requires coordination of activities among group members. Group living reaches its highest complexity in species that are classified as 'eusocial' (from Greek 'eu' = true). Typically an animal is classified as eusocial if it responds to 3 criteria, 1) eusocial animals display a reproductive division of labour with one or more individual in charge of the reproduction for the whole group while the other members of the group are more or less sterile; 2) a cooperative care for the young; and 3) an overlap of the adult generations (Wilson, 1971). Apart from insects, only two mammal species (the naked mole rat and the Damaraland mole-rat), and three species of shrimp (*Synalpheus regalis*, *Synalpheus filitigitus*, and *Synalpheus chacei)* are considered eusocial. Eusociality flourished in insects, where most eusocial species are found. The percentage of eusocial species is rather low in wasps and bees, but all ant and termites species are eusocial. Apart from eusocial species found in Hymenoptera and Isoptera, only one species of Coleoptera (the ambrosia beetle), one species of aphid (*Pemphigus spyrothecae)* and some species of thrips are eusocial.

The rest of this introduction will consider the case of insects, and especially social insects which are at the core of my PhD. The main ecological drivers leading to social insects' ecological success compared to solitary species are thought to be: enhanced survival through foraging efficiency, predator defence, reproduction, colonizing, and competitive abilities (Wilson 1980). Sociality however relies on a delicate balance between the costs and benefits provided by group living. In the first section we will review the current knowledge on one of the major costs of group living, which is the increased probability of pathogen spread linked to high densities and frequent contacts between individuals (Bull et al., 1991; Hochberg, 1991; Schmid-Hempel, 1998). In the second section we will review another major challenge faced

by insect colonies, which is the issue of nutrient provisioning, a task rendered highly complex by the strong division of labour and the heterogeneities in nutritional needs between colony members. How eusocial insects display coordinated behaviours allowing them to regulate those phenomenon both at the individual and colony levels, as if they were a single 'superorganism', has long been an intriguing question for biologists.

It is well-known that living and interacting closely with other animals increases the risk of parasites and pathogens spread (Chapter 5.7 Parasites and Pathogens in Ward and Webster, 2016, for invertebrates see Anderson and May 1981). Social insects live under crowded conditions with frequent physical contact, and the genetic diversity among individuals within the colony is usually low, especially in the haplodiploid social Hymenoptera (ants, bees and wasps). Both of those conditions favour an increased susceptibility to pathogens and parasites. The evolution of sociality is claimed to be dependent on the existence of a panel of strategies to cope with this issue (Brockmann, 1984). One of those strategies could be the collective modulation of nutrition with the onset of an infection in the colony. Numerous studies have indeed established the influence of nutrition on immunity in a wide range of species and contexts (reviewed in (Ponton et al., 2011)). Therefore, on top of providing foods for all colony members, foragers may have to modify food collection according to the presence of pathogens and diseases in the colony. The study of the interplay between nutrition and immunity in highly social animals is however still in its infancy. In this section, we will first review how the immune system of insects functions, then progress to the specific strategies used by social insects to cope with the increased pathogen loads, and finally summarise the current knowledge on the interplay between immunity and nutrition in insects.

## 3.a Humoral and cellular defence against pathogens in insects

The immune system of insects is often believed to be simpler than the immune system of vertebrates. There is indeed limited evidence of acquired immunity in insects, but they nonetheless have a panel of defences at their disposal. For instance, insects display cellular defences, mediated by hemocytes. Hemocytes, which are invertebrates' blood cells, can take a wide range of morphologies and functions. There are three main types of hemocytes: prohemocytes are stem cells that can differentiate in other types of hemocytes. Granulocytes release chemotactic factors mediating other cellular immune processes. Finally plasmatocytes are the equivalent to vertebrates' macrophages, and phagocyte foreign organisms (Lavine and Strand, 2002; Wilson-Rich et al., 2009). Hemocytes can aggregate against a foreign body and form nodules or encapsulations, accompanied with melanisation and sclerotisation (Gillespie et al., 1997; Wilson-Rich et al., 2009). Insects also rely on humoral responses, and secrete powerful antimicrobial compounds, with anti-microbial peptides and reactive oxygen species as key immune effectors (reviewed in Douglas, 2014). Insects are known to produce over 170 proteins (Saito et al., 2004), peptides and enzymes like phenoloxydases which have antibacterial and antifungal properties (reviewed in Gillespie et al 1997). Phenoloxydases are enzymes that transform dopamine precursors into quinones that neutralize pathogens. As is the case forvertebrates, insects are able to detect non-self, by recognising microbial molecules such as peptidoglycans and lipopolysaccharides specific to bacteria, or β-1,3-Glucans which are specific to fungi (Gillespie et al 1997, Janeway 1994). A trade-off appears to exist between the different types of defence, as antibacterial defence and hemocytes density is negatively correlated in the Egyptian cotton worm by Cotter et al. (2003), and a similar relationship is suggested in the caterpillar *Spodoptera littoralis* by Cotter et al. (2004). Microorganisms living in symbiosis also appear to have an important role in the defence of their host against pathogens, reviewed in (Douglas, 2014).

## 3.b Behavioural strategies used by social insects to fight parasites

Animals living in groups often display 'antiseptic behaviours', which decrease transmission and/or increase resistance to pathogens (Wilson-Rich et al., 2009). Social animals have the opportunity to reduce parasite loads due to allogrooming, which is the grooming of one another, and is more efficient at removing parasites than self-grooming. In termites the presence of nestmates increases the survival of individuals infected by a fungus, thanks to allogrooming (Rosengaus et al., 1998). Moreover, the immunity of naive termites is enhanced by the presence of immunized nestmates (Traniello et al., 2002), and this is also true for ants (Konrad et al., 2012; Ugelvig and Cremer, 2007). Termites also use a unique system of vibratory alarm to warn nestmates to avoid areas infected by *Metarhizium anisopliae* (Rosengaus et al., 1999). In honeybees some individuals even specialise in the task of allogrooming (Kolmes, 1989; Moore et al., 1995). In the leaf-cutting ant *Acromyrmex*, the transmission of a parasitic fungus was surprisingly inversely correlated to ant density, thanks to grooming and antibacterial secretions (Hughes et al., 2002). Allogrooming therefore appears as an efficient enough antiseptic behaviour to counterbalance the risk of increased transmission of pathogen when engaging in physical contacts. Another strategy relies on multiple matings, which increase genetic variability and resistance to diseases (Van Baalen and Beekman, 2006). Being inseminated with multiple males increases resistance to chalk-brood (Tarpy, 2003) and American foulbrood (Seeley and Tarpy, 2007) in honeybees. In the bumblebee *Bombus terrestris*, similar results are found regarding their most common parasite, the flagellate protozoa *Crithidia bombi* (Baer and Schmid-Hempel, 2001).

Social insects are also known for collecting antimicrobial substances, such as flower nectar rich in secondary metabolites (Baracchi et al., 2015) or resins, which are deposited inside the nest to prevent infections (Simone-Fintrom et al., 2012, Christe et al., 2003; Chapuisat et al., 2007; Simone et al., 2009). In addition, honeybees have developed a fascinating response to infection by the heat-sensitive pathogen *Ascosphaera apis*

(responsible for the 'chalk brood' disease): they increase their nest temperature to reduce infection. If the prevention of infection failed, many individuals who are parasitised isolate themselves (or are carried out by their nestmates), therefore reducing the chances of spreading the disease to other individuals (Baracchi et al., 2012; Moore, 2002; Rueppell et al., 2010; Schmid-Hempel, 1998; Schmid-Hempel, 2017; Ugelvig and Cremer, 2007; Waddington and Rothenbuhler, 1976). In a similar manner, infected larvae are detected and removed from their cells by honeybees, in a behaviour known as the honeybee 'hygienic behaviour'. This behaviour has been extensively studied, and its mechanisms revealed, providing a model for the understanding of antiseptic behaviours, from the neuronal pathways of individuals to the pattern of pathogen spread at the colony level. Hygienic behaviour is under genetic influence: some colonies perform it before the pathogen reaches the infectious stage (rapid-hygienic lines), preventing the dissemination of the pathogen, while others are slower and remove the larvae when they already carry infectious spores (slow-hygienic lines), leading to a faster pathogen spread (reviewed in (Wilson-Rich et al., 2009)). The difference between rapid-hygienic lines and slow-hygienic lines stems from higher detection levels of diseased brood odours in the rapid-hygienic lines, due to higher levels of octopamine, a neuro-modulator that increases the response of bees to olfactory stimuli. Interestingly, when a high enough density of rapid-hygienic bees is reached, task allocation between uncapping the diseased brood cells and removing infected larvae occurs, while when there are only a few rapid-hygienic bees, each bee performs both tasks.

## 3.c Physiological strategies used by social insects to fight parasites

Social insects are believed to have developed a stronger resistance to pathogens than solitary species, and/or have pathogens that might be less virulent; otherwise groups might not have survived and been maintained through time. Hochberg (1991) tested this prediction

empirically by comparing the survival of infected solitary *vs* social caterpillars, and found that social caterpillars where indeed more resistant to pathogens. Those results were confirmed by (Cotter et al., 2004) who found that *Spodoptera littoralis* has a different phenotype when living under crowded conditions, associated with higher melanisation and phenoloxydases than the solitary phenotype. Similar results are found in bees, where antimicrobial defences increase with sociality (Stow et al. 2007). In a seemingly contradictory manner honeybees have a low number of genes associated with immunity compared to other insects (Evans et al., 2006). This result is however probably a consequence of highly efficient antiseptic behaviours limiting the spread of pathogens in bee colonies (Evans et al., 2006). Finally, every social insect group can produce antimicrobial substances. For instance, termite secretions have antimicrobial properties (Chen et al. 1998), and their faecal pellets have antifungal properties (Rosengaus et al. 1998). They can also produce naphtalene to prevent parasite from invading their nests (Chen et al., 1998). Ants produce antibiotic secretions from their metapleural glands (review in Hölldobler and Wilson 1990, Beattie et al. 1986; Mackintosh et al. 1995), and the saliva of wasp larvae contains antibacterial agents (Gambino, 1993, Turillazzi et al 2004).

## 3.d Role of nutrition in insects' immunity

All of those mechanisms of defence against parasites are affected by nutrition, an aspect of immunity that is often overlooked. The nutritional state of an individual is crucial for the efficiency of their immune responses to pathogens (Ponton et al., 2011, and see Chandra 1996 for a review in humans and Lochmiller 2003 for a review in vertebrate). Animals can self-medicate by ingesting curative substances that are not usually part of their diets, such as toxins, minerals or secondary compounds (Clayton and Wolfe, 1993; de Roode et al., 2013). Immune responses are energetically costly, and NGF studies established in several species that a deficit or excess of macronutrients affects immunity. An increase of protein intake usually boosts the immune system of insects, and individuals actively modify their protein

intake to fight pathogens (Lee et al., 2006; Povey et al., 2009; Povey et al., 2014; Mason et al., 2014). When the caterpillars *Spodoptera littoralis* are infected with nucleopolyhedrovirus (a potent pathogen of insects), Lee et al. (2006) revealed that the cost of pathogens resistance are protein more than carbohydrate based, and that infected caterpillars switched their intake to a higher protein:carbohydrate ratio than control individuals. The same result is observed in the African armyworm *Spodoptera exempta* (Povey et al., 2014). However, in the caterpillar *Grammia incorrupta* carbohydrate consumption is positively correlated with melanisation, while protein consumption is not (Mason et al., 2014). The effects of nutrients appear to differ according to the type of immune challenges that the animal faces. A food that is optimal for a particular immune response might not be optimal for another. For instance, in the caterpillar *Spodoptera littoralis*, phenoloxydase, melanisation and lysozyme activities respond differently to macronutrient intake (Cotter et al., 2011), leading to a complex and not fully understood interplay between diet and immune system. Protein and carbohydrate intake and their interaction shape the immune response of a variety of solitary species (reviewed in Ponton et al., 2011). Therefore, studies that take into account food composition (such as NGF studies) are essential to build a full understanding of the phenomenon.

In social insects, NGF research is lagging behind, and the link between nutrition and immunity has rarely been studied. In honeybees pollen promotes the production of antimicrobial peptides (Alaux et al., 2011) and improves survival when individuals are infected with *Nosema ceranae* (Jack et al., 2016; Mayack and Naug, 2010). Carbohydrate consumption appears prevalent in some parasites like *Nosema ceranae* that cause an energetic stress by decreasing blood sugars (Martín-Hernández et al., 2011; Mayack and Naug, 2010). However, those studies did not control the ratio of nutrients ingested, therefore it is not possible to understand the potential compromises made by bees in a similar way to what was done in caterpillars. The effects of macronutrient on social immunity were first studied in ants. (Kay et al., 2014) measured the response of *Ectatomma ruidum* workers to the fungus

pathogen *Metarhizium anisopliae*. There was no noticeable difference between a low and a high protein: carbohydrate diet in ants kept in isolation, but workers kept in groups of 5 survived significantly longer on the high carbohydrate treatment. The benefits of social immunity came from the anti-microbial secretion of the metapleural gland. When those glands were blocked, infected groups no longer survived better than infected isolated ants (Kay et al., 2014). Those results highlight how social interactions can be intricately linked and how they can influence the immune and nutritional responses of a colony.

## 4. Nutrition in insect colonies

The organisation of an insect colony is highly elaborate and intricate (Hölldobler and Wilson, 2009), with more or less sterile individuals taking up tasks such as defence, construction, foraging and feeding the young, while only a few individuals are in charge of the reproduction. Typically, a division of labour occurs regarding food collection, where only 10% of the individuals (the foragers) forage for the entire colony, while other colony members such as larvae and reproductive are entirely dependent on the other colony members to receive food. This raises a number of challenges. How do those foragers, which do not possess direct information about the nutritional state of the whole colony and the level of food storage, meet the needs of the colony (Behmer, 2009; Feldhaar, 2014)? The field of collective nutrition has seen significant breakthroughs in the last two decades, but the question of how distributed systems such as eusocial insects maintain an optimal supply of multiple nutrients essential for life and reproduction remains to be fully elucidated. In this section we will first discuss the general principles which allow social insects colonies to take collective decisions, before further developing how such mechanisms are used to select the best food sources for the colony to exploit. We will then discuss what mechanisms might be used to distribute food

within the colony, and how nutritional ecology might be employed to better understand communal nutrition in social insects.

## 4.a. Self-organisation and foraging in social insects

Our understanding of how complex patterns can arise from simple local interactions, a process called 'self-organisation', has dramatically increased over the past four decades. Wheeler's description of social insect colonies as "superorganisms' in 1928 (Wheeler, 1928) emphasised the importance of interdependency and interactions among a colony. Further work describing division of labour set the framework for viewing insect colonies as decentralized systems (see for instance: Hölldobler and Wilson, 1990; Hölldobler and Wilson, 2009), until the concept of self-organisation was adopted. The term "self-organisation" was introduced to contemporary science from the 1950s by engineers, cyberneticians, and those associated with general systems theory. It was then applied to biological systems, as is reviewed in (Camazine et al., 2001). Self-organization is described in (Camazine et al., 2001) as *"a process in which patterns at a global level of a system emerge solely from numerous interactions among the lower-level components of the system. Moreover the rules specifying interactions among the system's components are executed using only local information, without reference to the global pattern."* It is observed in a wide range of biological, chemical and physical systems, such as crystallisation, molecular self-assembly, neural networks, thermal convection of fluids, planet movements, and our focus, animal collective behaviours. Self-organised phenomena are less easy for us to grasp than systems where the final pattern emerges through the directions of a leader and/or rules, such as an orchestra symphony that results from the musicians following their music sheets and the directions of the conductor. In self-organised systems, patterns result from simple, local interactions and feedbacks between components. Feedbacks can be positive, creating loops where a small change in the system is

amplified through snowballing effects. Negative feedbacks usually counteract the positive ones and stabilise the system.

A classic example of self-organisation in biology can be found in the foraging system of social insects. Social insects are known to recruit other colony members to food sources, and ensure its efficient exploitation through self-organised behaviours. A particular system of recruitment, mass recruitment, is mediated by chemical trails and is found in both termites (reviewed by Traniello and Leuthold in (Abe et al., 2000), and the chemical composition in *Nasutitermes* is described in (Sillam-Dusses et al., 2009)), and ants (reviewed in Hölldobler and Wilson, 1990; Hölldobler and Wilson, 2009). Foragers deposit pheromones between the food and the nest by laying the tip of their abdomen on the ground. A positive feedback occurs when recruits that follow this trail reinforce it by adding their own pheromones. The negative feedback occurs thanks to the volatile nature of pheromones, which evaporates quickly. A saturation effect when pheromone concentration is too high is another negative feedback. Despite distinct evolutionary histories, both ants and termites use the same system of mass recruitment, pointing to physical dynamic constraints acting on the system (Jaffe et al., 2012). Less famously, bumblebees in Amazonia also lay trails on the forest floor (Cameron and Whitfield, 1996), and stingless bees use polarised trails to indicate the direction, height and distance of the food (Nieh et al., 2004).

Trail-pheromone in ants allow the colony to select the shortest path leading to a food source (Deneubourg et al. 1990, Vittori et al. 2006, Beckers et al 1992, Dussutour et al 2009), through self-organisation processes. Ants using the shortest path spend less time to travel from their nest to the food, therefore after a certain amount of time the pheromone accumulated more quickly on this path through reinforcement than on the longest one. Once the shortest trail has been well established, the longest will eventually disappear. The mechanisms of recruitment of termites haven’t been as much studied as those of social Hymenoptera. In *Nasutitermes costalis*, soldiers start depositing a trail after discovering the

food, which attracts other soldiers, and when enough soldiers are present and have reinforced the trail to a certain threshold, workers are recruited (Traniello and Busher, 1985). In *Hodotermes mossambicus,* workers lay trails even during exploration, but modulate the concentration of the trail according to the distance from the nest, and appear to orientate by following a chemical gradient (Heidecker and Leuthold, 1984).

## 4.b Ending mass recruitment

Recruiting nestmates to a food source is only adaptive if it can be stopped when the food is exhausted. If the food source disappear workers simply stop laying pheromones and the trail disappear, leaving foragers free to move on to other food items. For instance ant foragers lay a trail only if they were not able to collect the entire food source they were presented with (Mailleux et al., 2000; Mailleux et al., 2003a; Mailleux et al., 2005). Similarly ants using direct recruitment choose to return to the nest and recruit other workers only if the food is of high value and not transportable in a single trip (De Biseau and Pasteels, 1994). Alternatively ants and termites can use 'negative' pheromones that have a repulsive effect and prevent foragers to follow the trail. Pharaoh ants use a "no entry" repellent pheromone to prevent foragers from following an unrewarding trail (Robinson et al., 2005; Robinson et al., 2008). The termite *Schedorhinotermes lamanianus* produces a labial secretion that is released in the food while eating, and induces a gnawing aggregation (Kaib and Ziesmann, 1992). This signal is non-volatile and highly persistent, therefore the presence of a negative feedback appears as necessary. This negative feedback comes from sternal gland secretions, that make-up the pheromone trail, but also serves as an inhibitor of the labial gland signal (Reinhard and Kaib, 1995). *Nasutitermes costalis* uses a combination of two pheromone, one is long-lasting (up to 4 hours in laboratory experiments) and is used as an orientation cue, while the other is short lasting (around 10 minutes in laboratory experiments) and serves as a recruitment signal. The same two pheromone systems were also observed in ants (*Pheidole megacephala*, Dussutour et al., 2009). Finally it is worth noting the honeybee "stop signal", where scouts inhibit the dance of scouts' indicating a different location than their own in the context of nest moving or indicating the presence of a danger in a foraging context (Nieh, 2010; Seeley et al., 2011).

## 4.c. Modulating recruitment according to food quality

As the environment has multiple food sources to offer, a colony needs to be able to tune its recruitment process to select the most profitable food. Ending or tuning recruitment rely on similar principles. Most of the studies investigating how a colony can select the best food have been conducted in ants. In *Lasius niger* foragers encountering a new food source do not fill their crop entirely, allowing them to sample a higher number of sources in one foraging trip (Mailleux et al., 2009b). Ants have been also known to modulate pheromone deposition according to the nutritional quality of the food discovered. This modulation can be done in two different ways: 1) an ant deposits pheromone or not as a function of food quality, as observed in *Lasius niger* (Beckers et al., 1993) and leaf-cutting ants (Roces, 1993). Thus, it is the proportion of trail-laying individuals that relays the information about food quality, 2) an ant deposits pheromone all the time, but modulates the quantity deposited as a function of food quality as seen in Pharaoh ants (Jackson and Chaline, 2007). In both cases ants modulate the overall concentration of pheromone deposited at the collective level. As foragers follow and reinforce the trail with the highest concentration of pheromone, this behaviour alone leads to the selection of the richest food (Beckers et al., 1993). Following the same principle, increasing speed with food nutritional value is another way to select the best food (*e.g.* Roces, 1993). Termites display analogous behaviours, as they increase trail deposition when highly profitable food items are discovered, which induces a fast recruitment and an increase in worker speed (Oloo and Leuthold, 1979; Reinhard and Kaib, 2001). The modulation of trail-laying according to the quality of the food discovered remains to be studied in termites, but it seems likely as many species are able to select their preferred wood species. Honeybees do not rely on trail-laying, but behave in comparable ways: recruitment and foraging speed are increased with nectar quality (Seeley et al., 1991), and there is ample evidence that foragers are able to select the best food sources among a panel of choices, in the field as well as in the lab (Nieh in Gadau and Fewell, 2009).

## 4.d. Information exchange and food sharing between castes

We have just reviewed how foragers recruit nestmates to collect food, and how they modulate recruitment according to food quality. The foragers also need to take into account how hungry the other colony members are when leaving the nest. The coexistence of several castes with different nutritional needs raises the challenge of regulating nutrition at the collective level. It is well established, for example, that larvae require more protein than workers, which need a diet richer in sugars (Cassill and Tschinkel, 1999; Dussutour and Simpson, 2009; Schmidt et al., 2012; Sorensen and Vinson, 1981; Weeks et al., 2004). Therefore, foragers, which hold information about the food characteristics, need to exchange information with inner-nest workers, which possess information about the colony nutritional status. Inner-nest workers are indeed in contact with the colony's food stores and the nutritionally dependent castes such as the larvae and the reproductives. Nutritional feedbacks are transmitted through a 'chain of demand': the nurses determine the nutritional status of the larvae, and in turn provide feedbacks to the foragers (in ants: Behmer, 2009b; Cassill and Tschinkel, 1995; Cassill and Tschinkel, 1996; Cassill et al., 1998). Larvae emit a 'hunger signal', letting the nurses know that they need to be fed. Acoustic hunger signals have been described in the group Vespa (Ishay and Landau, 1972; Ishay, 1975) and are common among wasps (reviewed in Maatsura & Yamane 1990). Certain ant larvae use the rocking movement of their heads to signal their hunger (Hölldobler and Wilson, 1990). The presence of a hunger signal in honeybee is less clear, but is suggested by Huang and Otis (1991). Honeybee brood produces a pheromone that stimulate pollen collection (Pankiw et al., 1998). Feeding of the larvae is positively correlated with the hive's pollen stores, but not with honey stores in honeybees (Schmickl and Crailsheim, 2002). The amount of proteinaceous trophallaxis from larvae to worker is used as signal. When pollen is plentiful, larvae are well fed and engage in proteinaceous trophallaxis with the nurses, which in turn pass the food to the foragers. This

food transfer from larvae to foragers inhibits pollen foraging (reviewed in Schmickl and Crailsheim, 2004). Apart from the larvae, reproductives can elicit foraging, as queen wasps do through behavioural interactions (Gamboa et al. 1990).

The feedbacks used by foragers to initiate recruitment and resume foraging depend on their nutritional state. Hungry honeybees (Pankiw et al., 2004) and ants (Mailleux et al., 2003b; Mailleux et al., 2006) are more likely to recruit other foragers. In *Lasius niger* foragers that come back to the nest are more likely to recruit other individuals if those are starved. If only the foragers are starved, no difference in recruitment is observed, therefore in this species recruitment relies on the behaviour and nutritional state of the recruits, and not on the state of the recruiters (Mailleux et al., 2009a). The other factor influencing foraging is the foragers' success at unloading food loads when returning to the nest. In honeybees, a forager resumes foraging depending on the likelihood of finding a food storer bee to accept the nectar and relieve the forager from its load (Seeley, 1989). The time a forager has to wait before being able to pass his food to a storer bee is a reliable indicator of the colony's nutritional reserves of pollen and honey. Therefore, foragers do not need to compare the quality of their foods with colony stores or other bees' food to decide whether to resume foraging, and can take decisions solely based on the absolute value of the food they collect (Seeley et al., 1991), as was repetitively showed in honeybees that display higher responses to good quality foods (*e.g*. Pankiw et al., 2004).

## 4.e. Distribution of nutrients in the colony

To explore how the food is shared within the colony, a different approach is to look at the nutrient flow. While studies described in the previous section help us understand the mechanisms of food sharing at an individual level, studies on nutrient flow give us a global picture of what happens at the colony level. In social insects liquid food is often shared by

trophallaxis (a mouth to mouth or mouth to anus food exchange): the forager ingests the food, and will regurgitate it to an inner-nest worker, who will in turn share it with another inner-nest worker and so on. The size of insect colonies often being consequent, it is usually not possible to monitor all the exchanges occurring. Experiments can nonetheless be carried out on subgroups or small colonies. Wasps for instance malaxate protein rich arthropods prey that they feed to larvae, while nectar is shared among workers (Hunt et al., 1987). To monitor the dynamic and pattern of food flow in ant colonies, several studies performed in the 80s used radioactive elements mixed with foods. They established that ants collect and transfer food quickly and to a high number of individuals depending on food quality. Proteins were distributed to larvae and queens, while carbohydrates were mostly shared between workers (Howard and Tschinkel, 1981; Sorensen and Vinson, 1981; Sorensen et al., 1985). The uptake and transfer rates were dependent on caste allocation (Sorensen et al., 1983; Sorensen et al., 1985). More recently, the analysis of the isotopic composition of each caste of *Pogonomyrmex badius* yielded analogous results. Larvae ingest more prey (protein rich) than seeds (carbohydrate rich) (Smith and Suarez, 2010). Similarly, Weeks et al. (2004) used rare earth elements to measure the distribution of protein, lipid and carbohydrates in field colonies of fire ants. They found that nutrients were discovered and distributed in less than 12 hours, and that protein were found in larvae, while carbohydrate and lipids were found in workers. Solid proteins were given solely to larvae for digestion (as workers lack the ability to process it), while proteins in liquid form were shared among workers. The distance between food baits and the nest influenced food flow as well: carbohydrates, the main energy source for the colony, were collected from farther distances than lipids and protein. Similar patterns are found in other honeybees, where food is shared by a few foragers to other colony members rapidly (Nixon and Ribbands, 1952). Micronutrients are also essential for nutrition, and may be shared according to the needs of each caste. For instance micronutrients are distributed in priority to caste with actively growing tissues, such as larvae in ants and young workers in termites (Judd and Fasnacht, 2007).

The limitation of this type of research is that the individuals need to be sacrificed at a chosen time point to measure their nutritional status (labelled or unlabelled, but see the use of Cobalt 60 that does not require sacrificing individuals (Suárez and Thorne, 2000)). Recent advances in technology allowed to solve this issue. For instance scintigraphy, a medical imagery technique that permits spatiotemporal monitoring of radiolabelled food, has brought insights into the spatiotemporal dynamics of nutrient flow (Buffin et al., 2012). Sugar water was shared among 200 workers (similar to the size of subnests observed in the wild) of the ant *Formica fusca* in about 30 minutes. This study showed that the storage of carbohydrate is centralised. The authors noticed that only a couple of workers received most of the trophallaxis and stored sugars for future redistribution in the colony (Buffin et al., 2012). Also using ants, (Greenwald et al., 2015) developed an innovative tracking system: using two cameras, they were able to record individuals' trajectory and interactions through the identification of miniature 2D barcodes, as well as food transfers thanks to a fluorescent marker diluted within the food. Therefore, the individual behaviour and the food flow could both be recorded. Their preliminary data shows that ants share high volumes of food in the first 20 minutes after food is introduced, and that those trophallaxis then decreased with time, while trophallaxis of small volumes appeared constant over the 3 hours after food introduction. Some ants shared food with 5 individuals, while other restricted their exchanges with a single partner. At the beginning of the experiment foragers shared on average 50% of their food with nestmates, and this volume decreased as more ants were fed. Inside the nest some workers seemed specialised in accepting and redistributing the food, while other filled their crops, a results in accord to the centralisation of resources found by Buffin et al. (2012). While those results are still preliminary, when taken together with other studies, they suggest that food distribution among ants is non-uniform over space, time, and between individuals. Noteworthy findings from this technique include that the direction of liquid flow exchanged during a trophallaxis can switch, the 'receiver' regurgitating food to the 'donor' as well, and

that a higher volume of food transfer comes from the numerous trophallactic events where small volumes are exchanged, rather than trophallaxis where larger amount of food is shared.

Termite nutrient flows and dynamics of food distribution have been very little studied in comparison. Termite engage less often in trophallaxis, and the speed at which nutrients are shared after feeding is slower and less efficient than in social hymenoptera (in the order of hours rather than minutes (Suárez and Thorne, 2000)), with only one third of individuals receiving food after 24h for instance (reviewed in La Fage and Nutting, 1978). An innovative technique of immunomarking used by Buczkowski et al. (2007) confirmed those findings: while most foragers discovered the food in less than 24 hours, after 72 hours only 50% of the workers and 30% of the larvae had received trophallaxis (in groups of 10 donors and 15 receivers, either larvae or unlabelled workers). Workers and nymphs acquired food faster than larvae and soldiers. As a comparison, with the same technique around 50% of the group was labelled after 5 hours, and more than 80% after 2 hours in an entire honeybee hive (DeGrandi-Hoffman and Hagler, 2000). This slower food flow likely reflects the longer time required to process food in termites that feed on ligno-cellulose compared to the simple sugars exploited by ants and bees, and perhaps the lack of an anatomical structure specialised for trophallaxis exchanges. Results seem to vary according to the study and species considered regarding food exchanges between castes (reviewed in La Fage and Nutting, 1978), and who is sharing food with whom and in what amounts remains to be investigated.

Numerous studies investigated how nutrient dynamics are affected by starvation. Howard and Tschinkel (1980) using radioactive sugar water revealed that ant foragers cope with starvation periods by increasing both food collection and transfer. In their next study, they revealed that the effect of starvation was dependent on food type (Howard and Tschinkel, 1981). Workers in normal condition collected sugar in high quantity, protein in intermediate quantity and oil in small quantity. The effects of selective carbohydrate starvation strongly increased foraging activity towards sugars, while no noticeable increase in oil or protein

consumption was recorded after 2 weeks of the corresponding starvation. However, when deprived of proteins, workers cannibalised their larvae, which could explain the subsequent lack of a protein hunger. Larvae cannibalism is common is social insects, and is often linked to periods of protein shortage. When bees suffer a shortage of pollen (their source of protein), workers feed in priority older larvae, which represent a stronger investment for the colony (Schmickl and Crailsheim, 2002), and if pollen is still lacking they consume some of the larvae (Schmickl and Crailsheim, 2001). The frequency of trophallaxis was highlighted as a mechanism regulating food distribution in the ant *Lasius niger*, as it depends on starvation levels and food type: the frequency of trophallaxis is higher for sugar solutions than for protein solutions (the duration of trophallaxis are constant) (Buffin et al., 2011). In honeybees, starvation induces higher amounts of food to be collected, and the nutrient flow among castes is more uniform than in a satiated colonies (Feigenbaum and Naug, 2010).

In conclusion the patterns of food flow and the mechanisms leading to it would benefit from additional studies, in particular in termites. Specifically how food quality affects the dynamics of food transfer remains understudied.

## 4.f. The insight brought by NGF studies in social insect nutrition

Recent key advances in social insect nutrition come from the application of the NGF: for instance the P:C ratio of foods is now a well-established key determinant of food intake and lifespan in social Hymenoptera.

Studies in multiple species of ants revealed the toxicity of high protein diets, and the associated regulation of P:C ratios to minimize those costs (*Linepithema humile* (Arganda et al., 2014; Arganda et al., 2017)*, Rhytidoponera sp* (Dussutour and Simpson, 2009), *Lasius niger* (Dussutour and Simpson, 2012; Dussutour et al., 2016), *Ectatomma ruidum* (Cook and Behmer, 2010), *Solenopsis invicta* (Cook et al., 2011), *Nylanderia sp.* (Cook et al., 2012),

*Odontomachus hastatus* (Bazazi et al., 2016), *Iridomyrmex suchieri* (Christensen et al., 2010), and the leaf-cutting ant *Mycocepurus smithii* (Shik et al., 2016)). Protein quality and protein type appear to be of importance on top of the ratio. Egg white was twice as harmful as a protein mix composed mainly of whey and casein in the ant *Lasius niger* (Poissonnier et al., 2014). Ingested in the same ratio, amino-acids (AA) are more toxic than protein in ants (Arganda et al., 2014; Arganda et al., 2017) and bumblebee (Stabler et al., 2015). Casein yielded better survival than pollen and royal jelly in honeybee (Pirk et al., 2010), and ovary development was highest in bees fed royal jelly. Finally the effects of macronutrient intake can vary according to caste: inner-nest workers are more resistant to nutritional stress than foragers in *Lasius niger*, thanks to their higher fat content (Dussutour et al., 2016).

When given a choice between diets, ants regulate their intake to a specific P:C ratio, that is specific to each species depending on their nutritional niche, but also depends on season (Cook et al., 2011). Ants that feed mainly on prey such as *Rhytidoponera sp* (Dussutour and Simpson, 2009) have a protein biased intake target while ants that feed mostly on honeydew such as *Lasius niger* have carbohydrate biased IT (Dussutour and Simpson, 2012). In fire ants, colonies increased their carbohydrate consumption in summer when more carbohydrate-biased foods are available in the environment (Cook et al. 2011). When restricted to a single diet, most ants tested so far prioritize carbohydrate (Bazazi et al., 2016; Dussutour and Simpson, 2008; Dussutour and Simpson, 2009). A carbohydrate biased diet is common in social insects, and has been claimed to be one of the factor of insect colonies' competitive dominance, possibly due to increased aggressiveness and activity under high sugar diets (e.g. Grover et al., 2007). A recent NGF study deciphered that the increased dominance under high sugar diet was more likely due to highest longevity and therefore group sizes than to behavioural changes in ants (Kay et al., 2012).

To maintain low P:C intakes, ants are capable of extracting required carbohydrates and rejecting surplus protein in the form of pellets that are discarded outside the nest (Cook et al., 2010; Dussutour and Simpson, 2009). Most of the previous experiments were done in the lab to precisely control what the ants were eating, but the regulation of P:C ratio appears to hold true in the field (Christensen et al., 2010; Cook and Behmer, 2010). Similar results were found in honeybees: foragers selected a specific P:C ratio, that is highly carbohydrate biased (Kay et al., 2014; Pirk et al., 2010). Young workers defended an IT slightly higher in protein than older workers did (Paoli et al., 2014). NGF studies were also carried in two species of bumblebee. The ratio of protein and lipid in pollen defined foraging decisions, and results are consistent in the field where bumblebees selected flowers with high protein: lipid ratios in *Bombus impatiens* (Vaudo et al., 2016a). *Bombus terrestris* regulated their intake according to P:L ratio as well (Vaudo et al., 2016b). Ant colonies are able to compensate for previous imbalances by increasing their intake of a previous nutrient shortage (Christensen et al., 2010). Remarkably, honeybees are able to do so for specific amino-acids (AA): if they were previously deprived of a single AA, they selected the diet containing the AA they were previously lacking (Hendriksma and Shafir, 2016).

The importance of protein for reproduction and for larval development into adults has long been known, but NGF studies allowed the precise quantification of those effects. In ants the IT defended by foragers is P:C 1:2 for colonies without larvae, but switches towards protein with a ratio of 1:1.5 for colonies with larvae in *Rhytidoponera* sp (Dussutour and Simpson, 2009). The role of protein in the development of reproductive organs was measured in honeybees: the greatest ovarian activation of queenless young workers was observed in bees kept on 1:3 ratio diets. This study highlights the trade-off that exists between maximising lifespan or reproduction, as bees lived longest on almost pure carbohydrate diets (Pirk et al., 2010).

The NGF has never been applied to termites (but for micronutrients see Judd et al., 2017), one of the major group of social insects, which drastically differs from insects studied so far in their biology and ecology. Application of NGF in termites would be highly valuable and informative in our understanding of their nutrition, as well as addressing major hypotheses of nutritional ecology regarding the effect of diet specialisation on nutrient intake regulation.

## 5. Aims and objectives

The general aim of this PhD was to study the mechanisms of communal nutrition in social insects and their impact on colony survival. Our objectives were separated in two broad parts: the first focused on nutrition and social immunity, and the second on macronutrient intake regulation in termites as a model of extreme dietary specialisation.

### *Nutrition and social immunity*

Thanks to the NGF, we know that the ingested macronutrient ratio affects defence against pathogens in solitary or isolated insects. Proteins in particular seem to boost immune defences, and infected insects successfully modify their intake to promote their immune defences. It remains unknown whether collective nutrient balancing in social insects, where foragers would alter their feeding decisions according to the infectious state of other colony members, could act as an efficient social immunity mechanism and prevent the spread of pathogens. The first part of this PhD was dedicated to modifying an existing individual based model of nutrition to address this question. We simulated a pathogen that spread by contact between colony members, and workers that could defend against the infection by increasing their intake of protein. Next, we investigated whether foragers would be able to meet the modified nutritional needs of infected colony members using simple rules of interactions where individuals accept food and resume foraging according to the distance between their nutritional state and their IT. We also explored whether impaired foraging in infected workers was advantageous for the colony by isolating them (via a quarantine effect) or deleterious, as forager shortage might hinder the colony's effort to defend against the pathogen. We varied additional parameters to explore in which cases social immunity could be beneficial for the colony's health. We investigated the effects of pathogen spread speed, the probability of detecting infected nestmates and engaging into an immune response, and the duration of the

immune response. We expected the protective effects of social immunity to be most relevant when individuals engage in short-lived immune responses as observed in insects.

## *Macronutrient intake regulation in termites*

The rest of this PhD was dedicated to applying the NGF to study macronutrient regulation for the first time in termites, the only social insect group whose nutritional ecology remains largely unexplored. The NGF has only been applied once in termites, in a study which showed that micronutrient intake is actively regulated in *Reticulitermes flavipes* (Judd et al., 2017). Very little is known about termite macronutrient requirements and the elements used in their feeding decisions. Contrary to other social insects studied, termites are specialist feeders. Nutritional ecology theory predicts that extreme specialist feeders should not display an active regulation of their macronutrient consumption to defend a specific intake, contrary to generalist feeders. We designed experimental diets to test this hypothesis in termites by controlling diet composition and measuring whether termites defend an intake target. In the first step, we restricted termites to a single diet to decipher the effect of lipid, protein, and carbohydrate on their intake, lifespan, body composition, and foraging behaviour. In the second step, we gave termites the choice between complementary diets to investigate whether they would defend a specific intake of macronutrient when given the alternative between different foods. We also varied the caste composition of the groups to examine their nutritional needs and their potential role in the regulation of food intake. As termite nutrition is highly specific and integrated, to introduce our experimental work, we will start this thesis by reviewing the interplay between termite diet, symbionts and communal life. Termites are indeed one of the few insects that can efficiently digest wood in their adult form, and to do so they require symbionts, that need to be acquired during development and after each moult.

# Chapter 2

# Termite nutritional ecology:

# taking an integrative approach to study the nutritional role of symbionts

*"One cannot think well, love well, sleep well, if one has not dined well."*

Virginia Woolf

# Chapter 2: Nutrition and symbionts: taking an integrative approach in termites as a model organism


## Abstract

The impact of nutritional symbiosis on host life history is increasingly recognised. The role of gut symbionts in providing metabolic pathways and nutrients to their hosts has been long known, but our understanding of the interplay between nutritional ecology and nutritional symbiosis remains in its infancy. An integrative approach is required to fully grasp the link between host diets and symbionts, and how this link influences ecological niches and species success through evolutionary time. Here we use termites as a model system to illustrate the impacts of symbionts in the evolution of diet, ecological success, and sociality. We start by reviewing how wood-feeding constitutes an extreme nutritional challenge, first by discussing wood nutritional composition before considering how to digest it. We then show how the complex relationship between termites and their symbionts plays a key role in turning this otherwise unexploitable diet into a highly successful ecological niche for termites. Next, we discuss what the implications of this obligate symbiosis might be on collective behaviour and group living. Finally, we introduce the nutritional geometric framework as the basis for an integrative approach combining concepts of nutritional ecology and symbiosis, and identify key research themes which will improve our general understanding of the role of nutrition in symbiosis evolution.



**Keywords**: *termite, microbiota, symbiosis, nutritional ecology, diet, collective nutrition*

# Introduction

From invertebrates to mammals, only a few animals do not harbour microorganisms[1,2]. The degree of co-dependency between host and symbionts varies from obligate (primary symbiosis) to facultative (secondary symbiosis) depending on the significance of the services exchanged. Among those services, the nutritional benefits provided by gut symbionts to their hosts is the most studied. For instance, in human nutrition, gut microbiota provides short chain fatty acids, micronutrients, and metabolic pathways (review in [3], and see [4] for a review on micronutrients). Nutritional symbiosis has been mostly investigated in insects because they are especially inclined to form primary associations. Ten to twenty percent of species are claimed to rely on obligate nutritional prokaryotic symbionts [5]. A well characterized example is the mutualism between aphids and their primary bacterial endosymbiont, *Buchnera aphidicola* (hereafter: *Buchnera*). Buchnera lives only within aphid bacteriocytes and provides the host with nutrients lacking in the phloem diet [6]. Elimination of *Buchnera* with antibiotics drastically reduces aphid fitness. A second well known example of nutritional symbiosis is found in termites. Termite symbionts allow their hosts to thrive on a diet unexploitable by most animals. Termite symbionts are more abundant and diverse than what is commonly found in other insects, rendering termites a fascinating model for nutritional symbiosis, which has captivated scientists for more than a century. Termite symbiotic systems are well known for their obvious importance for the host. Bacteria and archaea are present in a special paunch (an enlarged portion of the digestive tract) in the gut of all termites, while cellulolytic flagellates occur exclusively in the evolutionarily basal lineages, which are referred to as 'lower termites'. 'Higher termites' lost their flagellate during the Eocene period, and now rely mainly on bacteria. It is worth noting the case of the Macrotermitinae, which evolved special symbiosis with a basidiomycete fungus that they cultivate in gardens. The fungus provides the termites with both

fungal biomass and preprocessed wood. All termite species harbour a high number of symbiotic microorganisms in their guts. 61% of the hindgut weight was reported to be microbial cells in *Reticulitermes flavipes* [7]. The number of symbionts was estimated around $10^5$ per individual in *Reticulitermes speratus* [8]. In Zootermopsis protozoan symbionts represent 1/7 to 1/3 of the total termite body weight [9]. All those species are lower termites, but the pattern appears to be similar in higher termites. For example, in *Nasutitermes walkeri* workers the bacteria comprise about 17% of the total weight and 43.6% of the weight of the hindgut [10]. In light of these figures, it is not surprising that symbionts are essential to termite survival, a well-known fact for almost 100 years. Pioneer work by Cleveland in the 1920s revealed that the symbiotic protists of *Reticulitermes* were essential to digest wood, when they were removed the termites died after a few weeks, except if the symbionts were reinoculated or the termites were fed starch [11–13]. Likewise, *Mastotermes darwiniensis* cannot survive on a cellulose diet without its protozoa [14]. Other studies have shown that termites deprived of their symbionts died as quickly as if they were deprived of food [15]. Termite symbionts where thus classified as primary symbionts, and are a classic example of nutritional symbiosis, allowing their host to thrive on an otherwise inaccessible wood-based diet [16,17].

In order to fully understand the interactions between hosts and their microbiota, factors such as diet, foraging behaviour and ecology of hosts must be taken into consideration. The lack of information regarding termite nutritional requirement and feeding habits is rendering this task difficult. A number of studies have investigated the termites preference based on wood species [18], wood hardness [19], and presence of beneficial fungi [18]. More recently, the size of the wood log offered, assessed thanks to vibration emitted by the termites, was also revealed as a selection criteria in *Cryptotermes domesticus* [20]. Despite providing us with useful information on termite ecology and behaviour, those studies are not sufficient to build a global understanding of the nutritional requirements and foraging behaviour of one of the most dominant groups of insects on the planet. Termite biomass can reach up to 95% of the insect biomass in savannah, sometimes surpassing that of grazing mammals, with densities reaching 100 kg/m2 [21]. Through their role in the

cycling of organic matter, they modulate the availability of resources for other species. Brauman [22] claims that 'In tropical forest, they could consume half of the vegetal litter and in some savannahs, they can consume up to 49 % of the grass'. A better understanding of their nutrition would be highly valuable, through the improvement of our understanding of nutritional symbiosis, but also by increasing our knowledge of an understudied group of insects that has a strong environmental impact. We believe that nutrition in termite is a fully integrated and complex process, and that at least 3 main characteristics should be looked upon when studying the nutritional symbiosis of termites. First of all, termite live on ligno-cellulose based diets, which raises a number of nutritional challenges. We will show how the difficulties associated with a wood diet led to a strong association between termites and microorganisms. This leads to our second point, which is the role of symbionts in the nutrition of their host, and how the host and symbionts interact regarding nutrition. We will describe how nutritional symbiosis have a tremendous impact on the life history traits of their hosts, as termites xylophagy and association with symbionts has been suggested to be responsible for the transition from a subsocial ancestor to the contemporary termite eusocial way of life. Lastly, we will stress the fact that termite nutrition is communal and needs to be considered at the colony level, and not only at the individual level.

## The challenges of termite diet: the nutritional and physical hurdles

Symbionts provide new enzymes, metabolic pathways and nutrients, allowing their hosts to live on a diet formerly unavailable to them (*e.g.* in insects in [23]). Herbivory is a classic example of this phenomenon. Animals can prosper on a plant diet only through symbiosis with microbes supporting the digestion of plant materials and providing missing food components.

*The poor nutritional value of plant-based diets*

One of the main challenges of living on a plant diet is to cope with its low nitrogen content, essential to the synthesis of proteins. The nitrogen content of the wood is lower than what termites require. The percentage of nitrogen relative to carbon in termite tissues is 10-20% [15,24,25], whereas most wood tissues contain only 0.2-0.3% of nitrogen [26]. Xylophagous termites therefore had to evolve mechanisms to acquire and/or retain nitrogen to live solely on plant-based materials. It is worth mentioning that all nitrogenous compounds do not have the same nutritional value. Insects are unable to synthesize 9 amino acids which are only present in low quantity in plants [5,27–29]. At the physiological level, termites can either recycle their own metabolic waste (uric acid) a character they share with cockroaches, or accumulate uric acid as seen in termites [30]. At the behavioural level, mechanisms such as coprophagy, feeding on exuvia, cannibalism (including oophagy), and trophallaxis [25] ensure that little nitrogen leaves the colony. We will describe in details how the main source of nitrogen for the termites comes from their symbiosis with microorganisms in the next section.

In addition to nitrogen, wood is of poor nutritional value regarding a number of other nutrients, including lipidic compounds such as sterols, minerals and vitamins. Sterols are essential to insects. The inability to synthesize sterols is found in all insects studied so far, due to the lack of enzymes for the cyclization of isoprene units, it is therefore likely in termites, but remains to be specifically demonstrated [28]. Thus, termites probably need to get sterols from their diet [31]. As wood is poor in sterols, they could increase their sterol intake by consuming other organisms that develop on wood, such as fungi that produce ergosterols and are also rich in nitrogen. Likewise, micronutrients can be acquired from sources other than the wood. For example, calcium, iron, manganese and magnesium can be obtained from the soil in *Reticulitermes flavipes*. A recent study [32] showed that *R. flavipes* actively regulate their intake of KCl, MgSO4, and FePO4. When only

imbalanced sources are offered, *R. flavipes* prioritize their intake of MgSO4. These results illustrate the fact that while wood is a relatively predictable food source in terms of macronutrient, termite might forage from multiple sources to satisfy their nutritional requirements.

Along with their poor nutritional value, plant cell walls are notoriously hard to digest. Indeed, plant materials are mainly constituted of a complex of cellulose, hemicelluloses and lignin, which is highly recalcitrant to enzymatic attack. Wood is especially hard to degrade and very few species in the animal kingdom are able to survive on it throughout their life cycle. Ligno-cellulose is the most abundant biomass on earth, and understanding how natural systems are able to efficiently decompose these resources would be highly beneficial to humans in the context of energy production. Termites (and closely related wood roaches) are one of the only animals that can live solely on wood, thanks to the primary symbiotic relationships they display with a number of microorganisms. The ancestral diet of termites was wood. However, higher termites now feed on a wider range of diets (grass, herbivore dung, and humus [15]). This change has been suggested to be linked to the diversification of the termite symbiotic systems over time. We will first review what is known about termite digestive capacities, then move on to the role of their symbionts in nutrition in the next section.

*Digestion of recalcitrant carbohydrates*

Termites are able to break down the plant fibres into acetate and methane, with hydrogen as a central intermediate. They are highly efficient and exploit 74–99% of the cellulose. Surprisingly, termites are more efficient at digesting wood than ruminant that feed on less lignified grasses. By comparison cows use only 30-40% of the plants polysaccharides (Hyodo et al. 1999 and Katsumata 2007 in [33]). Most of the research so far has been focusing on the digestion of cellulose, and little is known about the other wood components. Cellulose is however not the sole component of plants, in eucalyptus wood for example it represents only 50% of the plant fibres, the rest being mostly hemicellulose and lignin.

*Degradation of lignin and other wood components*

Lignin is a non-negligible component of wood, ranging from 18 to 35% of the fibres [34]. Lignin degradation has been investigated in about 10 species of termites, and the results vary greatly, from 0.33 to 83% degradation [35]. However the method used in those studies were very different from one another (different times, humidity condition etc…), and the variation observed might reflect a difference in the acid solubility of lignin rather than actual degradation [35]. The work of Cookson [35–37] using $^{14}$C-lignins gives us more reliable data on lignin degradation by *N. exitiosus*: 8-10% of $^{14}$C-lignin was degraded to $^{14}CO_2$ by *N. exitiosus* over a 2-week period. Wood is also composed of hemicellulose that termites exploit at a rate of 65 to 87% [18].

*Implication of the diet in termite's life history*

Termite ancestors were wood eaters, resembling the wood roaches *Cryptocercus*. Being xylophagous implies a nutritional dependency of young instars on their parents, as juveniles do not possess the microbiota nor the sclerotized mouth parts necessary to process wood, and must be fed by their parents [38]. This dependency differs from classic hemimetabolous development, and was a first step towards the transition to eusociality in the termite ancestors [39,40].

## Diet and Symbionts interplay

The ability of termites to thrive on cellulose based diets is due to their remarkable symbiosis with other organisms. Termite microorganisms are notably hard to cultivate in vitro, which renders their study difficult. However, this famous symbiosis has fascinated scientists since the beginning of the 20th century, and numerous studies using advances in genetics have extended our knowledge.

*Role of symbionts in the degradation of plant materials*

Those studies revealed that termites need the help of symbionts to access and process efficiently the complex carbohydrates of plants. It was believed that their main role was to break down cellulose. But we now know that termites secrete their own cellulase (a general term for a mixture of enzymes of 3 major classes: endo and exoglucanases and cellobiases). They endogenously produce two known types, beta-1,4-endoglucanases from glycosyl hydrolase family 9, and beta-glycosidases, in large enough amount (at least in higher termites) to break down cellulose on their own [17,41], pointing to other roles for the symbionts, especially in higher termites. Cellulose degradation by symbionts has been the focus of many studies, and we will not go into details about the mechanisms as they were reviewed recently by Brune [42]. In short, Brune described the breakdown of lignin matrix and the access to cellulose as a 'dual system that combines activities of both the host and its intestinal symbionts'. On the contrary, studies investigating the role of symbionts in the digestion of other wood components are less abundant, while symbionts appear more crucial in these processes than in cellulose degradation. For instance, gut protists have been shown to be important for the decomposition of xylan, one of the main hemicellulose [43]. Hemicellulose degradation even depends entirely on symbionts in *Reticulitermes flavipes* [44]. The extent to which lignin, which represents 20-25% of the eucalyptus that *N.exitiosus* feed on for example [45], is degraded with the help of symbionts and used by termites is still not entirely clear. Yet, lignin degradation in *N. exitiosus* was greatly reduced by the antibiotics erythromycin, tetracycline, and metronidazole, and by an atmosphere of 100% $O_2$, supporting the role of gut bacteria in this process [37]. This was also substantiated by Pasti et al. 1990, who demonstrated that Actynomycetes symbionts can solubilise lignin. In fungus-farming termite, the fungus allows the degradation of lignin and complex polysaccharides, while the symbionts process smaller sugars [46].

In short, complementarity in digestion and task distribution between host and symbionts has emerged in termites to allow or improve wood consumption.

*Role of symbionts in providing lacking nutrients and digestion pathways*

As described above, termites have to cope with the lack of nitrogen in their diet. Symbionts have proven to be essential to solve this issue [47]. This is not restricted to termites, as insects with endosymbiotic bacteria have higher C:N ratios in their diet (from a study comparing 117 insect species [48]). Two different ways can be used to rebalance the C:N ratio: 1) termites can acquire additional nitrogen from other sources, 2) termites can eat more to reach their requirement in nitrogen and discard the excess of carbohydrates . The first method is well documented: termite can for instance recycle their own nitrogen waste, thanks to uric acid-degrading microbes [30,49]. They can digest symbiotic bacteria acquired by trophallaxis as suggested in [50], which provide the termite with nitrogen [51]. Most famously, termites have been shown to obtain nitrogen from the atmosphere, by harbouring N2 fixing microbes [52,53]. Those microbes have a decisive role in nitrogen acquisition: for example 50% of N acquired was derived from the atmosphere in workers of *Neotermes koshunensis* [54]. The second method is less documented, but methanogenic bacteria and respiration can offer a way to discard excess of carbohydrates [55]. Nitrogen is the most studied but not the only nutrient provided by symbionts. Mauldin's [56,57] research revealed the role of protozoa in lipid metabolism by defaunating *Coptotermes formosanus* and *Reticulitermes flavipes* and measuring the resultant lipids stores. They found that synthesis of triglycerides and oleic acid (the main fatty acid in those termites) from acetate rely on protozoa and bacteria in those species.

*Interplay between diet and microbiota*

We have shown how the symbionts help their hosts to cope with their difficult diet, but it is also well established that the gut microbiota of animals is in turn affected by what the host ingests. Numerous studies have been carried out in humans and the interplay between micronutrient and microbiota has been reviewed in [4].

Diet is known to also affect the symbionts in insects [reviewed in [58]]. In termites, there is evidence that diet affects the microbiota, but its influence is still not well understood. The symbionts of wood-feeding termites are separated from humus and soil feeders, irrespective of the host position in the phylogenetic tree, 'offering compelling evidence that diet is the primary determinant of bacterial community structure' [59]. Accordingly, the protistan fauna of*Coptotermes formosanus* changes with the wood attacked [60], and the type of carbohydrates eaten [61]. On the other hand, some authors claim that diet is only a secondary factor, affecting the relative abundance only, and that phylogeny and inheritance or acquisition of the symbionts is the most relevant factor [62]

Dietrich et al. [63] have shown that many genera of bacteria are present across both lower and higher termites, as well as cockroaches. The relative abundance of these bacteria has changed significantly throughout the evolution of cockroaches to termites, and from lower to higher termites. It appears that termites have continued to acquire bacteria from the environment (or other termite species) throughout their evolutionary history [64]. Studies where termites are fed diets with varying composition need to be carried out to reveal the possible changes in their microbiota. The reasons behind the loss of flagellate symbionts in higher termites and its subsequent effects on the diet breadth remain obscure and its apprehension could bring valuable insights to our understanding of the relationship between host diet and their microbiota. Did the loss of the flagellates allow the settlement of new beneficial bacteria? Could a mutualistic relationship with certain organisms prevent the formation of future associations and impose restriction on the host' feeding abilities? The description of the microbiota of the higher termite *Gnathamitermes* that retained or reacquired ciliates [62], together with a comparison with other higher termite species and their ecology might

help to get a better grasp of this phenomena. Positive as well as negative interactions between microbiota species have been described in insects [27]. Some flagellate symbionts such as *Trichomitus trypanoides* are feeding on bacteria in the termite *Reticulitermes santonensis* [65]. Moreover, removing a dominant protozoa lead to the multiplication and domination of another one, without any noticeable effects on the host in *Termopsis* [12]. In *Mastotermes darwiniensis* a change from a cellulose to a starch diet let to the loss of the large protoza and the subsequent development of bacteria [14].

*Symbiont requirements*

To understand the link between termite diet and their microbiota, an extended knowledge of termite symbiont ecology and requirements is needed. Termites harbour a high number of species and genera of symbionts, leading to intricate interactions: for instance in lower termites protists have developed an endosymbiosis with bacteria, such as spirochaetes which improves the protist motility and allow their displacement in the termite gut [15]. The complexity of the complete symbiosis is amplified by distinct and sometimes opposite requirements for different microbial processes necessary to termite survival. For instance, oxygen is required for the degradation of lignin and the fixation of N that requires a large amount of ATP, while the digestion of cellulose and the fixation of nitrogen depend on anaerobic conditions. The gut of termites is extraordinary in its complexity and offers a variety of physico-chemical conditions for the symbionts, providing gradients of pH and redox potential along its anterior to posterior axis [66–68]. Brune studies [69,70] revealed that the hindgut paunch, that was thought anoxic, actually has an oxygen gradient, from micro-oxic in the periphery to a complete anoxia in the centre. This variety of conditions allows the coexistence of a large panel of microbes, which in turn provide the termite with a number of new biological functions. Mikaelyan *et. al.* [71] revealed that bacterial community was linked to the heterogeneity of gut compartments in nine species of higher termites.

In addition to abiotic factors, symbionts need to acquire their nutrients from their hosts, but this is very little studied to date. Starvation induces a 9 fold reduction of *Acetobacter* and *Lactobacillus* in *Drosophila* [72], and it is known to deplete protists in termites as well [12,57]. Interestingly not all protists are affected in the same way, with the largest ones dying first.

Finally, symbionts need to survive their hosts' defence mechanisms. The interactions between host and immune system have been investigated in the fruit fly Drosophila and two species of mosquitoes (*Anopheles gambiae* and *Aedes aegypti*), where alterations of the immune system lead to changes in the gut bacteria community . The molecular mechanisms of this interplay rely on the production of uracil in Drosophila. Symbionts do not produce it, while pathogenic bacteria do, which triggers an immune response . In the weevil *Sitophilus*, antimicrobial peptide prevent the overgrowth of bacteria out of their specialised cells, the bacteriocytes . It is suggested that immune effectors are suppressed in those bacteriocytes [27]. A similar phenomenon might exist in the termite paunch where the symbionts are housed, however virtually nothing is known about the processes by which the immune system of termites and their microbiota interact.

*Foraging*

What termites eat and what their encounter in their environment will affect their gut conditions and in turn the microbiota. For instance, symbiont community varies according to the geographical repartition of termites [65], and we have seen previously how diet might affect the microbiota. Finding, selecting and eating foods in appropriate amounts is therefore a crucial part in the proper functioning of termites and their symbiosis. The microorganisms present in an animal's gut can also affect their food selection, as was revealed in Drosophila [72,73].

Termites life styles are divided into two categories: "one-piece types" and "separate-pieces types" [15]. In one-piece termites, the colony spends its all life in the same piece of log. Food selection is a single decision event resting entirely on the royal pair when they select a nesting site to establish a new colony (*e.g.* Zootermopsis royal pair chooses the nest according to quality of the

wood in [15]). Colony members will never leave the nest except for the nuptial flight, and therefore they only have to decide on the quantity of food to eat. Separate-pieces termites on the contrary forage away from their nests, and have to find and select their food. Those two contrasting ecologies are unsurprisingly linked with different microbial associations. One-piece termites cannot overeat to acquire more nitrogen, as wood is too low in nitrogen. Separate-pieces types on the other hand can rely on both overeating and/or gaining nitrogen from N-fixing bacteria, which is reflected in their symbiont communities

In separate-pieces termites many species (*Paraneotermes simplicicornis, Heterotermes aureus, Gnathamitermes perplexus, Amitermes spp, Reticulitermes spp, Coptotermes formosanus, Macrotermes bellicosus, Macrotermes michaelseni* etc…) are able to select their preferred wood species [15]. Termites show a preference for foods with a higher nitrogen content (wood with fungal decomposition, lichens, and lower parts of the litter). Secondary plant compounds, carbohydrate content (glucose, fructose, starch, Abushame and Kambal in [15]) and digestibility also play a role in food selection. We postulate that species that forage away from their nest and have a wider range of diets would have been be more likely to encounter new microorganisms and form new symbiosis than the one-piece termites.

The precise mechanisms and dynamics of those food choices have received very little attention in termites. Contrary to other social insects, the role of each caste in foraging is poorly understood. In *Nasutitermes*, soldiers are the first observed on a newly discovered food, and are later joined by major workers of the later stages [15]. In several species bigger workers have been shown to be more efficient at tunnelling than smaller workers [74–76], which might explain their prevalence as foragers. As regards to the onset of foraging, and the initiation of food search, it has been showed that starvation induce more tunnelling [77,78], as well as the discovery of food . Despite those studies, mechanisms by which termites choose their food and recruit other nestmates are still poorly understood. Ants are known to select the foods of higher nutritional quality [79–82] through trail-laying, by modulating the deposition of pheromones. Termites that forage away from their

nests rely on trail-laying as well, and increase trail deposition when food items are discovered , suggesting that the mechanisms regulating foraging are similar to the ones found in ants. On top of trail laying, other methods might exist in termites, see for example hardwood termites that use vibration signals to assess wood log size and select a log accordingly [20]. Termites' cryptic way of life renders the study of their foraging behaviour difficult. As a consequence, what termites feed on and their optimal diets are still poorly understood.

## Implications of symbiosis on social behaviour and collective dynamics

Symbiosis can have tremendous consequences on the life history of host species, and are recognised as driving forces in the evolution of eukaryotic organisms [83,84], as is evident in termites. In termites, new generations need to acquire their symbionts from other individuals, encouraging them to stay within the nest as helpers. This indispensable transmission of symbionts from parents to offsprings led to co-evolution [85], and nutritional co-dependency: termites have become entirely dependent on their symbiosis to acquire the necessary nutrients for their survival, but the reverse is also true as the genome of some microorganisms have been reduced (e.g. Blattabacterium in *Mastotermes darwiniensis* [86]). Symbionts have for instance lost some essential amino acid pathways that they now get from their host [87]. A number of symbionts have thus co-speciated with their host for millions of years, and are now found only in termites [88]. The biology of termites renders the co-dependency of individuals even stronger: termites are hemimetabolous, and moult throughout their lives. This is a crucial characteristic, as the symbionts are lost after each moult, and need to be reacquired to ensure survival. A challenging diet and a necessity to share symbionts are among the main determinants that led termites to transit from a subsocial ancestor to eusociality [89]. Today all

termite species known are classified as eusocial, and the division of labour and interdependency of colony members forces us to consider their nutrition at a collective level.

*Collective nutrition in insect colonies*

Eusocial insects (mainly composed of ants, bees, termites and wasps) are defined by a division of labour among their members. Only a small fraction of the colony, the foragers, are collecting the food, which is transferred from one individual to another. This renders the regulation of nutrition more complex than in solitary species, as the foragers need some knowledge or feedback about their nestmates' nutritional status or the food stores of the colony to take appropriate decisions in term of food selection. Moreover, a colony comprises individuals belonging to different castes (which can be morphological or reproductive) with different nutritional needs. In social Hymenoptera (most eusocial insects except termites) for example, workers feed mainly on carbohydrates, while queens and larvae require a diet richer in protein, for growth and reproduction [82,90–92]. The challenge for a forager is to find a food source that would address its own nutritional requirements as well as those of all colony members. The use of mechanistic approaches using principles adapted from statistical physics has revealed how complex collective phenomena can emerge from simple interactions between individuals, without the necessity that any of them possess a global information or act as a leader [88–90]. Following this principle, various studies investigated how insect colonies maintain a balanced supply of macronutrients at the colony level (ants [82,93,94]; honeybees [95], bumblebee [96]). In social Hymenoptera, a 'chain-of-demand' from the brood to the nurses provides the foragers with information on the nutritional state of the larvae for example (ants [97–99]; bumblebee [100], honeybees [101]). Studies using an integrative approach of nutrition have led to the fascinating finding that the presence of larvae and their feedbacks allows a more precise regulation of food intake (in ants [82,102]). In most ants, workers produce limited amount of proteases and have a narrow petiole that prevent them from swallowing solid protein. Thus, the proteins brought back to the nest by the foragers need to be digested by the larvae which do not present such constraints. Then, the larvae share an

easy to assimilate source of nitrogen with the other colony members. In termites, there is evidence that nutritional deficiencies can be evened out among the colony members through trophallaxis exchanges. Individuals suffering from a shortage of nitrogen can regain appropriate levels of N after being reintroduced with well-fed individuals [51,103]. This nutritional co-dependency between members of an insect colony, implies that their nutrition has to be considered at a colony level and not only at an individual level.

Collective nutrition has been a productive field in the last decade, and a lot of advances have been made in social Hymenoptera. However very little is known about the special case of termites. Termites present a number of characteristics that could affect their collective nutrition and make them unique and different from the other social insects studied so far: 1) they are specialist feeders and do not exploit foods which vary in their nutritional composition; 2) wood is a diet that requires a slow digestion process, probably rendering the food flow and nutritional feedbacks way slower that in social Hymenoptera; 3) their survival and digestion rely on symbionts that need to be reacquired by trophallaxis after each moult; 4) termites have a unique caste system, where not only the larvae but also the soldiers are nutritionally dependent on the workers, and have to be fed by trophallaxis and 5) the caste system is highly flexible, with individuals being able to switch from worker to reproductive or soldier (depending on the species and the stage of the individuals).

*Caste and nutrition interplay*

The eusocial insects studied so far are all holometabolous, where the major transformation is the transition from larvae to imagos, which is linked to a great need of protein. For example in ants, larvae require more protein than workers, and their presence in a colony increase the foraging effort towards protein rich food [82]. In termites the nutritional needs of different castes have not been investigated. As they are hemimetabolous, we could postulate that the needs of the larvae, who are mobile and resemble the adults, would not be as markedly different as in social hymenoptera. While data are not available on this subject, we however know that the body content profiles differ among

castes. Not surprisingly, the alates have a higher fat content than the other castes (in Nasutitermes spp ). Moreover, the royal adipose tissue has very different histochemical characteristics than the normal fat body of insects, marked by an absence of lipids but a richness of ribonucleotides instead [18]. A difference between the worker and the soldier caste has also been noted in the higher termite *N. exitiosus*, where soldiers have higher protein and lipid contents [104]. Likewise, the free amino acid concentration was higher in soldier in 3 species of higher termites [105], and fat levels were higher in soldiers of Nasutitermes spp [106]. Those differences are likely to affect the pattern of food flow in the colony, as was shown by Machida et al. : Trophallaxis is one of the means through which termite gain nitrogen, and different stages and castes receive different numbers of trophallaxis in the Japanese damp wood termite. We know that the trophallaxis pattern is altered according to the nutritional state of individuals, but there are still a number of points that need to be investigated to fully understand the mechanisms leading to the regulation of nutrition in termites: What are the key factors initiating trophallactic behaviour? Do nutritional feedbacks follow a chain of demand, similar to ants, where nurses detect larvae hunger and elicit food collection by foragers ? Is foraging regulated by the success or failure of termites at unloading their gut content to other termites as seen in bees [107]?

Termite is the only group where soldiers are so completely dependent on the colony for their nutrition [108]. As they only receive food from trophallaxis, one might postulate that their microflora would differs from the workers, as the food they receive is already processed. Soldiers indeed have a reduced digestive system in a lot of species (Coptotermes, Rhinotermes, Globitermes, most Macrotermitinae [18]). However, what is exactly transferred to soldiers during trophallaxis is not known. Lower termites have 2 types of stomodeal (mouth to mouth) trophallaxis, one mainly composed of salivary secretion, and the other containing wood particles (Grasse and Noirot 1945 [18]). Proctodeal (anus to mouth) trophallaxis consists of a liquid from the rectal paunch, containing 'flagellates, products of digestion, and also fragments of wood' [18]. Interestingly, soldiers display proctodeal trophallaxis in the lower termite family Kalotermitidae. It is noteworthy that

reproductives, who are fed processed food through salivary secretions from workers, lose their protists over time [13]. On top of this complex caste system, individuals moult throughout their lives, and moults can be progressive, regressive, or stationary. This will influence food flows within the colony, as individuals need extra feeding before moulting, and are inactive for a few days [108]. It is not known whether the production of different castes induces significant costs to the colony. An important ecological factor expected to influence the course of termite development is food availability. Interestingly, termites start moulting towards alates when the size of the nest decreases [109], individuals having to leave and reproduce before the nest conditions are too deteriorated to offer appropriate food and shelter for the next generation.

## Towards an integrative approach

From their food specialisation and symbiosis to their complex social structure, it is evident that termite nutrition is highly complex, and its understanding necessitates an approach that has the power to integrate all those disparate aspects [28,110] .

Nutritional ecology brings together a wide range of fields, such as ecology, nutrition, behaviour, physiology, life history and evolutionary biology. Nutritional ecology allows a proper understanding of life history phenomena such as food selection, habitat distribution, and success or decline of species over time. One of the most recent and successful approach in nutritional ecology is the Nutritional Geometric Framework (NGF) [48]. The NGF is a state-space modelling platform in which the foods are represented in a nutritional space as a ratio of nutrients (*i.e.* a line), and the nutritional status of individuals are represented as points that move in this space according to time and intake. When an animal feed on a food, its nutritional state changes along the rail of the chosen food. One of the key strengths of the NGF is that the nutrient intake associated with the highest fitness values (intake target), can be experimentally determined. The NGF also provides an

extensive toolset to determine how animals regulate their feeding behaviour to reach their intake target (IT). Typically, animals can reach their IT by eating a food that is nutritionally balanced, or by mixing foods that are imbalanced but complementary. When an animal is confined to a single imbalanced food, it cannot reach its IT and it has to make compromise between over-ingesting or under-ingesting some nutrients. Several behavioural rules of compromise can be followed, such as minimising the Euclidean distance between the nutritional state and the intake target (known as the 'closest distance rule of compromise'), or simply giving priority to one nutrient, to the detriment of others. Knowing the position in the nutrient space of an individual's nutritional state and intake target, we can make predictions about the animal physiological, behavioural and fitness responses to the foods present in the environment. This integrative approach has been successfully applied to a broad range of fields and taxa [48], and brought new perspectives in a number of fields, including neurobiology [111], collective behaviours [112,113], obesity [114], animal production [115] and conservation [116].

While examples of successful NGF applications in vertebrates now abound, it was originally designed to study nutrition in insects (reviewed in [117]). The nutritional requirements of many insects have been deciphered, as well as the impact of intake on a number of life history traits such as survival [118,119], reproduction [120], and resistance to pathogens [121,122]. The NGF provides tools to determine the nutritional requirements and rules of compromise used by animals when they are faced with imbalanced foods. The macronutrient requirements of many herbivorous insects have been successfully quantified. Recently, the case of social insects has attracted notable attention, and the mechanisms by which ants and bees regulate their intake of macronutrient both at an individual and a colony level have been investigated, resulting in the emergence of collective nutrition as a productive field in nutritional ecology. In sharp contrast to social Hymenoptera, the number of studies on the nutritional ecology of termite remains very low, and the NGF has not been applied to this elusive group (but see [32] for micronutrients).

Termite nutritional ecology is highly integrated and complex (Figure 1). In this article, we have reviewed the various levels at which nutrition and the relationship between termites and their

symbionts are intricately linked, from the level of metabolic pathways to the level of the termite society. Here, we propose that the NGF provides a general framework towards an integrative approach to nutrition in symbiosis. We have identified key future research questions (box 1) which we think will lead to major advances in our understanding of the role of nutrition in shaping the evolution of interactions between symbionts and termites, which represent a key model system for symbiotic relationships.

## Box 1: Future directions

**Characterising the nutritional ecology of termites and their symbionts**

Termite optimal nutrient intakes and rules of compromise need to be established following a NGF approach. Nutritional needs of individuals from different caste can be assessed either by constraining individuals to imbalanced foods or by offering pairs of complementary foods. Due to their hemimetabolous development the difference between castes might be less pronounced as compared to social Hymenoptera.

Termite symbionts are notoriously hard to cultivate in vitro, hence little is known about their nutritional requirements. Thanks to advances in genetics, the modifications of symbiont communities of termites fed different diets can be analysed through sequencing of specific genes fragments (for instance the 16S rRNA gene specific to bacteria). From such experiments, we can infer the nutritional needs of symbionts.

The metabolic capabilities and nutritional function of symbiotic microorganisms and their insect hosts can be studied to understand the underlying molecular processes of the symbiosis using new advances in genetics such as next generation sequencing and metagenomic analyses.

**Quantifying nutrient flow and trophallaxis networks**

The social network of interactions and the behaviours controlling food collection and exchange are unknown in termites. We must know 1) if there is a chain of demand from the dependent castes (larvae, soldiers and reproductives) to the foragers; 2) what controls the initiation and ending of trophallaxis; 3) the role of different caste in digestion; 4) whether and how quickly symbionts are shared, and how a lack of certain symbionts in "deficient termites" (*e.g.* newly moulted or young instars) is compensated by favouring trophallaxis with "non-deficient termites". Answers to these questions will be achieved by using recent advances in technologies such as automated tracking of both diets marked with fluorescent dyes and individuals tagged with miniature barcodes [123] combined with the NGF.

**The role of nutrition and symbiosis in termite evolution**

Fitness performances of termite species on various diets can be quantified and linked to the species evolutionary success, using the NGF approach. Basal phylogenetic group Mastotermes might be less efficient at exploiting their foods due to different symbionts and/or digestive systems than more recent termite genus that outcompeted them, including Nasutitermes.

Combining the NGF with advances in genetics will greatly improve our understanding of the role and evolution of nutritional symbiosis. For example by selectively removing or adding symbionts we can assess precisely how symbionts affect termite fitness on various diets. This will help to identify which symbionts are responsible for termite adaptation to different nutritional niches. To go a step further, we need to test whether some groups of symbionts prevent the development of others and if the loss of flagellates allowed the settlement of new bacteria in the higher termites.

Another key point to consider is the interplay between symbiont and host immune systems. We need to better understand how some symbiosis are established while other microorganisms are eliminated by the hosts.

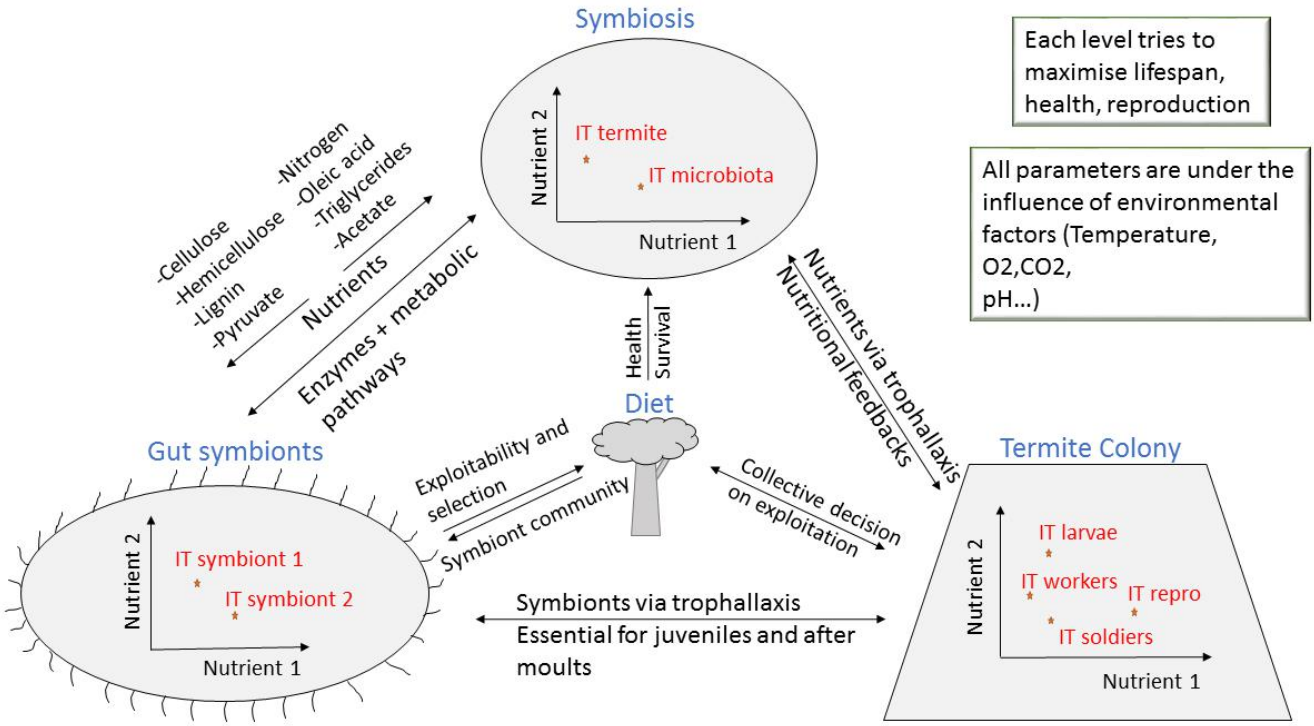


Fig. 1: Schematic summarising termite nutritional ecology and the interplay between termites, their symbionts and their diet. Based on the Nutritional Geometric Framework, we represented the intake targets for 2 hypothetical nutrients at the microbiota, termite, and colony levels. At the microbiota level, different species (restricted to 2 on the figure for simplicity) of symbionts likely require different amount and types of nutrients. At the termite level, the microbiota and the termite might have conflicting needs. Finally at the colony level, IT probably differ according to caste. Interactions between the diet and each level, as well as exchanges between levels are represented by arrows, and those interactions are under the influence of the environment.

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# Chapter 3

# A theoretical exploration of dietary collective medication in social insects

"*I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me.*"

Isaac Newton

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# A theoretical exploration of dietary collective medication in social insects

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ABSTRACT

Animals often alter their food choices following a pathogen infection in order to increase immune function and combat the infection. Whether social animals that collect food for their brood or nestmates adjust their nutrient intake to the infection states of their social partners is virtually unexplored. Here we develop an individual-based model of nutritional geometry to examine the impact of collective nutrient balancing on pathogen spread in a social insect colony. The model simulates a hypothetical social insect colony infected by a horizontally transmitted parasite. Simulation experiments suggest that collective nutrition, by which foragers adjust their nutrient intake to simultaneously address their own nutritional needs as well as those of their infected nestmates, is an efficient social immunity mechanism to limit contamination when immune responses are short. Impaired foraging in infected workers can favour colony resilience when pathogen transmission rate is low (by reducing contacts with the few infected foragers) or trigger colony collapse when transmission rate is fast (by depleting the entire pool of foragers). Our theoretical examination of dietary collective medication in social insects suggests a new possible mechanism by which colonies can defend themselves against pathogens and provides a conceptual framework for experimental investigations of the nutritional immunology of social animals.

## 1. Introduction

Animals select foods in order to reach physiological states maximising growth, reproduction, metabolic health and survival, depending on their sex, age, and reproductive status (Lee et al., 2008; Solon-Biet et al., 2015). Nutrient regulatory behaviours have been most effectively studied using nutritional geometry, a conceptual framework for modelling the nutritional interactions between animals and their environments (Simpson and Raubenheimer, 1993, 2012). In this approach, the challenge for the animals is to regulate their intake of multiple nutrients simultaneously (typically but not necessarily, the macronutrients protein, carbohydrates and fat) at amounts and balances enabling them to maintain nutritional states maximising fitness traits. This optimal intake is known as the nutrient 'intake target' (Simpson et al., 2015a). The multi-dimensional aspect of nutritional geometry is critical for capturing the complexity of animal nutritional decisions, by breaking down food intake into specific amounts and ratios of nutrients that can have independent and/or interacting effects on the physiology and behaviour of animals (Simpson et al., 2015b).

Several recent studies based on this framework show how animals can dynamically adjust their nutrient intake following an infection in order to boost their immune system and combat parasites or pathogens (Ponton et al., 2011, 2015). For instance, many insects increase their intake of dietary protein required for the synthesis of peptides in immune pathways (Lee et al., 2006; Povey et al., 2009; Povey et al., 2014; Mason et al., 2014). This behavioural response is analogous to self-medication, when sick animals ingest specific substances that are not usually part of their diets (Clayton and Wolfe, 1993; de Roode et al., 2013). In these individuals, the selective ingestion of curative substances in food produces measurable benefits to host fitness and negative effects on the pathogen.

While most research on dietary self-medication has been conducted on solitary animals (Abbot, 2014) or isolated individuals in the lab (Lee et al. 2006; Peck et al., 1992), nutrient balancing may also constitute a highly efficient, yet unexplored, mechanism to limit pathogen spread in animal groups, where the increased rate of contact between individuals induces a higher susceptibility to pathogens (Schmid-Hempel, 2017). In social animals the nutritional decisions of an individual not only depends on its own nutritional needs but also critically on the needs of other group members, as individuals will either cooperate or compete to access food (Lihoreau et al., 2015). Recent applications of nutritional geometry to social species indicate that animals can efficiently track

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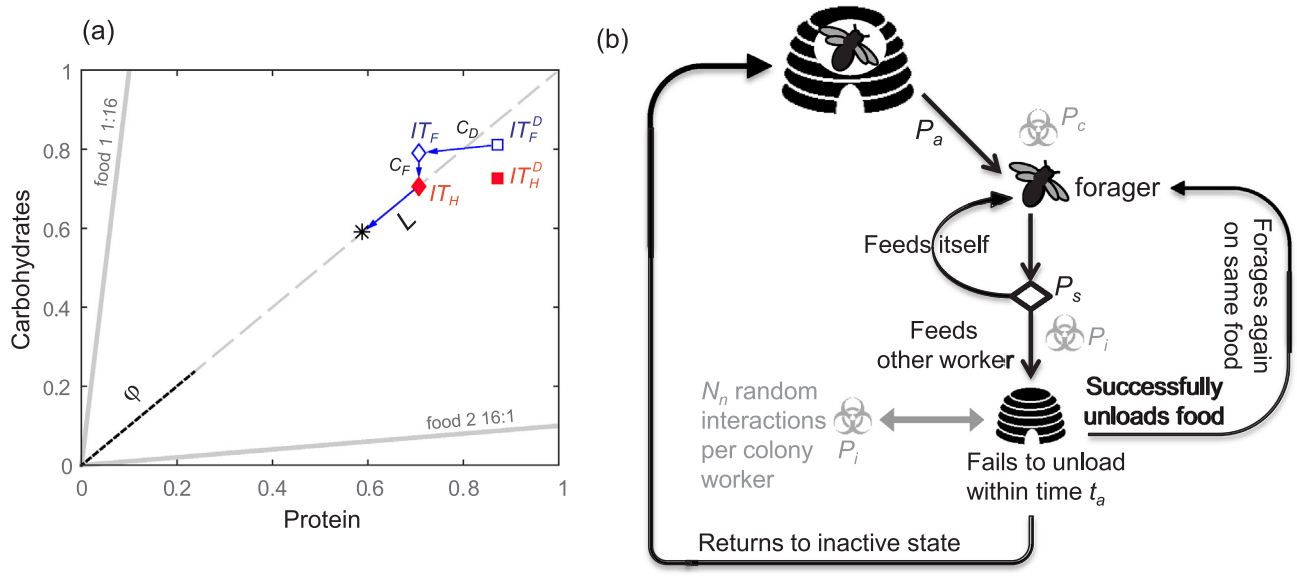


**Fig. 1.** Schematic overview of the model. All model parameters and variables are defined in Table 1. (a) Nutritional rules. Individual workers are defined by their nutritional state (NS, black star) and intake target (IT) in a two-dimensional nutrient space containing two foods. Non foragers have a P:C 1:1 intake target $IT_H$ of magnitude 1. Foragers have an intake target $IT_F$ composed of $IT_H$ to which an extra amount of carbohydrates $C_F$ is added. Workers can adopt a defence intake target, $IT_H^D$ for non foragers or$IT_F^D$ for foragers, by adding $\overrightarrow{C_D}$, a protein rich component (P:C 8:1, magnitude $m_D$) to their respective IT. A forager brings back an amount of food $\varphi$ per time step. At each time step, the NS of a worker is decreased by an amount that depends on its role and whether it has activated its immune response. The blue arrows illustrate the hypothetical path of a NS of a forager with activated immune response who managed to have its NS exactly on its $IT_F^D$. At the end of the step, this worker would see its NS decreased by $C_D$, $C_F$ and $L$. (b) Foraging rules. Any mature worker has a probability $P_a$ to start foraging in response to the nutritional demands of the colony. Foragers have a probability $Ps$ of collecting foods for themselves, otherwise they will offer the collected food to other colony members first. Only foragers that have been able to completely unload their crop after a set time $t_a$ continue to forage, otherwise they return to the status of non-foragers. If the forager was foraging for itself and continues to do so, it has a probability $P_l$ to change food. Parasite transmission can occur in three different ways: foragers have a probability $P_c$ of getting contaminated during the first simulation step in the initial infection regime or every time they successfully forage on a food source in the continuous infection regime; when food is unloaded from a forager to a non-forager, there is a probability $Pi$ that a state 0 worker will be contaminated by a state 2 worker; at the end of every time step, each non-forager randomly interacts with $N_n$ other non-foragers and there is a probability $Pi$ that a state 0 worker will be contaminated by a state 2 worker during this interaction. All parameters and variables are defined in Table 1.

their own nutritional needs in various social contexts, as evidenced by self-selection diet experiments (Dussutour and Simpson, 2009; Hendriksma and Shafir, 2016) and simulations of individual-based models (Lihoreau et al., 2014, Senior et al., 2015; 2016a). However, whether social animals can respond to the infection states of their social partners by adjusting their nutrient intake in order to feed conspecifics and combat pathogen invasion has not been established.

The question of dietary collective medication is especially relevant in social insects, such as bees and ants, that exhibit high levels of social complexity (Holldobler and Wilson, 2009). In these animal groups, individuals specialised in food collection (foragers) must regulate a nutrient intake target to a colony level comprising the needs of all the workers (e.g. nurses, guards), the breeders (e.g. queens, drones) and the brood (larvae), without a global knowledge of the intake targets of their nestmates, nor of the food stores in the colony (Behmer, 2009; Feldhaar, 2014). Disease transmission has been mainly explored in bees due to the fact that parasites and pathogens are major causes of population declines (Goulson et al., 2015; Klein et al., 2017). Social bees are infected by a wide range of viruses, protozoans, bacteria, mites, fungi and parasitoid insects, against which they have evolved behavioural strategies to prevent infection and limit contamination (Schmid-Hempel, 1998). At the individual level, foragers can adjust food collection, for instance by prioritising flowers with nectar rich in secondary metabolites to combat gut parasites (Baracchi et al., 2015) or collecting resins containing antimicrobial substances to limit the spread of fungus within colonies (Simone-Fintrom et al., 2012). At the collective level, bees also display a range of social immunity responses (Cremer et al., 2007) that include grooming (Büchler et al., 1992), social exclusion of infected nestmates (Waddington and Rothenbuhler, 1976, Baracchi et al., 2012), spatial segregation (Naug and Smith, 2007) and thermo-modulation to heat-kill pathogens (Starks et al., 2000). Although there is no direct evidence for a change of nutrient intake target in infected bees (de Grandi-Hoffman and Chen, 2015), honey bees and bumblebees are able to accurately balance their intake of multiple nutrients (*i.e.* carbohydrates and free amino acids in nectar, protein and lipids in pollen) (Altaye et al., 2010; Hendriksma and Shafir 2016; Vaudo et al., 2016a; Stabler et al., 2015) and protein intake modifies baseline immune-competence levels in adults (honey bees: Alaux et al., 2010), thus setting the stage for dietary collective medication in these social insects. Recently, it was shown that honey bees infected with microsporidians *Nosema* spp. increase their intake of sugar syrup containing amino-acids, presumably to support their immune responses (Martín-Hernández et al., 2011; Lach et al., 2015).

Here we developed an individual-based model of nutritional geometry to explore the impact of dietary collective medication on a pathogen spread in a social insect colony. The model simulates a hypothetical social insect colony infected by a hypothetical horizontally transmitted parasite, inspired from the well-described interactions between honey bee (*Apis mellifera*) and *Nosema ceranae* (Higes et al., 2013). In this host parasite system, infected workers start foraging earlier in life (Goblirsch et al., 2013; Natsopoulou and McMahon, 2014), increase their number of daily foraging trips (Alaux et al., 2014; Wells et al., 2016) and spend more time outside the colony (Alaux et al., 2014; Wolf et al., 2014; Kralj and Fuchs, 2010). The reduced foraging workforce is compensated with the premature onset of foraging by young workers that have cognitive deficits (Vance et al., 2009; Ushitani et al., 2016), potentially leading to colony collapse (Russel et al., 2013). Using our model, we examined the influence of the immune response duration on the efficiency of collective dietary medication. Next, we described the impact of foraging failure related to pathogen virulence on parasite spread. Our aim was not to provide a quantitative model of bee-parasite interactions but rather to propose a new theoretical approach for social insect nutritional immunology that could guide future experimental research.

**Table 1**
Model parameters, variables, their notations and values.

| variable/parameter | notation | description | Value |
|---|---|---|---|
| Total number of steps in a simulation | $Ts$ | Duration of a simulation in steps | $Ts = 2000$ |
| Number of mature workers | $n_m$ | Number of mature workers, which can either be foragers or remain in the nest | $n_m = 400$ |
| Number of foragers | $n_f$ | Number of mature workers which are foraging | Variable |
| Number of immature workers | $n_j$ | Number of workers in the nest which can never engage in foraging activity | $n_j = 100$ |
| Nutritional state | NS | An agent's nutritional state, as tracked by its ($p$,$c$) position in the nutrient space, and denoting total intake of the two nutrients. | Variable ($p$, $c$) and initialised at the value of the individual's intake target |
| Intake target | IT | An agent's IT is the ($p$, $c$) coordinate in the nutrient space that maximizes fitness | $IT_H = (0.707, 0.707)$<br>$IT_F = (0,707, 0.715)$<br>$IT_H^D = (0.724,0.709)$<br>$IT_F^D = (0.724,0.717)$ |
| Nutritional performance | $D$ | The Euclidean distance between an agent's NS and the IT. | Variable |
| Food rail slope | $V$ | The nutritional composition of a food in terms of the amount of carbohydrate ($c$) in a given food relative to single equivalent unit of protein ($p$). | Food 1: $V = 16$Food 2: $V = 1/16$ |
| Appetite | $A$ | The amount of a given food that an agent would consume to minimize $D$. | Variable |
| Current/ideal food rail angle | $\alpha_f, \alpha_{ideal}$ | $\alpha_{ideal}$ and $\alpha_f$ are the angles associated with the ideal rail joining the NS and the IT of the individual and with the food rail f respectively | Variable |
| Food ingested | $\varphi$ | The maximum amount of food that an agent is able to consume at a given time. | $\varphi = 0.2$ |
| Cost of life | $L$ | Amount by which the NS are decreased at each time step along a food rail of P:C 1:1 | $L = \varphi/12 = 0.0167$ |
| Cost of foraging | $C_F$ | Additional carbohydrate cost for foraging | $C_F = L/2 = 0.0083$ |
| Cost of immune response | $C_D$, $m_D$ | $C_D$ represents the cost of the immune response and is a vector along a food rail of P:C 8:1 and of magnitude $m_D$ | $m_D = L = 0.0167$ |
| Probability to start foraging and associated constant | $P_a$, $K_a$ | $P_a$ is the probability to start foraging | $P_a = e^{<D> \times K_a} - 1$<br>$K_a = 2$ |
| Probability for a forager of collecting foods for itself | $P_s$, $K_S$ | $P_S$ is the of collecting foods to address a forager's own requirements | $P_S = e^{D \times K_S} - 1$<br>$K_S = 4.5$ |
| Foraging giving up time | $T_a$ | Time after which a worker will give up foraging and return to the status of worker in the nest if it did not unload the food it brought back to the nest | $T_a = 6$ |
| Probability for a forager to leave a food while foraging for itself | $P_L$ | Probability Pl to change food according to the difference between the current food rail it exploits and the ideal angle linking its NS and IT | $P_L = \frac{\lvert\alpha_{ideal} - \alpha_f\rvert}{\pi/2}$ |
| Probability of forager's contamination | $P_c$ | Probability for a forager to be contaminated with the pathogen when it successfully brings food back to the nest | Initial contamination:<br>$P_C = 0.2$Continuous contamination:$P_C = 0.001$ |
| Probability of becoming contaminated during interactions | $Pi$ | Probability of becoming contaminated (state 1) by an interaction with an infected worker (state 2) | $P_i = 0.0025$ (default), 0.01 (fast pathogen spread simulations) |
| Probability for the infection to worsen | $P_W$, $K_W$ | Probability that an individual's state worsen from state 1 to 2 | $P_W = e^{D \times K_W} - 1$<br>$K_W = 6$ |
| Probability for the infection to ease | $P_R$ | Probability that an individual in state 2 returns to state 1 | $P_R = e^{-D \times K_R}$<br>$K_R = 25$ |
| Immune response time | $T_d$ | Time during which an individual engages its immune response (workers cannot recover or die) | $Td = 2, 5, 20, 200, 500$ |
| Probability of engaging in a social immune response | $P_D$ | Probability that an individual in state 0 or 1 activates its immune response when interacting with a state 2 individual | $P_D = 0.0, 0.01, 0.05, 0.25, 0.5, 0.75, 1$ |
| Probability of failing to forage | $P_f$ | The probability that an infected (state 2) bee will fail to forage for a simulation step | $P_f = 0.25$ (default), 0,0, 0.1, 0.35, 0.5 0.75 |

## 2. Model

### 2.1. Model overview

We developed an individual-based model of nutritional geometry to explore dietary collective medication in a social insect colony infected with a horizontally transmitted parasite (Fig. 1). In the model, the pathogen is transferred by passive contacts between individuals inside the colony. Each individual can be in a pathogen free state (state 0), contaminated at low level (state 1) or seriously infected (state 2). State 2 individuals are contagious and can fail foraging. All individuals have a normal intake target for protein and carbohydrates. Once infected, however, individuals adopt a defence intake target that is higher in protein. This defence target provides individuals with a better resistance to the pathogen. Uninfected foragers can detect infected nestmates and also adopt a defence intake target to engage in social immunity (Fouks and Lattorf, 2011). In what follows we describe the rules for nutritional decisions, foraging choices, pathogen spread and defence response. All parameters and variables are defined in Table 1.

### 2.2. Nutritional decisions

Individual workers are defined by their nutritional state (NS, the amount and ratio of nutrients ingested and available to the worker at a certain time, which changes every step) and nutrient intake target (IT, the amount and ratio of nutrients that the worker must reach to maximize fitness, according to its activity and infectious status) in a two-dimensional nutrient space defined by protein P (x-axis) and carbohydrates C (y-axis; Fig. 1a). Note that while we focused on two nutrients for which social insect regulatory behaviour have been best characterised (Dussutour and Simpson, 2009; Altaye et al., 2010; Hendriksma and Shafir, 2016), a similar approach could be readily extended to different nutrients or more nutrients simultaneously (Simpson and Raubenheimer, 2012). The nutritional environment is

composed of two foods each represented by a nutritional rail $V$ with a specific ratio of P to C (P:C). Instead of simulating natural food resources (Nicolson, 2011; Vanderplanck et al., 2017), in this theoretical exploration we used a C-rich food (P:C 1:16) and P-rich food (P:C 16:1) with extreme P:C ratios in order to cover a broad nutrient space.

The colony is composed of 500 individuals. To account for the aged-based division of labour of many social insects (Holldobler and Wilson, 2009), we simulated two types of individuals. The immature workers ($Nj$) stay in the nest and never undertake foraging. The mature workers ($N_m$) can dynamically shift between staying in the nest and foraging, in accordance with the nutritional demands of the colony. Workers have different ITs depending on their foraging activity and whether they defend themselves against the pathogen (Fig. 1a). Non-foraging workers (mature and immature workers) have a P:C 1:1 intake target $IT_H$ of magnitude 1. Foragers (mature workers) have an intake target $IT_F$ composed of $IT_H$ to which an extra amount of carbohydrates $C_F$ is added, to account for their increased need of energy to travel and collect resources. When insects defend themselves against the pathogen they adopt a defence intake target, $IT_H^D$ for non-foraging workers or $IT_F^D$ for foragers, by adding $\overrightarrow{C_D}$, a protein rich component (P:C 8:1, magnitude $m_D$) to their respective IT. This increase of protein replicates the observed behavioural response of many insects following an infection (Lee et al., 2006; Povey et al., 2009; Povey et al., 2014; Mason et al., 2014). Note that similar simulations could have been run with an increase of carbohydrate as some parasites can also induce an energetic stress to the host (Mayack and Naug, 2010). At the beginning of a simulation all workers have their NS initialised at their respective IT. Their NS can then increase (the bees acquire food) or decrease (the bees used part of their resources) at each time step. Again these values were chosen for the sake of simplicity and do not necessarily reflect the intake target of real workers.

When foraging, insects carry an amount $\varphi$ of food which is a fraction of the initial IT magnitude $|IT_H|$ ($\varphi = |IT_H|/5$ in our simulations) (Fig. 1a). The amount $L$ by which the NS are decreased at each time step is itself set as a fraction of $\varphi$ ($L = \varphi/12$ in our simulations). In effect, this means that a fully loaded forager could provide food for itself and for another nestmate for six time steps before being empty if these individuals were at a distance $L$ from their IT (*i.e.* they were previously on their IT and had their NS decreased by $L$ for one step before being fed). Alternatively, the forager could provide enough food for one nestmate to return to its IT if it had not received food for 12 steps (provided that the food itself would point directly to the IT). The magnitude of the extra-costs for foraging and the immune defence were also set in relation to $L$ with $C_F = L/2$ and $m_D = L/k_D$ (with $k_D = 1$) in all simulations.

$D$, is the Euclidian distance between the NS and IT of an individual. $D$ is a proxy of the nutritional performance of the individual (*i.e.* ability to track its IT). In the context of nutritional geometry, the lower $D$ the higher the fitness of the individual (Senior et al. 2015, 2016b). In our model, insects use a 'closest distance' rule of compromise (ROC), meaning that foods are consumed to minimize $D$, to decide when to stop eating or receiving a meal. This parsimonious rule, in which individuals can over eat one nutrient while under eating the other up to a certain point, has been observed in many animals and has the advantage of making the same assumption for both nutrients (Simpson and Raubenheimer, 2012). The amount of food that an individual ideally needs to consume from a given food, its appetite $A$, is calculated as follows:

$$A = \min\{\varphi, D|\alpha_{ideal} - \tan^{-1}V|\}$$

where φ is the maximum amount of food an individual can eat on one time step, $\alpha_{ideal}$ is the angle of a hypothetical 'ideal' food rail connecting an individual's NS to its IT, and $V$ is the food rail of the food being consumed.

### 2.3. Foraging decisions

Any mature worker has a probability $P_a$ to start foraging in response to the global nutritional demands of the colony (Fig. 1b). $P_a$ increases with average $D$ as follows:

$$P_a = e^{<D> \times K_a} - 1$$

where $K_a$ is a constant.

Foragers have a probability $P_S$ of collecting foods for themselves. Otherwise they will offer the collected food to other colony members first (Fig. 1b). $P_S$ increases with the forager's $D$ as follows:

$$P_S = e^{D \times K_S} - 1$$

When an individual forages for others, it first attempts to unload its crop to a randomly chosen worker in the nest. This individual accepts as much food as allowed by its ROC. If the individual does not accept the whole meal, the forager can consume part of the load according to its own ROC. If its crop is still not empty the forager waits until the next step to unload any remaining food using the same procedure, to another randomly chosen worker in the nest and then potentially consuming part of the remaining food for itself again. The outcome of these interactions between foragers and non-foraging workers in the nest is what provides a social feedback for the foragers to choose whether to continue the task. Only foragers that have been able to completely unload their crop after a set time $t_a$ continue to forage, otherwise they return to the status of workers in the nest (Fig. 1b). If the forager was foraging for itself and continues to do so, it has a probability $P_l$ to change food according to the difference between the current food rail it exploits and the ideal angle linking its NS and IT:

$$P_L = \frac{|\alpha_{ideal} - \alpha_f|}{\pi/2}$$

where $\alpha_{ideal}$ and $\alpha_f$ are the angles (in radians) associated with the ideal rail joining the NS and the IT of the individual and with the food rail $f$ respectively.

### 2.4. Pathogen spread

Each worker can be in a pathogen free state (state 0), contaminated but with low levels of infection (state 1), or seriously infected (state 2). In the model, no individual can die or definitely leave the colony. Pathogen spread was simulated in three different ways.

Infection initially occurs through direct contacts between foragers and food. Contamination could be either initial or continuous. In the 'initial contamination' regime, foragers have a probability $P_c$ ($Pc = 0.2$) to become contaminated only during the first simulation step. In the 'continuous contamination' regime, foragers have a probability $P_c$ ($Pc = 0.001$) to become contaminated at each simulation step.

Infection can also happen through interactions between foragers and workers in the nest during food exchanges. When state 0 workers interact with state 2 workers (see below), state 0 workers have a probability $P_i$ of getting infected.

Finally, infection can occur via passive contacts between workers in the nest. On each time step, each worker in the nest randomly draws a number $Nn$ of other nestmates to interact with, with a constant probability $P_i$ to infect or to be infected by their partner following the same rules as with interactions between foragers and non-foraging workers.

### 2.5. Defence rules

On each time step, state 1 workers have a probability $P_w$ of seeing their infection state worsen to state 2.

$$P_W = e^{D \times K_W} - 1$$

Individuals that turn into state 2 then immediately adopt their defence intake target $IT_D$. State 2 workers have a probability $P_R$ to

decrease their pathogen load and revert to state 1, and this probability is inversely proportional to $D$.

$$P_R = e^{-D \times K_R}$$

When this happens, the recovering worker continues to engage its immune response for $T_d$ time steps. During this time $T_d$ the infection state of workers cannot worsen to state 2. However workers can revert from state 2 to state 1. The higher $T_d$ the longer the immune response duration. Using different values of $T_d$ is analogous to triggering different components of the insect immune system. A low $T_d$ replicates an innate immune response whereas a high $T_d$ is more similar to an adaptive immune response (Schmid-Hempel, 1998).

State 2 workers also potentially suffer from a decreased foraging efficiency due to their heavy infection rates. On each time step, foragers have a probability *Pf* of failing to bring back food to the nest. In this case, workers must wait for the next step until they attempt foraging again. This simulates the impaired foraging performances of insects infected with parasites (Gómez-Moracho et al., 2017).

At the collective level, workers that are not yet infected (state 0) or just contaminated (state 1) have a probability $P_D$ to engage in a social immune response by adopting their defence intake target $IT^D$ whenever they interact with a seriously infected worker (state 2). Through social immunity, foragers can adjust their nutrient intake to prepare themselves to fight a potential contamination and to address the needs of infected nestmates and help them recover.

### 2.6. Simulations and analyses

All simulations and statistical analyses were conducted in Matlab. We ran simulations with either an initial contamination regime (contamination during the first simulation step only) or a continuous contamination regime (contamination at each simulation steps).

Each simulation ran for 2000 steps and was replicated 250 times (26250 simulations in total). We used populations of 500 insects (100 immature workers, 400 mature workers) with 40% of foragers at the beginning of the simulation (200 workers in the nest + 200 foragers). For each simulation, we measured variables related to pathogen spread (number of infected individuals in state 1, state 2 and state 1 + 2), colony nutritional performance (average $D$) and colony foraging effort (final number of foragers $n_f$, total number of foraging trips accomplished by all foragers $n_T$).

We conducted two-way analyses of variances (ANOVAs) on these results to test the effects of the immune response duration ($T_d$), the probability to engage in a social immune response ($P_D$) and their interaction on state 1, state 2, state 1 + 2, contamination latency $L$ (*i.e.*, the time elapsed until half of the colony was in state 1 or 2), $D$, $n_f$, and $n_T$. For the initial infection regime, some simulations never reached a state where half of the bees were contaminated or infected. In this case we used one-way ANOVAs to test the effect of $P_D$ on contamination latency $L$ for each value of $T_d$ (Table S1).

## 3. Results

### 3.1. Effect of immune response duration

We explored the effect of the immune response duration ($T_d$ = 2,5,20,200,500) and the probability to engage in a social immune response ($P_D$ = 0.0,0.01,0.05,0.25,0.5,0.75,1) on pathogen spread (state 1, state 2, state 1 + 2, contamination latency $L$), colony nutritional performance (the lower $D$ the higher the performance) and colony foraging effort ($n_f$, $n_T$). Here we focussed on the initial contamination regime only (Table 2) and a relatively slow pathogen spread ($P_s$ = 0.0025), where a majority of the workers is contaminated or infected within the simulated time (Fig. 2). Simulations with the continuous infection regime yielded similar results (Fig. S1, Table S2).

The immune response duration ($T_d$), the probability to engage in a social immunity ($P_D$) and their interaction had a significant effect on pathogen spread (Table 2; for the effect of $P_D$ on contamination latency $L$ see Table S1). Long immune responses ($T_d$ = 200, 500) resulted in the lowest level of pathogen spread (Fig. 2) and the lowest colony foraging effort (Fig. 3). In these conditions, there was no clear effect of engaging in social immune response.

By contrast, short immune responses ($T_d$ = 2, 5, 20) resulted in a much stronger pathogen spread, with larger numbers of contaminated workers (state 1 + state 2 individuals) due to the higher proportions of infected workers (state 2 individuals) and the shorter contamination latencies ($L$) than for long immune responses (Table S1). The colony foraging effort ($n_f$ and $n_T$) was also higher (Fig. 3). In these conditions, increasing the probability to engage in a social immune response ($P_D$) led to a significant reduction of pathogen spread and foraging effort, down to similar values to those obtained for long immune responses (Fig. 3). The average nutritional performance of insects ($D$) was always higher (lower $D$) in the presence of social immunity ($P_D > 0$) than without social immunity ($P_D = 0$). Therefore, social immunity, by which foragers adjust their nutrient intake to address the needs of their infected nestmates, is an efficient mechanism to reduce pathogen spread when immune responses are short.

### 3.2. Effects of pathogen virulence

Social insect infected by parasites often show impaired foraging behaviours (Gómez-Moracho et al., 2017), which suggests a reduced food intake at the colony level. Here we explored the consequences of foraging impairment on pathogen spread by varying the probability for infected foragers (state 2 workers) to fail foraging ($P_f$ = 0.0, 0.1, 0.35, 0.5, 0.75) for slow pathogen transmission ($P_s$ = 0.0025, *i.e.* same value as in the previous section), and fast pathogen transmission ($P_s$ = 0.01).

At a slow pathogen transmission rate ($P_s$ = 0.0025), increasing the probability of failing foraging ($P_f$) was generally beneficial to the colony. Colonies experienced reduced numbers of contaminated individuals (state 1 and 2 workers; Fig. 4a) and longer contamination latencies ($L$; Fig. 4c). Here, the impaired activities of foragers only induced a slight decrease of the colony nutritional performance ($D$; Fig. 5a) and an increase of the foraging effort ($n_f$ and $n_T$; Fig. 5b and c).

Interestingly the results were markedly different at a fast pathogen transmission rate ($P_s$ = 0.01). In these conditions, the pathogen almost always contaminated the entire colony (average number of state 1 + 2 workers: 498.64 ± 2.95 (mean ± SD)) within the simulated time. Increasing the probability of failing foraging ($P_f$) led to an increased number of infected workers (state 2 workers; Fig. 4b), up to nearly the whole population (average number of state 2 workers: 489.32 ± 7.35 (mean ± SD)) being infected for frequent foraging failure of contaminated individuals ($P_f$ = 0.75). The contamination latency ($L$) was generally low and increased with $P_f$ (Fig. 4c), indicating that while the final spread of the pathogen reached catastrophic levels, the early stages of the contamination were still partly delayed when infected foragers often failed to bring food back to the colony. Frequent foraging failure ($P_d$ = 0.75) led to a very marked decrease in colony nutritional performance (higher $D$) up to an average level that was approximately five times lower than for other values of $P_f$ (Fig. 5a). The effect of $P_f$ on the foraging effort was also very pronounced. Increasing $P_f$ led to a recruitment of much higher numbers of foragers ($n_f$; Fig. 5b) and an exponentially growing number of cumulated foraging trips throughout the simulations ($n_T$, Fig. 5c). Therefore, while high probabilities of failing foraging were beneficial to colonies at low transmission rates, by removing infected individuals and limiting pathogen spread, high probabilities of failing foraging were highly detrimental at fast transmission rates, by reducing social immunity and accelerating colony declines.

**Table 2**
Results of two-way ANOVAs testing the effect of $P_D$ and $T_d$ on the number of contaminated individuals (state 1 + state 2), contaminated individuals (state 1), infected individuals (state 2), number of foragers $n_f$ and total number of foraging trips $n_T$, for an initial contamination regime. Parameters and variables are defined in Table 1.

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Variable: number of contaminated and infected individuals (state 1 + 2) | | | | | |
| $P_D$ | 237699.9 | 6 | 39616.6 | 77.08 | 3.391E−94 |
| $T_d$ | 627124.9 | 4 | 156781.2 | 305.05 | 5.56E−246 |
| Interaction | 174465 | 24 | 7269.4 | 14.14 | 2.921E−56 |
| Error | 4479056 | 8715 | 513.9 | | |
| Total | 5518346 | 8749 | | | |
| Variable: number of state 1 individuals | | | | | |
| $P_D$ | 427.9 | 6 | 71.317 | 1.08 | 0.3689 |
| $T_d$ | 2738.4 | 4 | 684.588 | 10.41 | 0 |
| Interaction | 3024.9 | 24 | 126.039 | 1.92 | 0.0045 |
| Error | 573019.9 | 8715 | 65.751 | | |
| Total | 579211.1 | 8749 | | | |
| Variable: number of state 2 individuals | | | | | |
| $P_D$ | 230031.5 | 6 | 38338.6 | 106.25 | 1.11E−129 |
| $T_d$ | 547890.9 | 4 | 136972.7 | 379.6 | 7.33E−302 |
| Interaction | 182613.1 | 24 | 7608.9 | 21.09 | 5.856E−89 |
| Error | 3144675 | 8715 | 360.8 | | |
| Total | 4105211 | 8749 | | | |
| Variable: $D$ | | | | | |
| $P_D$ | 0.00176 | 6 | 0.00029 | 14.68 | 0 |
| $T_d$ | 0.00051 | 4 | 0.00013 | 6.32 | 0 |
| Interaction | 0.00047 | 24 | 0.00002 | 0.97 | 0.5018 |
| Error | 0.17433 | 8715 | 0.00002 | | |
| Total | 0.17706 | 8749 | | | |
| Variable: $n_f$ | | | | | |
| $P_D$ | 4265.1 | 6 | 710.85 | 17.86 | 1.099E−20 |
| $T_d$ | 9059.4 | 4 | 2264.86 | 56.89 | 1.858E−47 |
| Interaction | 4065.2 | 24 | 169.38 | 4.25 | 1.572E−11 |
| Error | 346955.7 | 8715 | 39.81 | | |
| Total | 364345.4 | 8749 | | | |
| Variable $n_T$ | | | | | |
| $P_D$ | 5.10E+09 | 6 | 8.51E+08 | 39.45 | 1.297E−47 |
| $T_d$ | 1.69E+10 | 4 | 4.23E+09 | 196 | 3.46E−161 |
| Interaction | 3.94E+09 | 24 | 1.64E+08 | 7.61 | 4.611E−26 |
| Error | 1.88E+11 | 8715 | 2.16E+07 | | |
| Total | 2.14E+11 | 8749 | | | |

## 4. Discussion

Many insects adjust their nutrient intake to combat pathogens and parasites (Lee et al., 2006; Povey et al., 2009; Povey et al., 2014). Here we explored how such self-medication behaviour could scale up to a social insect colony, where individuals forage for their nestmates and dynamically balance their collection of multiple nutrients to reach a colony-level intake target. Based on recent advances in nutritional geometry modelling (Lihoreau et al., 2014, 2015), we developed an individual-based model to explore the consequences of dietary collective medication in hypothetical social insect colonies infected by a horizontally transmitted parasite that impairs the foraging behaviour of workers.

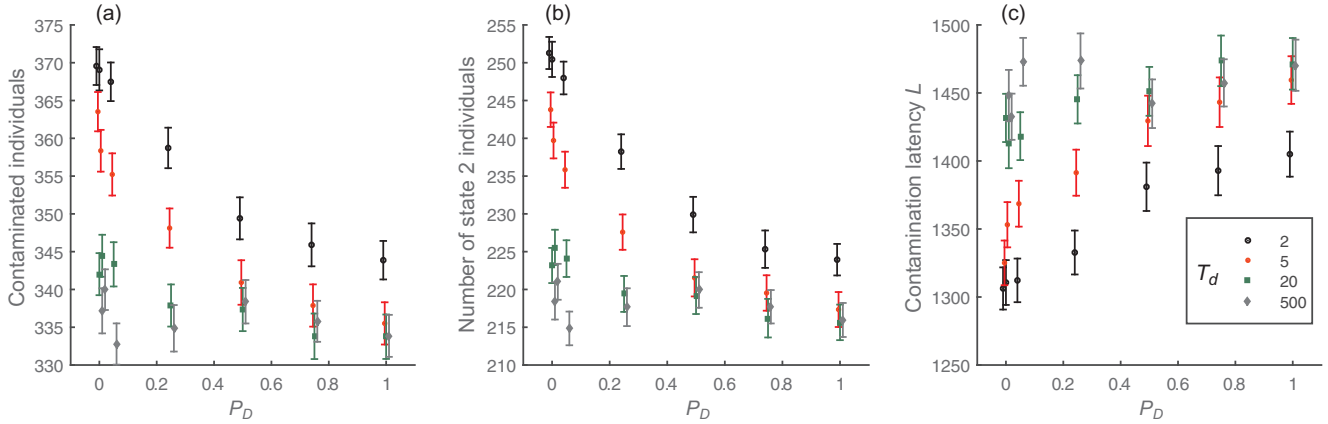


**Fig. 2.** Effect of the immune response duration ($T_d$, colour coded series) and the probability to engage in a social immune response ($P_D$) on (a) the total number of contaminated workers (state 1 + 2), (b) the number of infected workers (state 2), and (c) the contamination latency ($L$). All means are shown with their standard deviation. Parameters and variables are defined in Table 1.

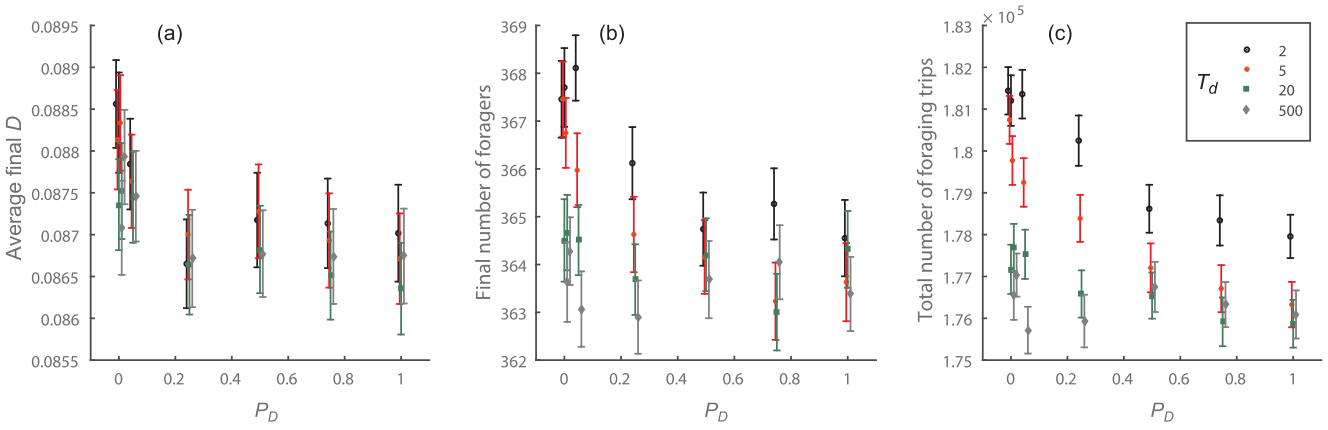


**Fig. 3.** Effect of the immune response time ($T_d$, colour coded series) and the probability to engage in a social immune response ($P_D$) on (a) the average nutritional performance of the colony ($D$), (b) the final number of foragers ($n_f$), (c) and the total number of foraging trips during a simulation ($n_T$). All means are shown with their standard deviation. Parameters and variables are defined in Table 1.

### 4.1. Collective nutrition can efficiently limit pathogen spread

Several recent models have considered the effects of pathogens and parasites on colony dynamics (Becher et al., 2014; Horn et al., 2016). Social insects are known to prophylactically collect anti-pathogen non-food materials such as resins to prevent contaminations of parasites and pathogens (Christe et al., 2003; Chapuisat et al., 2007; Simone et al., 2009; Simone-Finstrom et al., 2012). However, none of these studies have considered the potential role of nutrition, and in particular the interacting effects of specific nutrients, in mitigating these effects. Our theoretical exploration of dietary medication based on nutritional geometry suggests that collective nutrition is a potentially efficient social immunity mechanism to limit pathogen spread in a colony. This is true especially when immune responses are short, which is typically the case for innate immune responses in insects (Schmid-Hempel, 1998). Through social immunity, foragers can adjust their nutrient intake to address the needs of infected nestmates and prepare themselves to fight a potential contamination.

Although the basic assumption that social insects make dietary medication decisions remains to be empirically validated, several recent studies suggest that all the ingredients are met. In bees for instance, mounting evidence show that workers accurately balance their intake of macronutrients to address their nutritional needs at the individual (Altaye et al., 2010; Stabler et al., 2015) and collective (Hendriksma and Shafir, 2016; Vaudo et al., 2016b) levels. Additionally, foragers are known to exploit secondary metabolites in plant products with anti-microbial properties in order to combat pathogens (Erler et al., 2014; Manson et al., 2010; Baracchi et al., 2015). Similar observations have been made in ants (Christe et al., 2003; Chapuisat et al., 2007; Dussutour and Simpson, 2009; Cook et al., 2010). Importantly, individual-based models such ours are a powerful mean to generate well-defined predictions for empirical testing using experiments with nutritional geometry designs (Lihoreau et al., 2015).

### 4.2. Foraging impairment limits pathogen spread at slow transmission rates

Our theoretical exploration of dietary collective medication also suggests interesting consequences of behavioural changes in infected foragers for the colony health. For instance, infected honey bees often spend more time outside the colony and sometimes even never come back (e.g. Kralj and Fuchs, 2010; Dussaubat et al., 2013). Such behaviour may reflect impaired cognitive capacities that reduce the orientation performances of honey bee foragers or a host manipulation by the pathogen to favour its own spread (Schmid-Hempel, 2011). Reduced homing rates have also been interpreted as an altruistic 'self-removal' strategy by which infected honey bees remove themselves from the colony to prevent disease transmission (Rueppell et al., 2010). Interestingly, our model suggests contrasted influences of impaired foraging on pathogen spread depending on its transmission rate. Inefficient infected foragers can benefit the colony provided that the pathogen does not spread fast enough, because their inability to feed nestmates or to return to the colony can act as a de facto quarantine mechanism. But if that quarantine effect of failing foragers is insufficient to stop the initial spread of the pathogen, there is a risk of failing to provide the colony with the adequate intake requirements necessary to engage its immune defence response. Therefore, affecting the foraging activity can either hinder or help its progression, depending on the specific effects of a pathogen, suggesting that different selection pressures may lead to similar behaviour depending on the host-pathogen system under consideration.

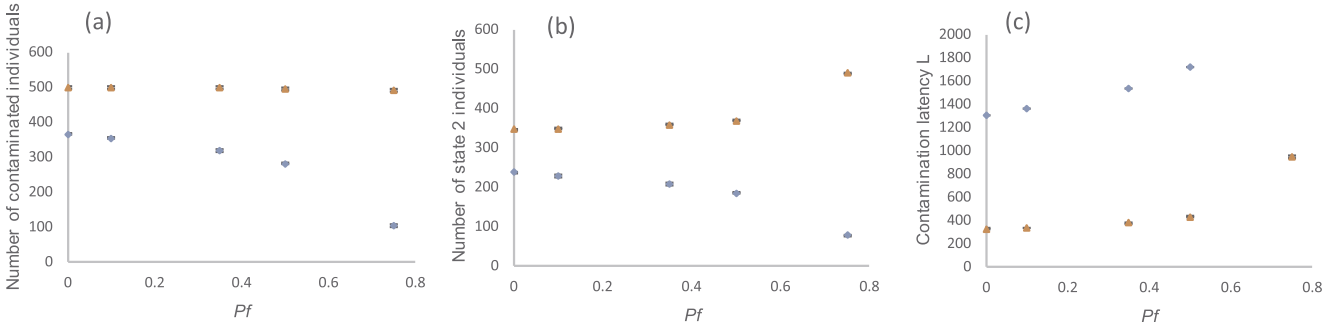


**Fig. 4.** Effect of the probability of infected individuals to fail foraging ($P_f$) for slow (blue: Ps = 0.0025) and fast (red: Ps = 0.01) transmission rates on (a) the total number of contaminated individuals in state 1 or 2, (b) the number of infected individuals (state 2), (c) and the contamination latency ($L$). All means are shown with their standard deviation. Parameters and variables are defined in Table 1.

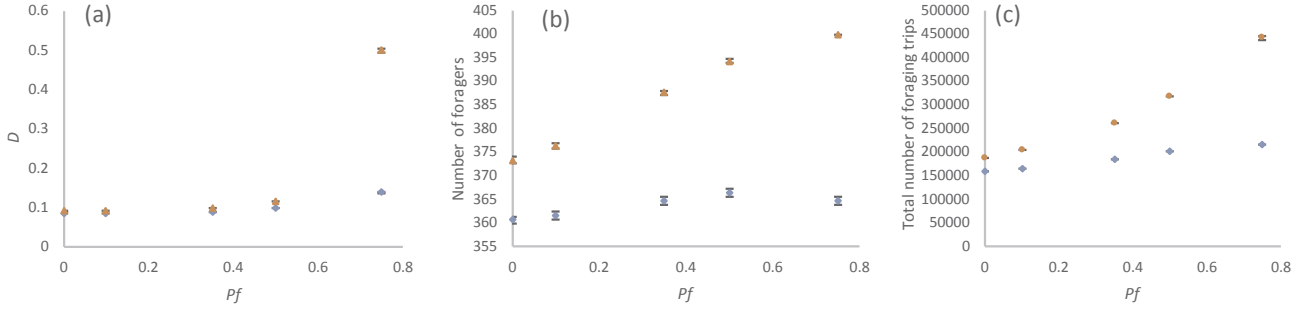


**Fig. 5.** Effect of the probability of infected individuals to fail foraging ($P_f$) at two different pathogen transmission probabilities (blue: Ps = 0.0025; red: Ps = 0.01) on (a) the average nutritional performance of the colony ($D$), (b) the final number of foragers ($n_f$), (c) and the total number of foraging trips during a simulation ($n_T$). All means are shown with their standard deviation. Parameters and variables are defined in Table 1.

### 4.3. Implications for colony health

An imbalanced nutrition is known to induce high mortality rates and colony collapse in social insects (Dussutour and Simpson, 2012). Over recent years, malnutrition has become particularly concerning in bees and is now considered a major cause of population declines, due to the lack of key nutrients for development, cognition or survival (Goulson et al., 2015; Klein et al., 2017). For instance, honey bees fed omega-3 poor pollen have reduced learning abilities, potentially incurring important foraging costs to colonies (Arien et al., 2016). Under natural conditions, when foragers fail in their collect food or disappear, more hive bees tend to become foragers and cease their hive-related activity, potentially translating into reduced brood care, defence and hygiene, as well as less efficient foraging efficiencies by the inexperienced foragers. Above a certain rate of forager disappearance, colony population may decrease dramatically and lead to an inevitable colony collapse (Khoury et al., 2011, 2013; Russell et al., 2013; Perry et al., 2015). Although purely theoretical, our study suggests that a failure in addressing the different nutritional needs of nestmates with various infection levels can have similar consequences.

Ultimately, accurate mapping of nutrient intake by infected bees and its consequences on colony functions, using our models, may allow for designing constructive interventions to limit these dynamics, for instance by providing infected colonies with appropriate plant resources that produce nectars and pollens that enable bees to self-medicate. Of course this would require some more development and precise parameterisation of our exploratory model, based on observations and experimental work. For instance, honey bee workers may use an "asymmetrical quadratic" rule of compromise when balancing carbohydrates and essential amino acids instead of the parsimonious nearest distance rule of compromise used here (Paoli et al., 2014). Bees also appear to balance their intake of lipids in pollen (Vaudo et al., 2016a,b) and mineral salt in water (Lau and Nieh, 2016) in addition to carbohydrates and protein. Importantly, all these adjustments are readily available in classical state-space models of nutritional geometry (Simpson and Raubenheimer, 2012) and can be implemented in our individual-based platform.

### 4.4. Nutritional immunology of social animals

Beyond identifying novel predictions about social insect-pathogen interactions, our model introduces a new framework for studying the nutritional immunology of social animals, by integrating models of epidemiology (Fefferman et al., 2007) and social nutrition (Lihoreau et al., 2014, 2015). This approach, here developed for a superorganism, could be expanded to a wider range of social species in which individuals make nutritional decisions for others, for instance when adults provision their brood or choose nesting environments that will provide nutrition for offspring. In principle, nutritional geometry models can be applied to many host-pathogen interactions in order to derive new empirically testable predictions, with only minimal fine tuning of the nutritional rules (number of nutritional dimensions, intake targets, rule of compromises, non-dietary foods), social rules (nature and frequency of interactions), and fitness consequences (impaired development and behaviour) for the hosts and the pathogens (Simpson et al., 2015a). A major challenge for host-pathogen research is to study these interactions from both perspectives (Schmid-Hempel, 2011). In the present case, the potential overlap of the nutritional changes required by the defence of the hosts and the nutritional requirements of the pathogens themselves may be critical. In honey bees, for instance, diets with higher pollen quantities increase *N. ceranae* intensity but also enhance the survival of honey bees (Jack et al., 2016). Models of nutritional geometry have already begun to explore these aspects by integrating the nutrient intake and fitness responses of multiple actors (hosts, pathogens, symbionts) and how they influence each other (Ponton et al., 2011; Wong et al., 2015). How these complex interactions scale up at the level of animal groups, where hosts interact with each other, remains an open question.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2017.08.005.

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# Chapter 4

# Nutrition in extreme food specialists: an illustration using termites

*"Once the ants and termites jumped the high barrier that prevents the vast variety of evolving animal groups from becoming fully social, they dominated the world."*

E. O. Wilson

# Chapter 4 - Nutrition in extreme food specialists: an illustration using termites

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# Abstract

1. Recent nutritional ecology theories predict that an organism feeding on a single, highly predictable food should lack the typical active regulation of nutrient balance observed in all other organisms studied so far. It could instead limit itself to controlling the amount of food eaten alone. Such an animal would however be strongly affected by nutrient imbalances.
2. Termites are an ideal model animal to test those predictions.
3. We investigated how the nutritional content of food affected termites' intake and performance by constraining groups of *Nasutitermes exitiosus* to artificial diets varying in their macronutrient ratios.
4. We showed that (1) termites, contrary to other insects, did not compensate for nutrient imbalance by adjusting food collection (2) longevity in workers was strongly influenced by carbohydrate intake, while in soldiers it depended almost entirely on the number of workers remaining to feed them (3) tunnelling activity increased with the quantity of food collected and (4) intake had very little influence on lipid and protein termite body contents.
5. We provide evidence that extreme food specialists might have lost the ability to regulate macronutrient intake.



**Keywords**: *caste, longevity, macronutrients, nutrition, termites, tunnelling*.

# 1. Introduction

All living organisms need a nutrient intake which accurately matches their needs to sustain their metabolism, growth, reproductive performance, and immune system. Hence a large number of studies and approaches have been developed to understand how living organisms might regulate their intake. The Geometric Framework (GF) is an approach which has recently brought new insights in the field of nutrition and foraging ecology, and has also been applied to a wide range of biological questions involving diverse taxa and fields (Simpson and Raubenheimer, 2012). The GF allows the relationship between any life history trait and nutrition to be mapped, providing the basis for integrative models of nutritional biology. It is a state-space modelling platform in which the food intake and the nutritional state of individuals are represented in a nutritional space where each axis represents key food components such as macronutrients. The GF provides a means to quantify whether and how individuals regulate the amount and ratios of specific nutrients they ingest to reach areas in the nutrient space where their fitness is maximised (the “intake target”). When faced with imbalanced nutritional environments that prevent the intake target to be reached, individuals must balance consuming excesses and deficits of different nutrients, with associated fitness costs (the so-called "rules of compromise"). These rule of compromise depend on the nutritional ecology of the organisms being studied. For example, a specialist feeder which is usually restricted to a narrow range of nutrient compositions will be less willing to overeat from an imbalanced food than a generalist because the chance that it will encounter a food with a complementary imbalance later on is relatively low (Behmer, 2009; Simpson and Raubenheimer, 2012).

In recent years collective nutrition has emerged as a new field of research, within the broader field of nutritional ecology (Raubenheimer et al., 2009). Numerous studies have investigated how insect colonies maintain the balanced supply of nutrients at both a collective and an individual level (review in Feldhaar, 2014). In an insect colony, only a small proportion of the individuals collects the food for the entire colony. Hence the regulation of

nutrition is more complex than for a solitary individual, as the foragers do not possess a global knowledge of the colony's nutritional status or food stores. Despite the challenge, bees and ants offered a choice of foods varying in nutrient composition have been shown to regulate their intake and collection of protein and carbohydrate to reach the intake target for the colony (ants (Cook et al., 2010; Dussutour and Simpson, 2009), honeybees (Altaye et al., 2010), bumblebees (Vaudo et al., 2016a; Vaudo et al., 2016b)). Notably, foragers can respond to the varying nutritional needs of the colony by increasing their collection of protein to meet the needs of developing larvae for example (ants: Audrey Dussutour & Simpson, 2009).

Termites differ in several important respects from the more intensively studied hymenopteran social insects, and offer new opportunities to test predictions arising from nutritional ecology and social foraging theory. According to nutritional ecology theory, the extent to which species have evolved the capacity to regulate intake of multiple nutrients depends upon three factors: 1) the degree to which specific nutrients are correlated in their concentrations within foods; 2) the extent of heterogeneity among foods in nutritional composition, and 3) the extent to which nutritional requirements change qualitatively (*i.e.* in target ratio) over time (Raubenheimer et al., 2009; Simpson and Raubenheimer, 2012). Nutrients for which specific appetite systems have evolved are expected to be those that are not reliably positively correlated with one another within foods (such that regulating the intake of only one nutrient does not ensure a balanced intake of the others), and which vary in their ratio between different foods (such that different foods need to be mixed to attain a balanced complement of nutrients). For example, where there are separate protein-rich and carbohydrate-rich foods, an animal must mix its intake of the two food types to satisfy its requirements for protein and carbohydrate; a process known as complementary feeding. To balance nutrient intake through complementary feeding requires that the animal has a means of assessing the composition of different foods and of relating this to current requirements for protein and carbohydrate (either its own needs, or the colony's). When faced with a single suboptimal food, this animal will reduce its intake if the food contains nutrients that can

become harmful if ingested in high volumes, or in the opposite it will overeat foods with low nutritional values to reach a minimum quantity of essential nutrients. If an organism specialises on a single food of invariant composition, it needs only evolve the capacity to find, select and process that type of food and then simply regulate the volume ingested to attain a balanced intake of all required nutrients. Such a species would be predicted not to have evolved specific appetites for different nutrients.

Termites offer a rare example which closely matches such a nutritional ecology. Whereas Hymenoptera alternate between foods that vary in their macronutrient composition to reach a balanced diet (*e.g.* prey *vs* honeydew in ants), termites feed mainly on a single type of food, wood, which is relatively invariant in its macronutrient composition (Pettersen, 1984). We chose the Australian termite *Nasutitermes exitiosus* as our model species because they are specialists of Eucalyptus trees. Like all trees, Eucalyptus are mostly composed of carbohydrates, and their composition in carbohydrate, protein and lipid is stable (Evtuguin and Neto, 2007). Therefore, we expect *N.exitiosus* foragers to regulate only the amount of food they collect to meet their macronutrient requirements.

Another reason to expect that termites may not have evolved specific appetites for macronutrients is that they are hemimetabolous. Juvenile and adult stages differ less in the qualitative nutritional requirements than is the case for larval and adult forms of holometabolous insects such as Hymenoptera, where larval and adult forms have very different intake targets ( Sorensen and Vinson, 1981; Cassill and Tschinkel, 1999; ; Weeks Jr et al., 2004; Dussutour and Simpson, 2009;). However termites are able to moult and change caste, which comes with a cost (Bernays, 1986), and different castes might display variation in their macronutrient requirements. A reason why animals might have evolved specific appetite systems, even when feeding on a relatively nutritionally invariant food type, is where nutritional requirements change markedly over time, for example, across larval and adult life-stages in holometabolous species, necessitating that consumption is adjusted to respond to the currently most limiting nutrient (Raubenheimer et al., 2009). Given their hemimetabolous development and their high degree of food specialisation, we therefore predicted that termite

foragers, in contrast to Hymenoptera, would be less able to regulate colony nutrition by adjusting foraging behaviour in response to experimental manipulation of their diet. In this paper, we used the GF approach to address this prediction, by investigating whether termites adjusted their food consumption when faced with foods varying in their macronutrient ratios, and how their longevity, physiology and behaviour were impacted by the diet composition.

## 2. Material and Method

### *a) Species studied and rearing conditions*

Four colonies of *Nasutitermes exitiosus* of similar sizes were collected in Adelaide (South Australia) in late spring, between the 22$^{nd}$ of November and the 2$^{nd}$ of December 2016, on a hill crest, where Eucalyptus trees were the main vegetation. The mother colonies were kept in the lab for at least 2 weeks with ad libitum wood, insect vitamins (Vanderzant vitamin mixture for insects - Sigma) and salt mixture W (MP biomedical) prior to the experiment to reduce eventual variability in colonies initial nutritional state. From these mother colonies, 76 experimental colonies of 100 individuals were constructed.

Each experimental colony consisted of 70 large workers (stage 2 to 5, see (McMahan and Watson, 1975)) and 30 minor soldiers. We used castes that are sterile so we could study the effects of nutritional challenges, independent of reproductive effort. Termites were housed in a 10*10 cm Petri dish. Two third of the Petri dish was filled with 4% agar gel, to provide humidity and allow tunnelling. This type of husbandry has been used previously in this species (Eutick et al., 1978). To prevent fungus infections, a solution of fungicide (10 drops of Zaleton per litre) was sprayed on the agar and allowed to dry before the termites were introduced. Each experimental colony was transferred to a new nest every 6 days to prevent infections and dryness. The nests were kept at room temperature (27ºC) under complete darkness.

## *b) Synthetic diets*

In the field, termites feed from wood that only varies slightly in its composition of macronutrients (Carbohydrate 65-75%, Nitrogen :0.03-0.1% (Pettersen, 1984)). For the experiment we used synthetic foods varying in their ratio of protein, lipid and carbohydrate. Sterols were used as a lipid as they are thought to be limiting, insects lacking the ability to synthetise them. Wood is typically poor in nitrogen content and varying protein in our diet was important to explore whether termites might specifically regulate its intake. Cellulose is the main energy source used by termites. The protein content of all the foods consisted of a mixture of whey (90%, Myopure) and egg white (10%, Myopure), lipids were added as 50% phytosterol (Bulk Supplements) and 50% ergosterol (Sigma), and cellulose (Sigma) was used as a digestible carbohydrate source. Each food contained 0.5% of vitamins (Vanderzant vitamin mixture for insects, Sigma) and 0.5% of mineral salts (salt mixture W, MP biomedical). The foods were presented to the termites as a powder (see Table 1 below) in 2 mL Eppendorf tubes, which they had access through a small plastic tube. We confined 76 experimental colonies to one of 11 diets differing in their ratio of protein (P), lipids (L) and carbohydrates (C). The P:C:L ratios used are indicated in Table 1. For each treatment, we used 6 to 7 experimental colonies originating from four different mother colonies. As our experimental setups and artificial diets had never been tested before, we also confined 7 experimental colonies to wood, their natural food that we know termite live well on, using the same experimental conditions as artificial diet groups, and measured mortality as a control. We used two species of Eucalyptus found locally on the Waite campus, Adelaide (*E. cneoriflora, E. kruseana*). The wood was cut in pieces of approximately 4 cm long (one of each species), and placed untreated in the nest. We did not observe fungus growth on the wood.

- Table 1: Proportion and ratio of macronutrient in the artificial diets (C=Carbohydrate, P=protein, L=lipid). P1, P2, P3 and P4 are diets presenting more proteins than lipids with increasing concentrations in proteins from P1 to P4. L1, L2, L3 and L4 are diets presenting more lipids than proteins with increasing concentrations in lipids from L1 to L4. S1 and S2 are diets presenting an equal balance of lipids and proteins with increasing concentration in carbohydrates. Ce is a diet composed of carbohydrate only and micronutrients.

| ***Diet name*** | ***C*** | ***P*** | ***L*** | ***Ratio P:C*** | ***Ratio L:C*** | ***Ratio P:L*** |
|---|---|---|---|---|---|---|
| ***p1*** | 0.99 | 0.007 | 0.003 | 0.007 | 0.003 | 2.333 |
| ***p2*** | 0.95 | 0.048 | 0.002 | 0.05 | 0.002 | 24 |
| ***p3*** | 0.9 | 0.09 | 0.01 | 0.1 | 0.011 | 9 |
| ***p4*** | 0.8 | 0.16 | 0.04 | 0.2 | 0.05 | 4 |
| ***l1*** | 0.99 | 0.003 | 0.007 | 0.003 | 0.007 | 0.428 |
| ***l2*** | 0.95 | 0.002 | 0.048 | 0.002 | 0.05 | 0.042 |
| ***l3*** | 0.9 | 0.01 | 0.09 | 0.011 | 0.1 | 0.111 |
| ***l4*** | 0.8 | 0.04 | 0.16 | 0.05 | 0.2 | 0.25 |
| ***s1*** | 0.99 | 0.005 | 0.005 | 0.005 | 0.005 | 1 |
| ***s2*** | 0.9 | 0.05 | 0.05 | 0.055 | 0.055 | 1 |
| ***ce*** | 1 | 0 | 0 | 0 | 0 | NA |

## *c) Experiment*

In this experiment, we investigated the link between nutrition and performance in terms of food collection, longevity, tunnelling activity and body composition in termites.

## Food collection

All experimental colonies had ad libitum access to food that was replenished every 3 days. Colonies never collected all the food offered before it was renewed. In order to evaluate the colony's intake the food was dried at 40ºC and weighed, before it was placed in the nest, and again after they were removed. We divided the colony intake by the number of termites in

each colony at the time the food was offered, to take into account differences in mortality between colonies.

## Survival

To assess mortality in all experiments, the number of dead termites within each experimental colony was counted every day for the first 2 weeks and then every 3 days until all termites had died. Corpses were removed from the colony and kept in Eppendorf tubes at -14ºC (for body composition analysis). Pictures of the nest were also taken whenever the nest was changed to check that mortality was accurately recorded and that no dead bodies were missed (by counting live termites).

## Tunnelling activity

During the whole experiment, we took a picture of the nest every six days (SI, picture 1), just before each colony transfer to a new nest. Tunnelling activity was quantified by measuring the total tunnel length using imageJ. A total of 562 pictures were analysed.

## Body composition

We measured the body composition of termites found dead in the nest during the experiment. First, we used a chloroform extraction protocol modified from (Marden, 1987) to extract whole-body lipids. Groups of 5 termites (5 workers or 5 soldiers) were dried for 24h at 50ºC (to attain a stable dry weight), weighed to nearest 0.01 mg and placed in an Eppendorf. Next, we added 0.3 mL of chloroform to each Eppendorf. After 24h we aspirated the chloroform and added fresh chloroform. A total of three chloroform soaks were completed. After these extractions, we allowed the termites to dry completely at 50ºC and we reweighed

each group of 5 termites. The difference in weight of termite bodies before and after lipid extraction gave us the weight of lipids in the samples.

Second, we used a protein extraction protocol described in (Rho and Lee, 2014). The remaining bodies from the lipid extraction were used, and a similar procedure was followed, using 0.35M sodium hydroxide solution instead of chloroform. The difference in weight of termites bodies before and after protein extraction gave us the weight of proteins in the samples. A total of 469 groups of workers and 264 groups of soldiers were analysed.

### Statistics

All statistical tests were performed using matlab. To compare food collection on each diet, we used a Kruskal-Wallis test. All generalized linear mixed models (using the function fitglme, with Distribution = normal, link function= identity) and surface regression (using the function fitlme) were done with colony as a random factor. All consumption were standardized ((value-mean)/standard deviation). This procedure reduces the covariation between linear variables and their interaction terms (Aiken 1991).

## 3. Results

### *a) Food collection*

First, we measured food collection of experimental colonies of termites forced to feed from a single diet which varied in its macronutrient composition. Thus, termites were confronted with the situation wherein there is a potential conflict between meeting their requirements for protein, lipids and carbohydrates. Termites did not modify food collection according to the proportion of macronutrient (SI, Fig. S1) but maintained the amount of food collected constant on all diets (Fig. 1, $\chi^2$=14.14, DF=10, P=0.167, SI, table S1).

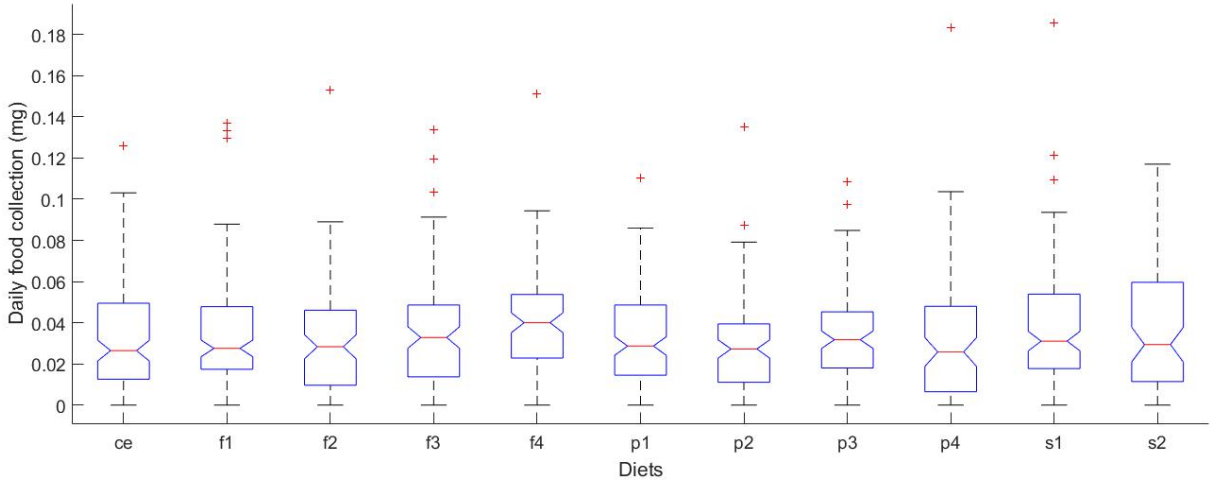


Fig. 1: Macronutrient collection: Notched boxplot presenting the average daily intake per individual (mg) when experimental colonies of termites were confined on one of 11 diets varying in macronutrient content and composition (76 experimental colonies in total, N=7 per diet, except for diet S2, N=6). The red central mark is the median, the edges of the box are the 25$^{th}$ and 75$^{th}$ percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually. Refer to table 1 for the definition of each diet.

## *b) Effect of macronutrient collection on lifespan*

We then investigated whether there was a ratio and quantity of protein, lipid and carbohydrate collected by workers that maximized worker and soldier lifespan. Experimental colonies confined to the control diet (wood) survived well (Mean colony half-life ± CI 95%= 57.25 ± 12.9 days) and similarly to what was reported in the literature ((Cookson, 1987)). This indicates that our experimental nests were appropriate for the study.

Taken together, our 11 diets allowed us to generate maps of the macronutrient intake space, on which lifespan could be represented and regressed (Fig. 2). Worker mortality was significantly affected by macronutrient composition, and was mostly influenced by the quantity of carbohydrate collected (P<0.001, t=4.72, DF= 68, SI, table S2). Termites lived longest when the daily collection of carbohydrate was around 0.03mg per individual (Fig. 2a and 2b). Survival also depended on protein collection, falling when it increased (P=0.002, t=-3.2, DF= 68, SI, table S2). Survival also decreased when the ratio L:C increased (P=0.002, t=-3.3, DF= 68, SI, table S2).

Soldier lifespan decreased slightly when the L:C ratio increased (P=0.009, t=-2.67, DF=68, SI, table S3). If we included the worker lifespan as an explanatory variable in the equation, it became the most significant factor affecting the soldier survival (Adjusted $R^2$=0.20 vs 0.39, SI, tables S3 and S4). In short, worker survival depended on food collection, while soldiers survival was mainly affected by the number of workers remaining in the colony to feed them (Fig. 2, $R^2$ for the soldiers' surface regression were very low, contrary to workers', SI, tables S5 to S10).

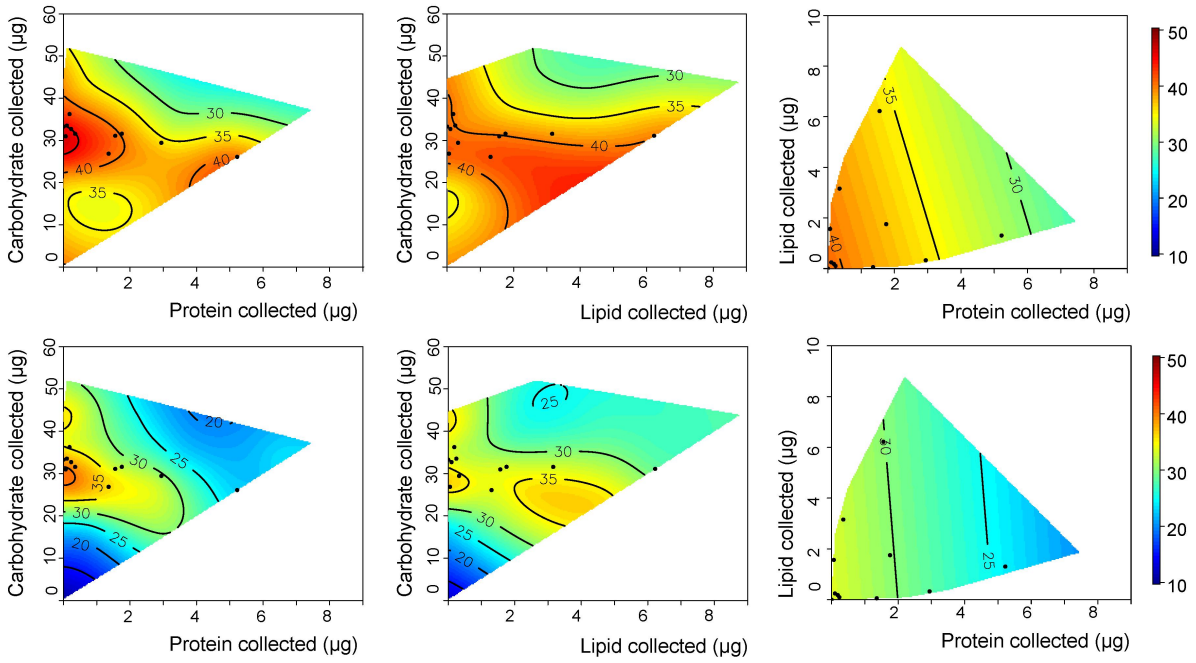


Fig. 2: Effects of nutrient collection on termite survival. Daily consumption per individual (µg) was recorded for each experimental colonies of termites (70 workers and 30 soldiers in each colony) confined for the whole duration of the experiment to one of 11 diets varying in macronutrient content and composition. Response surfaces were visualized using the function *tps* in the package fields in the statistical software R. Red indicates the highest values for the lifespan, while blue regions are associated with the lowest values. Black circles indicate the mean intake per individual on each of the diet. (a) Effects of nutrient intake on workers survival (Mean lifespan for each experimental colony). (b) Effects of nutrient intake on soldiers survival (Mean lifespan for each experimental colony). We adjusted intake to the number of termites still alive in each colony, to take into account differences in mortality between colonies. Tested from left to right as a function of protein and carbohydrate, lipid and carbohydrate, and protein and lipid. Adjusted $R^2$ of surface regression of lifespan as a function of nutrient collected are respectively 0.67, 0.64 and 0.35 for panel a), and 0.08, 0.1 and 0.1 for panel b) (ESM tables S5 to S10).

## *c) Effect of macronutrient collection on tunnelling activity*

We also measured how tunnelling activity was affected by food collection (Fig. 3). The tunnel lengths were positively correlated with carbohydrate ($P<0.001$, $t=8.97$, $DF=554$, SI, table 11) and protein collection ($P<0.001$, $t=4.87$, $DF=554$, SI, table 11), but not with lipid collection ($P=0.066$, $t=1.84$, $DF=554$, SI, table 11).

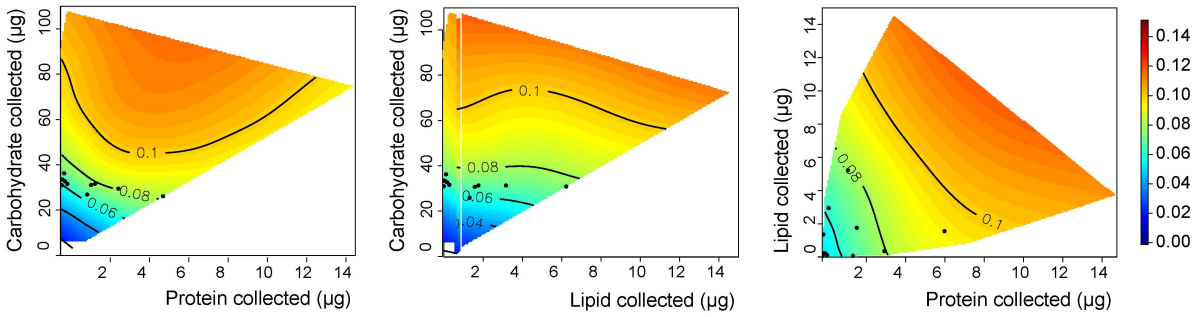


Fig. 3: Effects of nutrient collection on tunnelling activity. Daily consumption per individual (µg) was recorded for each experimental colonies of termites (70 workers and 30 soldiers in each colony) confined for the whole duration of the experiment to one of 11 diets varying in macronutrient content and composition. Response surfaces were visualized using the function *tps* in the package fields in the statistical software R. Red indicates the highest values for tunnelling activity, while blue regions are associated with the lowest values. The tunnel length was measured for each colony every six days until the end of the experiment, and associated with the consumption of food over those 6 days (N=562). We adjusted intake to the number of termites still alive, and tunnelling activity (cm dug per day) to the number of workers still alive in each colony (as soldiers do not dig), to take into account differences in mortality between colonies. Adjusted $R^2$ of surface regression of tunnelling as a function of nutrient collected are respectively 0.3, 0.28 and 0.11 (ESM, tables S12 to S14).

## *d) Effect of macronutrient collection on body composition*

We then examined the effect of macronutrient collection on lipid and protein body contents. Body lipid content was very low in workers (mean lipid proportion ± 95% CI = 0.73 ± 0.13), while it was relatively high in soldiers (20.9 ± 1.19) (Fig. 4a). However, when the soldier bodies were analysed without their heads, their lipid content was comparable to the ones of full worker bodies. Thus, heads alone accounted for the higher lipid content of soldier

bodies (Fig. 4b). Protein content was also higher in soldiers than in workers (mean protein proportion +- 95% C = 82.5+-3.2 vs 62.7 +-3.6, respectively).

In workers, both lipid and protein body content decreased with time (P=0.002, t=-3.12, DF=452, SI, table S15; P<0.001, t=8.01, DF=452, SI, table S16 respectively) meaning that workers became leaner during the experiment. This effect was not observed in soldiers (P=0.098, t=1.65, DF=246, SI, table S17; P=0.155, t=-1.42, DF=246, SI, table S18 respectively).

Body composition was slightly affected by macronutrient collection in workers but not in soldiers (Fig. S2 and S3, SI, tables S15 to 18). Lipid content increased with lipid collection (P= 0.037, t=2.09, DF=452, SI, table S15) and protein content increased with protein collection (P<0.001, t=3.9, DF=452, SI, table S16).

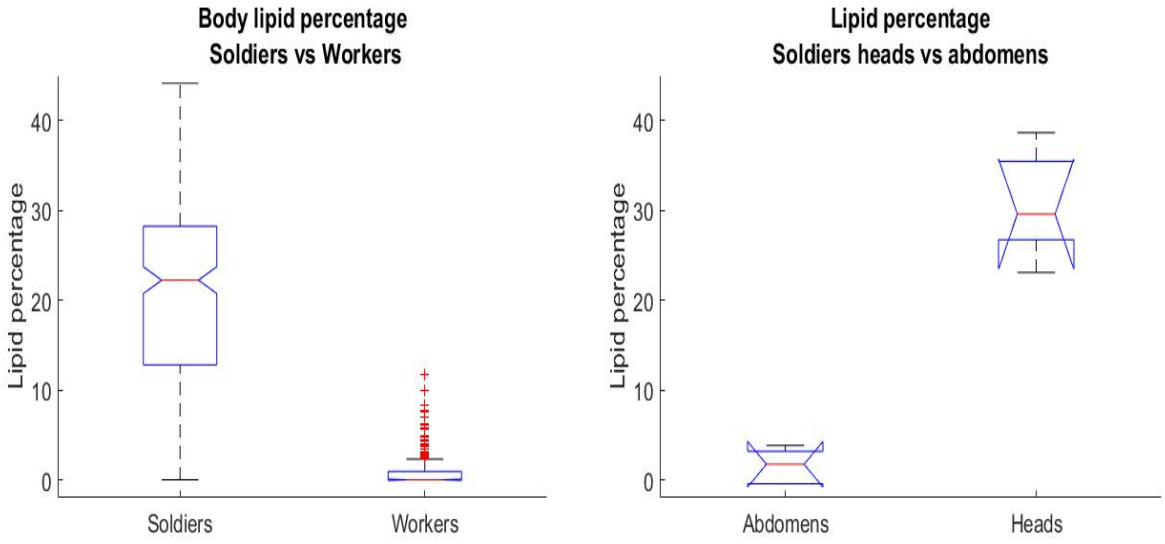


Fig. 4: Body composition of termites. Notched boxplot of the body lipid percentage of a) dead soldiers (N=264 groups of 5) and dead workers (N=469 groups of 5) from colonies confined from the start of the experiment until their natural death to one of 11 diets varying in macronutrient content and composition, and b) lipid percentage in the heads and the abdomens of soldiers sacrificed before starting the experiment (N=5 groups of 5). The red central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

# 4. Discussion

Nutritional ecology theory (Raubenheimer et al., 2009; Simpson and Raubenheimer, 2012) predicts that there would be no need for organisms to evolve (or retain) mechanisms to regulate their intake of separate nutrients (*e.g.* macronutrients) in the case of extreme food specialisation. All that would be required to ensure nutritional balance would be to control the volume eaten (or collected in the case of a social forager). In this study, we have demonstrated that, in agreement with these predictions, termites kept food collection constant across a wide range of P: C: L ratios. Contrary to termites, other social insects such as ants have a pronounced ability to compensate for carbohydrate deficiency or amino acids excess by adjusting the amount of food collected as a function of the imbalance. For example, they collect more food on low carbohydrate diet (Cook et al., 2010; Dussutour and Simpson, 2009), and less food on high amino acids diet (Arganda et al., 2017). The species of ants used in these studies were generalists from a nutritional point of view, while *Nasutitermes exitiosus* is a dietary specialist.

Nutrient regulation has been shown to differ between specialist and generalist feeders in non-social insects such as caterpillars and locusts (Lee *et al.* 2004, 2006; Raubenheimer and Simpson 2003; Simpson *et al.* 2002, reviewed in (Behmer, 2009)). Specialists experience a lower range of food compositions and lower nutritional variability than a generalist and are typically less likely to overconsume nutritionally imbalanced foods than are generalists. It has been hypothesised that generalists effectively mortgage the short-term costs of ingesting excess nutrients on an imbalanced food against the higher likelihood that they will encounter a nutritionally complementary food in the future, thereby balancing the previous excess (Simpson and Raubenheimer, 2012). However, none of the specialist species that have been studied to date is as limited in the range of foods eaten as are termites, and they have all evolved the capacity to regulate both protein and carbohydrate intake (reviewed in (Simpson and Raubenheimer, 2012). Here, we are

proposing that termites, because of their extreme specialism, have lacked the need for fine macronutrient regulatory mechanisms and hence do not respond to variation in the macronutrient content of their food. We postulate that they might have lost this regulatory ability secondarily, as they evolved from generalist cockroach like ancestors (Hunt and Nalepa, 1994), and cockroaches are known to regulate their intake of macronutrient accurately (Jones and Raubenheimer, 2001; Raubenheimer and Jones, 2006). An additional reason why termites may not have developed the ability to increase consumption in the face of nutritional imbalance in food is the physically challenging nature of their food, where the energy lost in processing more food might outweigh the gain of nutrients (Hunt and Nalepa, 1994). The impact of diet hardness is striking in *Blatella germanica*, where the growth of individuals raised on hard diets is delayed by 43% compared to those fed a crushed diet (Cooper and Schal, 1992).

Intriguingly, although termites are extreme food specialists when it comes to sources of macronutrients, several studies suggest that they gain their micronutrients from the soil (Janzow et al., 2015; Seymour et al., 2014). Unlike wood, soil composition in micronutrients is variable, and the correlation between individual elements is low (Heuvelink and Webster, 2001; Yavitt et al., 2009) – conditions which are hypothesised to lead to the evolution of regulatory feeding abilities. Indeed, previous studies from Judd *et al.* (Judd et al., 2017) and Botch *et al.* (Botch et al., 2010) have provided evidence of such an active regulation of micronutrients by termites. Other insects have been shown to balance their intake of minerals orthogonally to macronutrients by food selection (Simpson and Raubenheimer, 2015; Trumper and Simpson, 1993).

Because it is predicted that extreme specialists will not actively regulate their intake of macronutrients, it follows that their performance (*e.g.* life-history responses) will be sensitive to experimentally imposed changes in food macronutrient composition. Termites were indeed strongly affected by such a variation in the present study. The key determinant of the relationship between diet and longevity in termites was the quantity of carbohydrate and protein collected, and to a far lesser extent to the quantity of lipid collected. Termite workers

survived best when they collected a daily amount of carbohydrate comprised between 0.02 and 0.04mg of per individual and a low quantity of protein (0-0.001mg) and lipid (0-0.003mg). This optimum in term of lifespan is relatively narrow in comparison to other insects studied (Arganda et al., 2017; Dussutour and Simpson, 2012; Lee et al., 2008; Maklakov et al., 2008).

That the optimal macronutrient balance was carbohydrate biased reflects the nutritional composition of wood. Termites have evolved to rely on their gut bacteria to provide the necessary nitrogen from their low-protein food (review in (Hongoh, 2011)) and can even survive on a pure cellulose diet. For example, *Reticulitermes flavipes* lived for more than 4 months on cellulose (Cleveland, 1923). This ability relies on their association with a large community of gut symbionts, which help break down plant tissues. In addition, termites rely on the biosynthetic capacities of these symbionts as a nutritional resource. For example, wood-feeding termites such as *N. exitiosus*, can take up nitrogen from the atmosphere with the aid of N2-fixing gut bacteria to balance the low nitrogen content in their food (Hongoh, 2011). In our experiment termites could also survive on pure cellulose for a certain time but survived better on *Eucalyptus* wood, their natural diet (Fig. 5). A similar difference between wood and cellulose substrate was also recorded in *Coptotermes formosanus* (Su et al., 1985). The lower survival on cellulose and other artificial diets as well suggests that termites lacked some essential nutrients that can be found in *Eucalyptus* wood such as additional sugars (xylose, mannose, galactose, rhamnose, and arabinose), proteins, lipids as well as micronutrients (Evtuguin and Neto, 2007) which might not be provided by gut symbionts.

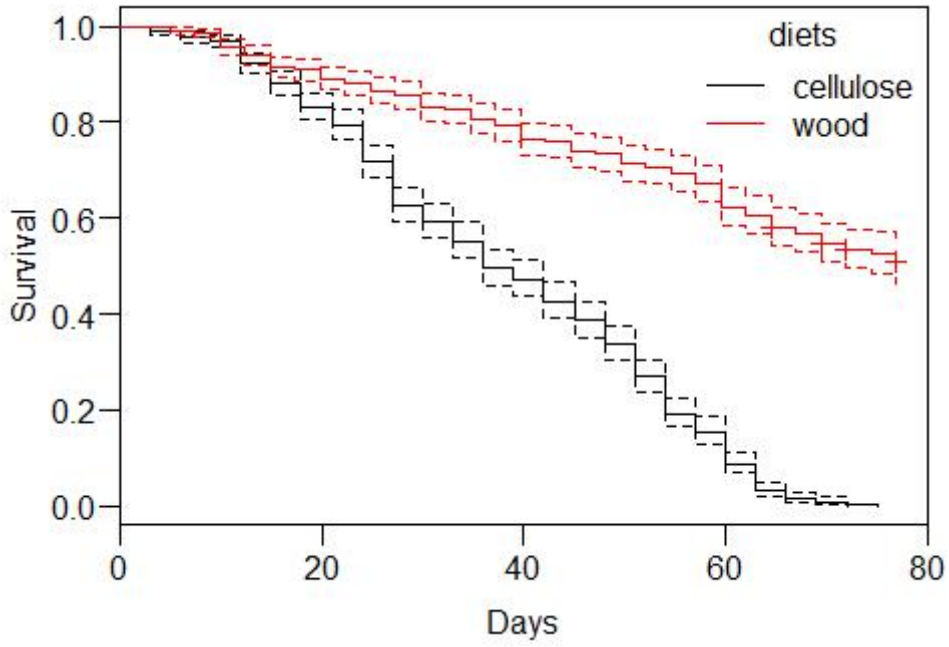


Fig. 5: Survival curves of workers kept on a wood diet vs a pure cellulose diet. The dashed lines are the 95% intervals.

In our experiments, using artificial diets varying in P: C: L, lifespan was reduced when there was a lack or excess of carbohydrates and/or an elevated intake of protein. High protein intake and carbohydrate excess have been shown to shorten lifespan in many animals from insects to mammals due to protein toxicity and various metabolic disorders (*e.g.* in ants (Arganda et al., 2017; Dussutour and Simpson, 2012), Drosophila (Lee et al., 2008), mice (Solon-Biet et al., 2014)). Interestingly, the survival of soldiers depended mostly on worker survival and was only marginally affected by macronutrient collection. Workers and soldiers have distinct eating habits, and the results obtained here may reflect this difference. Soldiers are unable to feed themselves and receive trophallaxis from workers (Grassé, 1984). Thus, our results suggest that secondary feeding by trophallaxis appears to protect the soldiers from the detrimental effects of nutritional imbalance. Soldiers represent a strong investment for the colony for several reasons: 1) they must be nutritionally supported, 2) they provide no energetic input into colony growth and 3) they are the sink of most energy brought back to the nest, as suggested by their high content in lipid and protein. However, these energy draining, sterile, and dependant individuals defend the colony against predators and

they require energy to synthesize the sticky terpenoids secretions they store in their head capsule as a defence mechanism (Prestwich, G and Collins, 1981; Prestwich, 1979). We found that lipid content in soldiers was indeed mostly localised in the head capsule. Thus, our results suggest that the colony, to protect its investment, may overcome the deleterious effects of macronutrient imbalance in soldiers by getting the workers to process the diet for them. This type of communal nutrition has been observed in ants where workers avoid the negative effects of macronutrient excess by getting the larvae to digest them (Dussutour and Simpson, 2009).

Food intake per se is only part of the complex and fully integrated feeding behaviour of termites. Foraging is an important component related to food intake that can also be regulated. Termites such as *N.exitiosus* explore and exploit their environment by tunnelling through soil and wood. Total tunnel length therefore gives an indication of general foraging activity (Mackay et al., 1985). Digging tunnels is an activity that is energetically costly (Mackay et al., 1985). Tunnelling is related to food discovery (Campora and Grace, 2004) and is stimulated under food deprivation (Gallagher and Jones, 2005; Hedlund and Henderson, 1999). Interestingly, in our experiment, where termites had unlimited access to food, tunnelling activity increased with carbohydrate and protein collection. The most likely explanation is that termites increased their foraging activity to maximize exploration and therefore chances of finding a better food. Elevated foraging activity under adverse nutritional conditions, such as food deprivation or confinement to imbalanced food, has been reported in many animals (*e.g.* in rodents (Pirke et al., 1993); in flies (Fanson et al., 2013; Isabel et al., 2005; Knoppien et al., 2000; Lee and Park, 2004; Meunier et al., 2007; Yang et al., 2015); in ants (Dussutour and Simpson, 2012; Dussutour et al., 2016)) and has been suggested to be the best way to find scarce or complementary food. An alternative or additional explanation could be that termites increased their foraging activity as a means to burn off excess nutrients (protein or carbohydrate) to rebalance their nutrient intake. By keeping their food collection constant on all diets, termites on imbalanced diets acquire certain nutrients in excess while other nutrients remain limiting. Were they to selectively metabolise the excess

nutrient to fuel increased tunnelling, they could rebalance the ratio of macronutrients post-ingestively (*e.g.* (Clissold et al., 2010; Woodring et al., 2009; Zanotto et al., 1993)).

In summary, our experiment showed that termites did not actively compensate for experimentally-imposed changes in the macronutrient content of foods by adjusting food intake, nor did they avoid the detrimental effect of macronutrient imbalances. We postulate that in species such as termites with highly specialised diets, the need to finely regulate macronutrient intakes is lost: regulating the amount of food ingested alone will be sufficient to attain nutrient balance when the composition of foods is invariant. An additional capacity to rely upon communities of gut symbionts has allowed termites to exploit wood, an otherwise nutritionally unpromising substrate.

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## Contribution

- L.A.P. conceived and performed all the experiments and statistical analyses and wrote the first draft of the manuscript
- S.A. assisted with the statistical analyses
- A.D. and J.B. co-supervised the work of L.A.P., and designed the study
- J.B. funded the experiments
- All authors contributed critically to the drafts and gave final approval for publication.

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# Supporting information

## Details on the statistics

All generalized linear mixed models (GLMM) were done in Matlab (using the function fitglme, with Distribution = normal, link function= identity), with colony as a random factor. All surface regression are done in Matlab (using the function fitlme), with colony as a random factor. All consumption data used in the models were standardized ((value-mean)/standard deviation). This procedure reduces the covariation between linear variables and their interaction terms (Aiken 1991). Except in table S1 where we only consider the global intake of food and are not interested in the effect of each macronutrient. Carbohydrate, protein and lipid are abbreviated C, P and L. P-values <0.05 are in bold.

## 1) Intake regulation

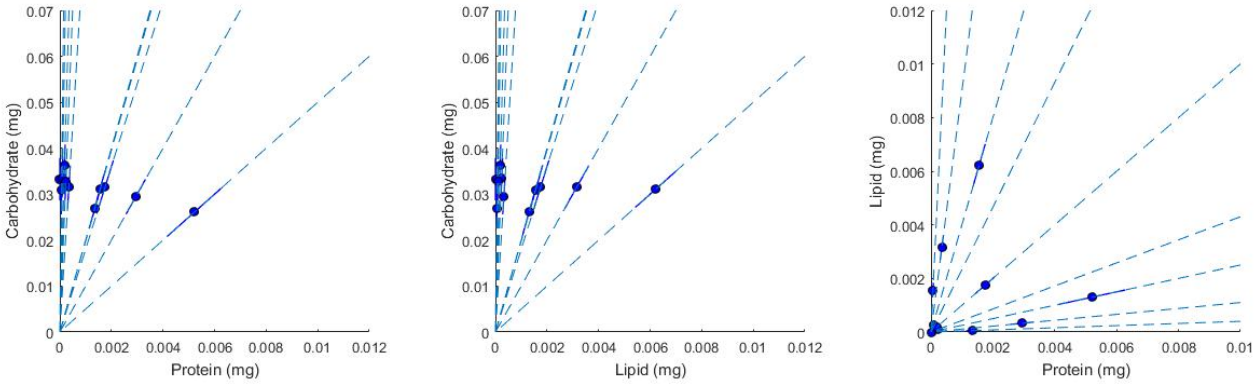



Fig. S1: The average daily intake (+- 95% CI) of protein, carbohydrate and lipid of experimental colonies of 70 workers + 30 soldiers for each of the 11 diets (76 experimental colonies in total, 7 per diet, except for diet S2, where 6 colonies were used – N ranges from 86 to 144 data points per diet).

**Table S1**: Kruskal-Wallis ANOVA Table of intake as a function of diet (per individual to feed in the nest)

| Source | SS | DF | MS | Chi-sq | Prob>Chi-sq |
|---|---|---|---|---|---|
| Groups | 1.77128E+06 | 10 | 177127.6 | 14.14 | 0.1667 |
| Error | 1.51675E+08 | 1215 | 124835.6 | | |
| Total | 1.53447E+08 | 1215 | | | |

# 2) Effects of macronutrient collection on lifespan

**Table S2**: GLMM of Worker Lifespan as a function of macronutrient daily average consumption per nest (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| **'(Intercept)'** | 31.6244 | 3.8234 | 8.2713 | 68 | **7.05E-12** | 23.9949 | 39.2538 |
| **L** | 0.0642 | 0.8109 | 0.0792 | 68 | 0.9371 | -1.5539 | 1.6823 |
| **C** | 4.0722 | 0.8617 | 4.7259 | 68 | **1.19E-05** | 2.3527 | 5.7917 |
| **P** | -2.4343 | 0.7613 | -3.1978 | 68 | **0.0021** | -3.9534 | -0.9153 |
| **L:C** | -2.7585 | 0.8449 | -3.2649 | 68 | **0.0017** | -4.4445 | -1.0725 |
| **L:P** | 0.5344 | 2.1302 | 0.2509 | 68 | 0.8027 | -3.7162 | 4.7851 |
| **C:P** | -1.7413 | 1.2311 | -1.4144 | 68 | 0.1618 | -4.1979 | 0.7154 |
| **L:C:P** | 3.6747 | 1.9911 | 1.8456 | 68 | 0.0693 | -0.2984 | 7.6478 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 512.18 | 535.49 | -246.09 | 492.18 | 0.73 | 0.702 |

Random effects covariance parameters:

Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 7.5017 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 5.6233 |

**Table S3**: GLMM of Soldier Lifespan as a function of macronutrient daily average consumption per nest (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| **'(Intercept)'** | 38.2154 | 2.252 | 16.9699 | 68 | **3.85E-26** | 33.7217 | 42.7091 |
| **L** | 1.2863 | 1.4471 | 0.8888 | 68 | 0.3772 | -1.6014 | 4.1739 |
| **C** | -0.5334 | 1.4855 | -0.3591 | 68 | 0.7206 | -3.4977 | 2.4309 |
| **P** | -1.6357 | 1.3521 | -1.2098 | 68 | 0.2306 | -4.3339 | 1.0624 |
| **L:C** | -4.0146 | 1.5039 | -2.6695 | 68 | **0.0095** | -7.0156 | -1.0136 |
| **L:P** | 7.1757 | 3.7848 | 1.8959 | 68 | 0.0622 | -0.3767 | 14.728 |
| **C:P** | -3.7405 | 2.178 | -1.7174 | 68 | 0.0905 | -8.0867 | 0.6056 |
| **L:C:P** | -1.9598 | 3.5074 | -0.5588 | 68 | 0.5782 | -8.9587 | 5.039 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 591.3 | 614.61 | -285.65 | 571.3 | 0.278 | 0.204 |

Random effects covariance parameters:

Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 3.6857 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 10.042 |

**Table S4**: GLMM of Soldier Lifespan as a function of macronutrient daily average consumption per nest + WorkerLifespan (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| **'(Intercept)'** | 17.2757 | 3.7119 | 4.6541 | 67 | **1.59E-05** | 9.8666 | 24.6847 |
| **Worker Lifespan** | 0.6631 | 0.1116 | 5.9398 | 67 | **1.13E-07** | 0.4403 | 0.886 |
| **L** | 1.2053 | 1.2647 | 0.9531 | 67 | 0.344 | -1.319 | 3.7297 |
| **C** | -2.1793 | 1.1819 | -1.8438 | 67 | 0.0696 | -4.5385 | 0.1799 |
| **P** | 0.2132 | 1.2307 | 0.1732 | 67 | 0.863 | -2.2433 | 2.6697 |
| **L:C** | -2.0112 | 1.3661 | -1.4722 | 67 | 0.1456 | -4.7381 | 0.7156 |
| **L:P** | 6.9865 | 3.2846 | 2.1271 | 67 | **0.0371** | 0.4305 | 13.5425 |
| **C:P** | -2.5495 | 1.8855 | -1.3522 | 67 | 0.1809 | -6.313 | 1.214 |
| **L:C:P** | -5.059 | 3.0795 | -1.6428 | 67 | 0.1051 | -11.2057 | 1.0877 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 568.08 | 593.72 | -273.04 | 546.08 | 0.456 | 0.391 |

Random effects covariance parameters:

Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.0012432 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 8.7906 |

**Table S5**: Surface regression of Worker Lifespan as a function of P and C eaten (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 2.5677 | 6.039 | 0.4252 | 70 | 0.672 | -9.4767 | 14.6121 |
| C | 1.49E+03 | 340.4828 | 4.3662 | 70 | **4.28E-05** | 807.5324 | 2.17E+03 |
| P | 2.19E+03 | 2.15E+03 | 1.02 | 70 | 0.3112 | -2.09E+03 | 6.48E+03 |
| C:P | -1.49E+05 | 6.35E+04 | -2.3418 | 70 | **0.022** | -2.75E+05 | -2.21E+04 |
| C^2 | -1.41E+04 | 5.98E+03 | -2.3603 | 70 | **0.0211** | -2.61E+04 | -2.19E+03 |
| P^2 | 1.80E+05 | 1.81E+05 | 0.9904 | 70 | 0.3254 | -1.82E+05 | 5.42E+05 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 516.18 | 534.82 | -250.09 | 500.18 | 0.697 | 0.675 |

Random effects covariance parameters (95% CIs):
Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 7.3674 | 3.5816 | 15.155 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 5.9495 | 5.0528 | 7.0052 |

**Table S6**: Surface regression of Worker Lifespan as a function of L and C eaten (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 7.6852 | 6.3502 | 1.2102 | 70 | 0.2303 | -4.9798 | 20.3503 |
| C | 877.5212 | 375.4733 | 2.3371 | 70 | **2.23E-02** | 128.6633 | 1.63E+03 |
| L | 3.38E+03 | 1.78E+03 | 1.898 | 70 | 0.0618 | -1.72E+02 | 6.93E+03 |
| C:L | -1.85E+05 | 5.72E+04 | -3.2418 | 70 | **0.0018** | -2.99E+05 | -7.13E+04 |
| C^2 | -1.77E+03 | 6.91E+03 | -0.256 | 70 | 0.7987 | -1.55E+04 | 1.20E+04 |
| L^2 | 4.02E+05 | 1.64E+05 | 2.452 | 70 | 0.0167 | 7.50E+04 | 7.29E+05 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 523.15 | 541.8 | -253.58 | 507.15 | 0.668 | 0.644 |

Random effects covariance parameters (95% CIs):
Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 8.004 | 3.8995 | 16.429 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 6.2165 | 5.2796 | 7.3196 |

**Table S7**: Surface regression of Worker Lifespan as a function of P and L eaten (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 33.4829 | 3.2766 | 10.2188 | 70 | **1.61E-15** | 26.9479 | 40.0178 |
| P | -2.73E+03 | 1.60E+03 | -1.7057 | 70 | 0.0925 | -5.93E+03 | 462.864 |
| L | -14.5135 | 1.35E+03 | -0.0108 | 70 | 0.9914 | -2.70E+03 | 2.67E+03 |
| P:L | 8.73E+04 | 1.03E+06 | 0.0846 | 70 | 0.9328 | -1.97E+06 | 2.15E+06 |
| P^2 | 1.97E+05 | 3.38E+05 | 0.5824 | 70 | 0.5622 | -4.78E+05 | 8.71E+05 |
| L^2 | 2.10E+04 | 3.09E+05 | 0.0679 | 70 | 0.9461 | -5.96E+05 | 6.38E+05 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 561.7 | 580.35 | -272.85 | 545.7 | 0.398 | 0.355 |

Random effects covariance parameters (95% CIs):

Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 5.7211 | 2.6724 | 12.248 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 8.2594 | 7.0161 | 9.7231 |

**Table S8**: Surface regression of Soldier Lifespan as a function of P and C eaten (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 27.0053 | 8.7386 | 3.0903 | 70 | **2.90E-03** | 9.5767 | 44.434 |
| C | 969.5868 | 600.1093 | 1.6157 | 70 | 0.1107 | -227.2934 | 2.17E+03 |
| P | 2.12E+03 | 3.92E+03 | 0.5416 | 70 | 0.5898 | -5.69E+03 | 9.93E+03 |
| C:P | -1.91E+05 | 1.16E+05 | -1.6559 | 70 | 0.1022 | -4.22E+05 | 3.91E+04 |
| C^2 | -1.48E+04 | 1.02E+04 | -1.4535 | 70 | 0.1505 | -3.52E+04 | 5.52E+03 |
| P^2 | 3.86E+05 | 3.36E+05 | 1.1486 | 70 | 0.2546 | -2.85E+05 | 1.06E+06 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 596.77 | 615.42 | -290.39 | 580.77 | 0.141 | 0.08 |

Random effects covariance parameters (95% CIs):

Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 2.4524e-15 | NaN | NaN |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 11.045 | 9.4212 | 12.948 |

**Table S9**: Surface regression of Soldier Lifespan as a function of L and C eaten (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 33.2936 | 8.5365 | 3.9001 | 70 | 2.18E-04 | 16.268 | 50.3192 |
| C | -60.8921 | 621.9755 | -0.0979 | 70 | 0.9223 | -1.30E+03 | 1.18E+03 |
| L | 6.03E+03 | 2.99E+03 | 2.0197 | 70 | **0.0472** | 7.54E+01 | 1.20E+04 |
| C:L | -2.69E+05 | 9.57E+04 | -2.8076 | 70 | **0.0065** | -4.60E+05 | -7.78E+04 |
| C^2 | 7.94E+03 | 1.14E+04 | 0.6993 | 70 | 0.4867 | -1.47E+04 | 3.06E+04 |
| L^2 | 4.60E+05 | 2.76E+05 | 1.67 | 70 | 0.0994 | -8.94E+04 | 1.01E+06 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 594.41 | 613.06 | -289.21 | 578.41 | 0.21 | 0.099 |

Random effects covariance parameters (95% CIs):

Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 4.334 | 1.6735 | 11.224 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 10.475 | 8.8945 | 12.337 |

**Table S10**: Surface regression of Soldier Lifespan as a function of P and L eaten (N=76 experimental colonies).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 41.1392 | 2.7606 | 14.902 | 70 | **2.10E-23** | 35.6332 | 46.6451 |
| P | -3.11E+03 | 2.10E+03 | -1.4825 | 70 | 0.1427 | -7.29E+03 | 1.07E+03 |
| L | -22.7594 | 1.76E+03 | -0.0129 | 70 | 0.9897 | -3.54E+03 | 3.49E+03 |
| P:L | 9.61E+05 | 1.35E+06 | 0.7133 | 70 | 0.478 | -1.73E+06 | 3.65E+06 |
| P^2 | 3.63E+04 | 4.42E+05 | 0.0822 | 70 | 0.9347 | -8.45E+05 | 9.18E+05 |
| L^2 | -2.37E+05 | 4.04E+05 | -0.5862 | 70 | 0.5596 | -1.04E+06 | 5.69E+05 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 597.92 | 616.56 | -290.96 | 581.92 | 0.159 | 0.099 |

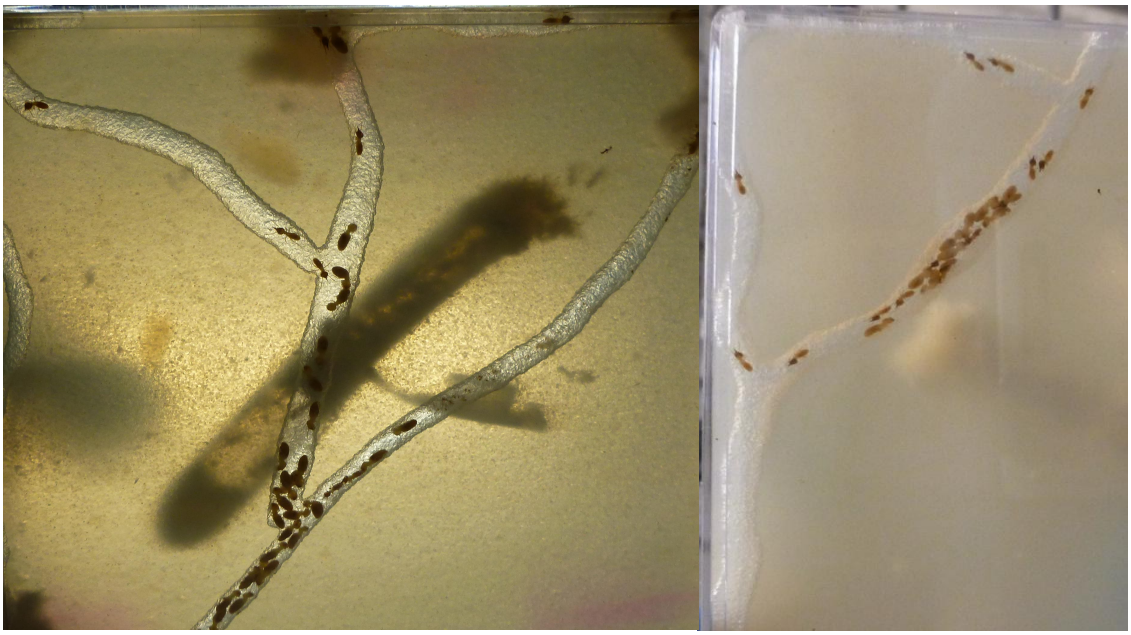
Random effects covariance parameters (95% CIs):
Group: colony (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 3.6382 | 1.3559 | 9.762 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 10.802 | 9.1786 | 12.713 |

# 3) Effects of macronutrient collection on digging activity



Picture S1: Example of the galleries built in the agar nest

**Table S11**: GLMM of gallery length (cm/worker) vs macronutrient average consumption over 6 days per nest until the end of the experiment (N=562).

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| **'(Intercept)'** | 0.3864 | 0.0305 | 12.6623 | 554 | 1.88E-32 | 0.3265 | 0.4464 |
| **C** | 0.1283 | 0.0143 | 8.9677 | 554 | **4.65E-18** | 0.1002 | 0.1564 |
| **P** | 0.0777 | 0.016 | 4.8685 | 554 | **1.47E-06** | 0.0463 | 0.109 |
| **L** | 0.0323 | 0.0175 | 1.8431 | 554 | 0.0658 | -0.0021 | 0.0668 |
| **C:P** | -0.0461 | 0.0158 | -2.9071 | 554 | **0.0038** | -0.0772 | -0.0149 |
| **C:L** | -0.0295 | 0.0146 | -2.0186 | 554 | **0.044** | -0.0581 | -7.93E-04 |
| **P:L** | -0.0018 | 0.0337 | -0.0545 | 554 | 0.9566 | -0.0681 | 0.0644 |
| **C:P:L** | -0.0028 | 0.0203 | -0.1361 | 554 | 0.8918 | -0.0427 | 0.0372 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 167.39 | 210.71 | -73.697 | 147.39 | 0.295 | 0.286 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.053849 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 0.2741 |

**Table S12**: Surface regression of gallery length (cm/worker) as a function of P and C collected per individuals (N=562)

| | Estimate | SE | tStat | DF | pValue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 0.4191 | 0.0317 | 13.2181 | 556 | 7.01E-35 | 0.3568 | 0.4813 |
| C | 0.1674 | 0.0148 | 11.3184 | 556 | 7.18E-27 | 0.1383 | 0.1964 |
| P | 0.0933 | 0.02 | 4.6596 | 556 | 3.97E-06 | 0.054 | 0.1327 |
| C:P | -0.0196 | 0.0177 | -1.1046 | 556 | 0.2698 | -0.0544 | 0.0152 |
| C^2 | -0.0359 | 0.0097 | -3.7189 | 556 | 2.20E-04 | -0.0549 | -0.017 |
| P^2 | -0.0132 | 0.0065 | -2.0259 | 556 | 0.0433 | -0.0261 | -4.03E-04 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 150.83 | 185.48 | -67.415 | 134.83 | 0.311 | 0.305 |

Random effects covariance parameters (95% CIs):

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.055056 | 0.023925 | 0.1267 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 0.271 | 0.25556 | 0.28738 |

**Table S13**: Surface regression of gallery length (cm/worker) as a function of L and C eaten (N=562)

| | Estimate | SE | tStat | DF | pValue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 0.4062 | 0.0335 | 12.1278 | 556 | 3.38E-30 | 0.3404 | 0.472 |
| C | 0.1758 | 0.0154 | 11.4055 | 556 | 3.20E-27 | 0.1455 | 0.206 |
| L | 0.006 | 0.0233 | 0.2588 | 556 | 0.7958 | -0.0398 | 0.0519 |
| C:L | -0.037 | 0.0169 | -2.1956 | 556 | 0.0285 | -0.0702 | -0.0039 |
| C^2 | -0.0415 | 0.0101 | -4.1033 | 556 | 4.68E-05 | -0.0614 | -0.0216 |
| L^2 | 0.0112 | 0.0084 | 1.3264 | 556 | 0.1853 | -0.0054 | 0.0277 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 168.71 | 203.37 | -76.357 | 152.71 | 0.289 | 0.282 |

Random effects covariance parameters (95% CIs):

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.057841 | 0.02532 | 0.13214 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 0.27529 | 0.2596 | 0.29193 |

**Table S14**: Surface regression of gallery length (cm/worker) as a function of P and L eaten (N=562)

| | Estimate | SE | tStat | DF | pValue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 0.3923 | 0.0319 | 12.2859 | 556 | 7.31E-31 | 0.3296 | 0.4551 |
| P | 0.1213 | 0.0253 | 4.796 | 556 | 2.08E-06 | 0.0716 | 0.1709 |
| L | 0.0363 | 0.0279 | 1.302 | 556 | 0.1935 | -0.0185 | 0.0912 |
| P:L | -0.0415 | 0.0364 | -1.1423 | 556 | 0.2538 | -0.1129 | 0.0299 |
| P^2 | -0.0128 | 0.0088 | -1.4446 | 556 | 0.1491 | -0.0301 | 0.0046 |
| L^2 | 0.0054 | 0.0129 | 0.4216 | 556 | 0.6735 | -0.0198 | 0.0307 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 285.05 | 319.7 | -134.52 | 269.05 | 0.122 | 0.114 |

Random effects covariance parameters (95% CIs):

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate | Lower | Upper |
|---|---|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.051435 | 0.022252 | 0.11889 |

Group: Error

| Name | Estimate | Lower | Upper |
|---|---|---|---|
| 'Res Std' | 0.3057 | 0.28829 | 0.32417 |

# 4) Effect of macronutrient collection on body composition

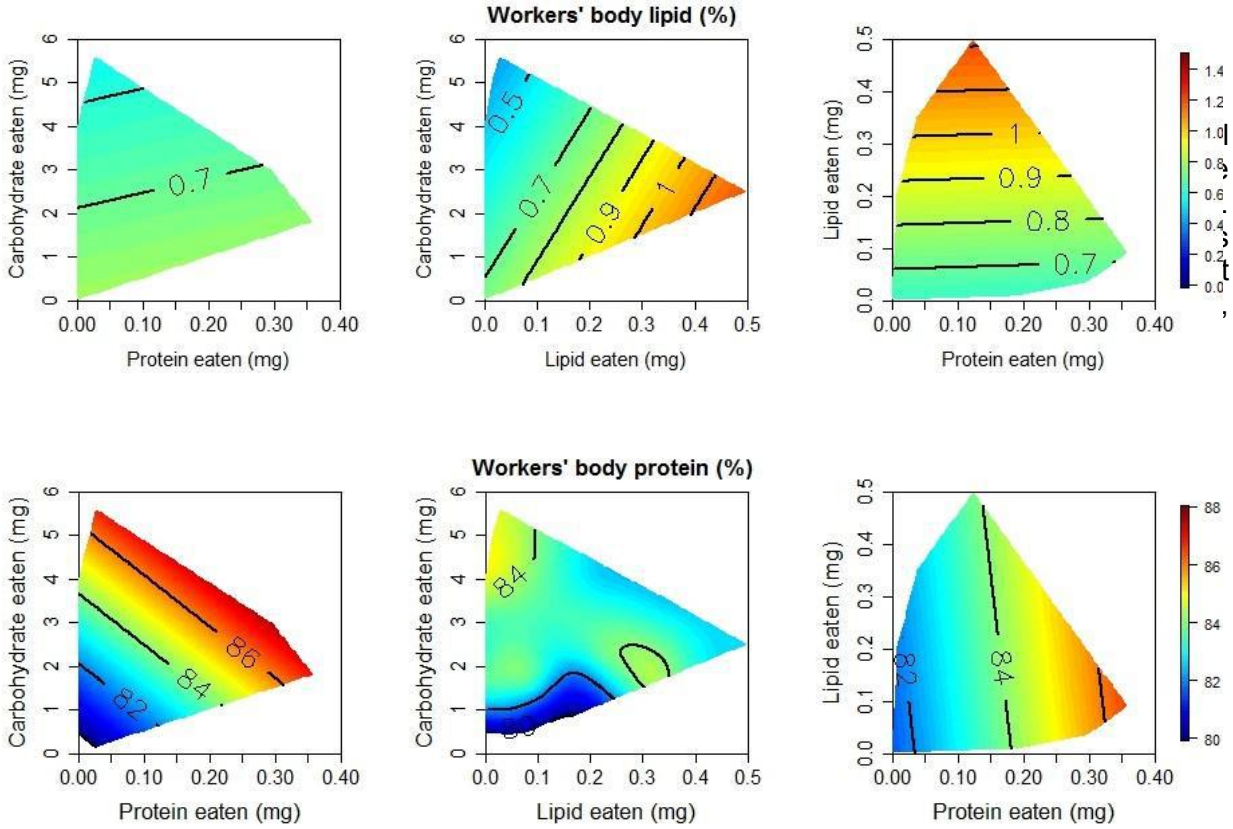



Fig S3: Effect of nutrient collection on body protein content. Daily consumption per individual (mg) was recorded on 469 group of 5 workers that died after being confined for the whole duration of the experiment to 10 diets varying in macronutrient content and composition. Response surfaces were visualized using fields in the statistical software R. Red indicates the highest values for tunnelling activity, while blue regions are associated with the lowest values. Adjusted $R^2$ of surface regression are respectively 0.095, 0.06 and 0.033 (ESM, tables S22 to S24).

**Table S15**: GLMM of worker body lipid proportion as a function of C, P, L intake and time (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| '(Intercept)' | 1.2976 | 0.223 | 5.8193 | 452 | **1.12E-08** | 0.8594 | 1.7358 |
| time | -0.0212 | 0.0068 | -3.1174 | 452 | **0.0019** | -0.0346 | -0.0078 |
| C | -0.5297 | 0.2994 | -1.7694 | 452 | 0.0775 | -1.118 | 0.0586 |
| P | -0.2332 | 0.2419 | -0.9642 | 452 | 0.3355 | -0.7086 | 0.2421 |
| L | 0.5596 | 0.2677 | 2.0904 | 452 | **0.0371** | 0.0335 | 1.0856 |
| time:C | 0.0189 | 0.0071 | 2.6557 | 452 | **0.0082** | 0.0049 | 0.033 |
| time:P | 0.0067 | 0.007 | 0.9521 | 452 | 0.3416 | -0.0071 | 0.0205 |
| C:P | -0.796 | 0.4649 | -1.7122 | 452 | 0.0875 | -1.7097 | 0.1176 |
| time:L | -0.0135 | 0.0075 | -1.7977 | 452 | 0.0729 | -0.0283 | 0.0013 |
| C:L | -0.7364 | 0.4341 | -1.6965 | 452 | 0.0905 | -1.5895 | 0.1166 |
| P:L | -0.4733 | 0.5386 | -0.8787 | 452 | 0.3801 | -1.5318 | 0.5853 |
| time:C:P | 0.0169 | 0.0111 | 1.5289 | 452 | 0.127 | -0.0048 | 0.0386 |
| time:C:L | 0.027 | 0.0118 | 2.2786 | 452 | **0.0232** | 0.0037 | 0.0502 |
| time:P:L | 0.0158 | 0.0152 | 1.038 | 452 | 0.2998 | -0.0141 | 0.0458 |
| C:P:L | -1.0752 | 0.6914 | -1.5552 | 452 | 0.1206 | -2.434 | 0.2835 |
| time:C:P:L | 0.0282 | 0.0194 | 1.4491 | 452 | 0.148 | -0.01 | 0.0663 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 1658.6 | 1733.3 | -811.31 | 1622.6 | 0.0527 | 0.0213 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 7.0515e-06 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 1.3698 |

**Table S16**: GLMM of worker body protein proportion as a function of C, P, L intake and time (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| '(Intercept)' | 76.3792 | 1.2109 | 63.0755 | 452 | **3.63E-226** | 73.9995 | 78.7589 |
| time | 0.2081 | 0.026 | 8.0097 | 452 | **9.85E-15** | 0.157 | 0.2591 |
| C | 0.4366 | 1.0153 | 0.43 | 452 | 0.6674 | -1.5587 | 2.4319 |
| P | 3.1276 | 0.8021 | 3.8995 | 452 | **1.11E-04** | 1.5514 | 4.7038 |
| L | 0.4981 | 0.9008 | 0.5529 | 452 | 0.5806 | -1.2722 | 2.2684 |
| time:C | -0.0429 | 0.0238 | -1.8051 | 452 | 0.0717 | -0.0896 | 0.0038 |
| time:P | -0.056 | 0.0234 | -2.3922 | 452 | **0.0172** | -0.102 | -0.01 |
| C:P | 1.5989 | 1.5366 | 1.0406 | 452 | 0.2986 | -1.4208 | 4.6186 |
| time:L | -0.0197 | 0.0252 | -0.7827 | 452 | 0.4342 | -0.0693 | 0.0298 |
| C:L | -0.4181 | 1.4365 | -0.291 | 452 | 0.7712 | -3.2411 | 2.4049 |
| P:L | 1.7368 | 1.7678 | 0.9824 | 452 | 0.3264 | -1.7374 | 5.211 |
| time:C:P | -0.0149 | 0.0364 | -0.4096 | 452 | 0.6823 | -0.0865 | 0.0566 |
| time:C:L | -0.0076 | 0.0391 | -0.1941 | 452 | 0.8462 | -0.0844 | 0.0693 |
| time:P:L | -0.0257 | 0.05 | -0.514 | 452 | 0.6075 | -0.124 | 0.0726 |
| C:P:L | -0.2595 | 2.3159 | -0.112 | 452 | 0.9108 | -4.8108 | 4.2918 |
| time:C:P:L | -0.0077 | 0.0645 | -0.1188 | 452 | 0.9055 | -0.1344 | 0.1191 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 2781.9 | 2856.6 | -1372.9 | 2745.9 | 0.272 | 0.248 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 1.7108 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 4.4926 |

**Table S17**: GLMM of soldier body lipid proportion as a function of C, P, L intake and time (N=264 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| '(Intercept)' | 17.8002 | 4.0005 | 4.4495 | 246 | **1.31E-05** | 9.9206 | 25.6799 |
| time | 0.0871 | 0.0525 | 1.6582 | 246 | 0.0985 | -0.0164 | 0.1906 |
| C | 1.0195 | 2.0108 | 0.507 | 246 | 0.6126 | -2.9411 | 4.98 |
| P | -3.0472 | 1.6754 | -1.8187 | 246 | 0.0702 | -6.3472 | 0.2529 |
| L | -2.3186 | 1.8498 | -1.2534 | 246 | 0.2112 | -5.9621 | 1.3249 |
| time:C | -0.0237 | 0.0499 | -0.4748 | 246 | 0.6353 | -0.122 | 0.0746 |
| time:P | 0.0916 | 0.0494 | 1.8536 | 246 | 0.065 | -0.0057 | 0.1888 |
| C:P | -2.2974 | 2.8302 | -0.8117 | 246 | 0.4177 | -7.8719 | 3.2772 |
| time:L | 0.058 | 0.0518 | 1.1193 | 246 | 0.2641 | -0.044 | 0.1599 |
| C:L | -1.8924 | 2.9203 | -0.648 | 246 | 0.5176 | -7.6444 | 3.8596 |
| P:L | -1.6634 | 3.3179 | -0.5013 | 246 | 0.6166 | -8.1986 | 4.8718 |
| time:C:P | 0.0406 | 0.073 | 0.5562 | 246 | 0.5786 | -0.1031 | 0.1843 |
| time:C:L | 0.0959 | 0.0869 | 1.1036 | 246 | 0.2708 | -0.0752 | 0.267 |
| time:P:L | 0.0147 | 0.0954 | 0.1544 | 246 | 0.8774 | -0.1731 | 0.2026 |
| C:P:L | -5.8193 | 4.8981 | -1.1881 | 246 | 0.2359 | -15.4669 | 3.8283 |
| time:C:P:L | 0.2001 | 0.1447 | 1.3831 | 246 | 0.1679 | -0.0848 | 0.485 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 1772.8 | 1837 | -868.4 | 1736.8 | 0.571 | 0.545 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 7.0267 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 6.4392 |

**Table S18**: GLMM of soldier body protein proportion as a function of C, P, L intake and time (N=264 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| '(Intercept)' | 65.9211 | 3.9839 | 16.547 | 246 | **7.59E-42** | 58.0743 | 73.7679 |
| time | -0.0899 | 0.0632 | -1.4232 | 246 | 0.1559 | -0.2143 | 0.0345 |
| C | -1.3941 | 2.4198 | -0.5761 | 246 | 0.5651 | -6.1602 | 3.3721 |
| P | 2.441 | 2.0174 | 1.2099 | 246 | 0.2275 | -1.5326 | 6.4146 |
| L | 0.9406 | 2.226 | 0.4226 | 246 | 0.673 | -3.4439 | 5.3252 |
| time:C | 0.0479 | 0.0601 | 0.7972 | 246 | 0.4261 | -0.0705 | 0.1663 |
| time:P | -0.0691 | 0.0595 | -1.1621 | 246 | 0.2463 | -0.1863 | 0.048 |
| C:P | 3.8404 | 3.4085 | 1.1267 | 246 | 0.261 | -2.8732 | 10.5539 |
| time:L | -0.013 | 0.0623 | -0.2086 | 246 | 0.8349 | -0.1357 | 0.1097 |
| C:L | 0.2987 | 3.5171 | 0.0849 | 246 | 0.9324 | -6.6286 | 7.2261 |
| P:L | 1.0471 | 3.9959 | 0.262 | 246 | 0.7935 | -6.8234 | 8.9177 |
| time:C:P | -0.0427 | 0.0879 | -0.4855 | 246 | 0.6278 | -0.2158 | 0.1304 |
| time:C:L | -0.0459 | 0.1046 | -0.439 | 246 | 0.6611 | -0.252 | 0.1602 |
| time:P:L | 0.0143 | 0.1149 | 0.1242 | 246 | 0.9012 | -0.212 | 0.2405 |
| C:P:L | 8.7988 | 5.8962 | 1.4923 | 246 | 0.1369 | -2.8146 | 20.4123 |
| time:C:P:L | -0.2272 | 0.1742 | -1.3045 | 246 | 0.1933 | -0.5703 | 0.1159 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 1868.2 | 1932.4 | -916.09 | 1832.2 | 0.438 | 0.404 |

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 6.5059 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 7.7553 |

**Table S19**: Surface regression of worker body protein proportion as a function of P and C eaten (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 78.9038 | 0.7598 | 103.8516 | 463 | 6.9000e-323 | 77.4108 | 80.3969 |
| C | 3.6242 | 1.1342 | 3.1954 | 463 | 0.0015 | 1.3954 | 5.8529 |
| P | 2.6471 | 16.0526 | 0.1649 | 463 | 0.8691 | -28.8978 | 34.192 |
| C:P | 15.8006 | 10.2884 | 1.5358 | 463 | 0.1253 | -4.4172 | 36.0183 |
| C^2 | -0.8101 | 0.391 | -2.0717 | 463 | 0.0388 | -1.5785 | -0.0417 |
| P^2 | -9.9114 | 61.9123 | -0.1601 | 463 | 0.8729 | -131.575 | 111.7526 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 2853.9 | 2887.1 | -1419 | 2837.9 | 0.105 | 0.095 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 1.2732e-07 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 4.9857 |

**Table S20**: Surface regression of worker body protein proportion as a function of L and C eaten (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 78.6838 | 0.7224 | 108.9256 | 463 | 0 | 77.2643 | 80.1034 |
| C | 5.5937 | 1.1965 | 4.675 | 463 | 3.86E-06 | 3.2424 | 7.9449 |
| L | 2.5884 | 11.8459 | 0.2185 | 463 | 0.8271 | -20.6899 | 25.8668 |
| C:L | -9.5352 | 8.5164 | -1.1196 | 463 | 0.2635 | -26.2708 | 7.2005 |
| C^2 | -1.3436 | 0.4153 | -3.2353 | 463 | 0.0013 | -2.1596 | -0.5275 |
| L^2 | 33.327 | 35.4106 | 0.9412 | 463 | 0.3471 | -36.2583 | 102.9124 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 2871.8 | 2905 | -1427.9 | 2855.8 | 0.0703 | 0.0603 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|

| | | | |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 4.9032e-10 |

Group: Error

| | |
|---|---|
| Name | Estimate |
| 'sqrt(Dispersion)' | 5.0818 |

**Table S21**: Surface regression of worker body protein proportion as a function of P and L eaten (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 81.6201 | 0.4131 | 197.5925 | 463 | 0 | 80.8083 | 82.4318 |
| P | 16.879 | 13.0276 | 1.2956 | 463 | 0.1957 | -8.7215 | 42.4796 |
| L | 1.416 | 8.7537 | 0.1618 | 463 | 0.8716 | -15.7859 | 18.6179 |
| P:L | 120.8098 | 186.5211 | 0.6477 | 463 | 0.5175 | -245.723 | 487.3425 |
| P^2 | -15.0044 | 74.72 | -0.2008 | 463 | 0.8409 | -161.837 | 131.8278 |
| L^2 | -25.2788 | 55.5706 | -0.4549 | 463 | 0.6494 | -134.481 | 83.923 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 2885 | 2918.2 | -1434.5 | 2869 | 0.0438 | 0.0334 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 2.0688e-06 |

Group: Error

| | |
|---|---|
| Name | Estimate |
| 'sqrt(Dispersion)' | 5.1538 |

**Table S22**: Surface regression of worker body lipid proportion as a function of P and C eaten (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 0.5991 | 0.2465 | 2.4299 | 463 | **0.0155** | 0.1146 | 1.0836 |
| C | 0.2877 | 0.3369 | 0.8538 | 463 | 0.3937 | -0.3744 | 0.9498 |
| P | 2.2039 | 4.4901 | 0.4908 | 463 | 0.6238 | -6.6195 | 11.0274 |
| C:P | -3.173 | 2.8658 | -1.1072 | 463 | 0.2688 | -8.8045 | 2.4585 |
| C^2 | -0.0925 | 0.1169 | -0.7913 | 463 | 0.4292 | -0.3223 | 0.1373 |
| P^2 | 8.7209 | 17.3051 | 0.5039 | 463 | 0.6145 | -25.2853 | 42.7271 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|

| 1658.9 | 1692.1 | -821.46 | 1642.9 | 0.0199 | 0.022 |
|---|---|---|---|---|---|

Random effects covariance parameters:
Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.23839 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 1.3858 |

**Table S23**: Surface regression of worker body lipid proportion as a function of L and C eaten (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 0.7591 | 0.2283 | 3.325 | 463 | **9.55E-04** | 0.3105 | 1.2077 |
| C | -0.0689 | 0.2189 | -0.3148 | 463 | 0.7531 | -0.499 | 0.3612 |
| L | 0.607 | 2.246 | 0.2702 | 463 | 0.7871 | -3.8066 | 5.0205 |
| C:L | 0.5443 | 1.001 | 0.5437 | 463 | 0.5869 | -1.4229 | 2.5114 |
| C^2 | -0.0025 | 0.0495 | -0.0514 | 463 | 0.959 | -0.0999 | 0.0948 |
| L^2 | -1.0024 | 4.4927 | -0.2231 | 463 | 0.8235 | -9.8311 | 7.8263 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 1655.2 | 1688.4 | -819.58 | 1639.2 | 0.0229 | 0.0123 |

Random effects covariance parameters:
Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.22683 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 1.3806 |

**Table S24**: Surface regression of worker body lipid proportion as a function of P and L eaten (N=469 groups of 5)

| | Estimate | SE | tstat | DF | Pvalue | lower | upper |
|---|---|---|---|---|---|---|---|
| (Intercept) | 0.6832 | 0.1635 | 4.1776 | 463 | 3.52E-05 | 0.3618 | 1.0046 |
| P | -3.5196 | 3.499 | -1.0059 | 463 | 0.315 | -10.3955 | 3.3563 |
| L | 2.1227 | 2.3503 | 0.9031 | 463 | 0.3669 | -2.4959 | 6.7413 |
| P:L | -5.273 | 50.1509 | -0.1051 | 463 | 0.9163 | -103.825 | 93.2785 |
| P^2 | 21.7245 | 20.1314 | 1.0791 | 463 | 0.2811 | -17.8358 | 61.2847 |
| L^2 | 1.3071 | 14.9556 | 0.0874 | 463 | 0.9304 | -28.0822 | 30.6965 |

Model fit statistics:

| AIC | BIC | LogLikelihood | Deviance | R squared ordinary | R squared adjusted |
|---|---|---|---|---|---|
| 1654.4 | 1687.6 | -819.21 | 1638.4 | 0.0325 | 0.022 |

Random effects covariance parameters:

Group: col (4 Levels)

| Name1 | Name2 | Type | Estimate |
|---|---|---|---|
| '(Intercept)' | '(Intercept)' | 'std' | 0.24015 |

Group: Error

| Name | Estimate |
|---|---|
| 'sqrt(Dispersion)' | 1.379 |

# Chapter 5

# Regulation of macronutrient intake in termites: a dietary self-selection experiment

*"The mind is not a vessel to be filled, but a fire to be kindled."*
Plutarch

# Chapter 5: Regulation of macronutrient intake in termites: a dietary self-selection experiment


## Abstract

Many animals have been shown to select among nutritionally complementary foods to reach a specific balance of nutrients that supports optimisation of key life history traits. Nutritional ecology theory, however, predicts that an animal with a diet that is very stable in its composition, and with nutritional requirements that do not vary in their balance through time, would not need to display such mechanisms of regulation. Here we use the Australian termite *Nasutitermes exitiosus* as a model to test this prediction for the first time. We used the nutritional geometric framework to investigate the regulation of carbohydrate and protein, as well as the effects on foraging behaviour of protein type and group caste composition and size. Our results confirm the prediction of nutritional ecology, as termites failed to actively defend a well-defined macronutrient ratio. They did however collect a similar amount of carbohydrate, which is their main source of energy. Protein type and group composition did not affect food collection.



**Keywords**: *termites, nutrition, protein, carbohydrate, caste, nutritional geometric framework*

# Introduction

A key factor in nutrition is its multidimensionality. Organisms forage to acquire a range of nutrients, each of which is needed at its own level. Nutritional requirements vary depending on growth, reproductive status, infections by pathogens, activity and so on. To meet their nutritional requirements, organisms need to assess their nutritional state (NS), select a suitable food, and regulate the amount of food eaten. However, whenever the balance of nutrients found in food does not match the nutritional requirements of the organisms, there is a need to trade-off over ingesting some nutrients against under ingesting others. Thanks to a recently developed integrative approach, the nutritional geometric framework (NGF), it is now well known that organisms from unicellular organisms to mammals are able to precisely regulate their food intake. The NGF is a state-space modelling approach, combining the organisms' current nutritional states, nutritional requirements, available food, feeding choices and their fitness consequences in a single model. This powerful method led to great advances in nutritional ecology and beyond (reviewed in Simpson and Raubenheimer, 2012). A key parameters of the NGF is the intake target (IT), which is the optimal balance and amount of nutrients that should be eaten by animals to maximize their fitness. ITs can be determined experimentally in 2 ways: 1) by constraining individuals to one of multiple diets differing in their macronutrient balance and measuring the animal performances in terms of longevity, reproduction, immune response, etc...; 2) by challenging individuals to achieve the same intake of various nutrients when facing different food pairings. Numerous studies have elucidated the regulatory strategies employed by organisms to meet their own IT (review in Simpson and Raubenheimer, 2012).

However, in social animals, especially eusocial insects, reaching the IT for the group as a whole is more complex than in isolated individuals due to division of labour. The task of foraging is performed by a subset of individuals: the foragers. These individuals collect the food for the entire colony and need to assess the nutritional state and the nutritional requirements of the colony as a whole. The field of collective nutrition emerged a decade ago with the first studies showing how ants maintain nutritional homeostasis at both individual and collective levels (Cook et al., 2010; Dussutour and Simpson, 2008; Dussutour and Simpson, 2009) and was

later extended to bees (Altaye et al., 2010; Vaudo et al., 2016) and very recently to termites (Poissonnier et al., 2018). An important factor to consider when studying social insect nutrients is the co-existence of several castes within a colony that have distinct nutritional needs. Larvae need protein to support their growth, while workers require mostly sugars to sustain energy expenditure (Cassill and Tschinkel, 1999; Sorensen and Vinson, 1981; Weeks et al., 2004). In ants, foragers increase protein collection and regulate nutritional intake better in presence of larvae (Dussutour and Simpson, 2008; Dussutour and Simpson, 2009), suggesting that larvae provide strong feedbacks to workers regarding the nutritional status of the colony. Studies on ants and bees nutrition are now building up and the regulatory strategies employed to meet the IT at the colony level are better understood (review in Lihoreau et al., 2018).

Yet, all social insect species studied until now using the NGF are food generalists that naturally feed from foods that vary greatly in their macronutrient composition (prey vs honeydew or nectar in ants, pollen vs nectar in bees). In a seminal paper, Simpson and Raubenheimer (1999) postulate that species which experience a relatively narrow range of food nutrient compositions would regulate food quantity but not quality while species that feed on a wide range of food types are more likely to regulate both food quantity and quality. The authors suggest that the extent to which an animal would overeat an unbalanced food depend on the probability of finding a complementary food later (Simpson and Raubenheimer, 1999). This probability is expected to be high for a generalist but low for a specialist. Thus, as specialists experience a narrow range of food composition, they only need to adjust the amount of food eaten and they would not show any compensation behaviour. The authors also propose that specialist would be much less able than generalist to reach the IT by eating from very different foods. This was indeed the case for locusts (reviewed in Simpson and Raubenheimer, 2012) and caterpillars (Lee et al., 2003; Lee et al., 2004; Warbrick-Smith et al., 2009), for a review see (Behmer, 2009; Machovsky-Capuska et al., 2016). However in caterpillars, specialists were able to regulate accurately their intake of protein and carbohydrates (Lee et al. 2003).

Within social insects, termites are extreme specialists and provide the perfect model to test those predictions at the social level. In a previous paper (Poissonnier et al., 2018), we have

already shown that termites did not modify their intake according to food composition, when subjected to a single diet of variable macronutrient composition, corroborating Simpson and Raubenheimer's hypothesis (1999). In this paper, our aim was to confirm the hypothesis that termites presented with food pairing would fail to reach a well-defined IT and instead eat randomly from the two unbalanced but complementary foods. We manipulated protein quality and colony demography as was done in the past with ants (Dussutour and Simpson, 2009; Dussutour and Simpson, 2012; Poissonnier et al., 2014) to show if regulation could be improved by larvae or by the use of more natural diets.

## Material and methods

*(a) Biological model and experimental colonies*

We collected two colonies of *Nasutitermes exitiosus* of similar size in Adelaide (South Australia) at the end of March 2018. The mother colonies were kept in the lab in their mound material with ad libitum wood available at 27°C. From these mother colonies, we constituted 71 experimental groups of varying caste composition and placed them in a 10*10 cm Petri dish with a layer of 4% agar gel, to maintain humidity. The agar was sprayed with a solution of fungicide (10 drops of Zaleton per litre) to prevent fungus infections which could be fatal for termites. The experimental groups were transferred to a fresh nest every 6 days to prevent infections and desiccation and kept in the dark at room temperature (27 °C).

*(b) Synthetic diets and feeding protocols*

We designed synthetic foods to control and manipulate their macronutrient composition. Various protein powders were used as nitrogen source and will be described below. Cellulose (Sigma) was used as a digestible carbohydrate, being accessible to termites because of their symbiotic microbiota. Each food also contained 0.5% of vitamins (Vanderzant vitamin mixture for insects, Sigma), 0.5% of mineral salts (salt mixture W, MP biomedical) and 0.03% of sterols (a 50:50 mix of ergosterol, Sigma and phytosterol, Bulk Supplements). Foods were placed in 2 mL Eppendorf tubes as a powder and offered to the termites.

In the experiments we confined the experimental colonies to various food pairings. Within a pair, diets differed in their protein (P) to carbohydrate (C) ratio. A pair always comprised a diet mostly comprised of cellulose (high C) with a C:P ratio of 3333, paired with a diet enriched in protein (high P) with a C:P ratio of 10 (food pairing 1) or a C:P ratio of 2 (food pairing 2).

All experimental colonies had ad libitum access to a pair of food types that was replenished every 3 days for the whole duration of the experiment (12 days). Colonies never collected all the food offered before it was renewed. To calculate colonies' food collection, we dried and weighed the food (to the nearest 0.1mg) before and after it was placed in the termite nests. We divided the colony intake by the number of individuals in each colony the day the food was offered, to take into account differences in mortality between colonies. The number of dead termites were counted and removed from the nest every three days for 12 days.

*(c) Effect of protein type on macronutrient intake*

Here we investigated whether protein type played a role in nutritional regulation. We tested two types of protein powders: whey (Myopure) (animal protein: a) or a 50:50 mix of rice and soy protein (Myopure) (plant protein: p). We used 50 groups of 70 large workers (stage 2 to 5, see McMahan and Watson 1975) and 30 minor soldiers (N $\geq$10 per treatment). If termites do not regulate their intake, foragers should collect the same amount of each food, both within and between pairings, irrespective of protein types.

*(d) Effect of group composition experiment on macronutrient intake*

In this second experiment, our objective was to see if food consumption depended on food pairing and colony composition. Using the same combination of diets as in the previous experiment (but only using plant protein), we manipulated group compositions to investigate the effect of food pairing, group size, presence of minor workers and presence of larvae.

The group compositions were:

- Control: 70 large workers and 30 minor soldiers (100 individuals, N ≥10)

- Effect of group size: 140 large workers and 60 minor soldiers (200 individuals, N = 4)

- Effect of minor workers: 45 large workers, 25 minor workers and 30 minor soldiers (100 individuals but presence of minor workers, N≥3)

- Effect of larvae: 70 large workers, 10 minor soldiers and 30 larvae (110 individuals and presence of larvae, N=3)

If termites do not regulate their intake, foragers should collect the same amount of each food, both within and between pairings, irrespective of group composition.

*(f) Statistics*

All linear mixed models were performed using the R function 'lmer'. All consumption data used in the models were standardized ((value-mean)/standard deviation). This procedure reduces the covariation between linear variables and their interaction terms (Aiken 1991). The models investigated the effect of diet (High P or High C), food pairing (1 or 2), protein type (a or p) or group composition on food consumption (with colony as a random factor). All the models used to compare food consumption presented in the paper do not include interactions between the parameters, as none of them were significant, and the AIC were lower in the models without interactions. We used a general linear mixed model with colony as a random factor, to test for the effects of protein type and group composition on mortality.

# Results

*(a) Macronutrient regulation: Effect of protein type*

In this experiment, we tested if termites have the capacity to regulate their intake of protein and carbohydrate when offered two different food pairings varying in their ratio of protein and carbohydrate. We manipulated protein type to test if it affected food regulation.

First, termites collected less of the protein biased foods, regardless of the food pairings and the protein types (glmm, R2=0.21, diet effect X2=42.73 P<0.001; food pairing effect X2=0.67 P=0.412; protein type effect X2=0.05 P=0.820; Figure 1).

Second, within a food pairing termites ate the same amount of food in total (sum of the food eaten from the two diets offered, High P + High C), regardless of the food pairing offered and the protein type (glmm, R2=0.01, food pairing effect X2=0.94 P=0.333, protein type effect X2=0.07 P=0.785, par table 1). However, we noticed that termites did not eat the same amount of protein (glmm, $R^2$=0.41, food pairing effect X2=35.58 P<0.001, protein type effect X2=0.38 P=0.538), while the intake of carbohydrate remained comparable (glmm, $R^2$=0.08, food pairing effect X2=3.65 P=0.056, protein type effect X2=0.41 P=0.524).

Third, termites did not maintain the same ratio in the face of two different complementary food pairings (glmm, food pairing effect X2=8.36 P<0.001, protein type effect X2=0.28 P=0.593; Figure 2), meaning that termites did not have the capacity to regulate both protein and carbohydrate collection regardless of protein type. In short, termites avoided eating highP diets, but did not actively eat to reach a particular intake ratio.

Mortality was higher for animal protein than for plant protein (protein type effect $F_{1,43}$=12.98 P=0.001) especially for the second food pairing (food pairing effect $F_{1,43}$=16.09 P<0.001, protein type*food pairing $F_{1,43}$=5.35 P=0.026; mean proportion of dead individuals ± CI95 : 0.11±0.03, 0.22±0.04, 0.09±0.03, and 0.12±0.04, for a1, a2, p1 and p2 respectively).

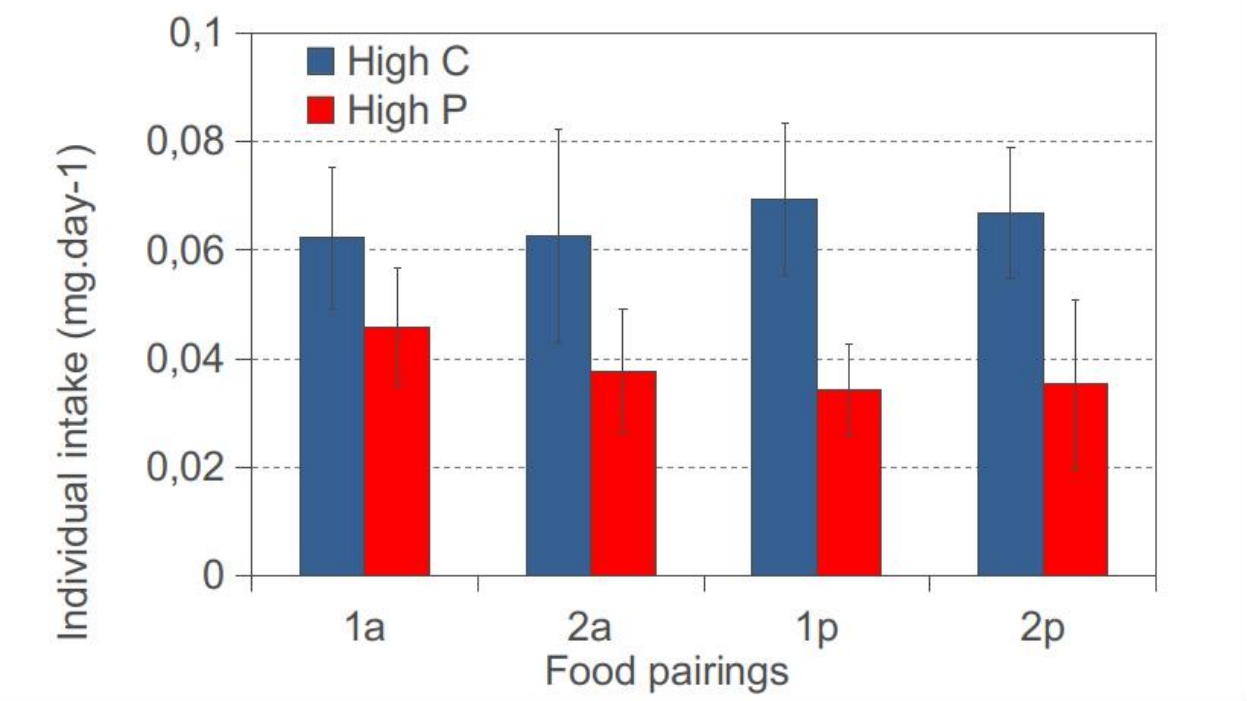



Figure 1: **Food Collection and effect of protein type on macronutrient intake**.The bars represent the daily intake per individual (mg) for each food pairing. Food collection was measured over 12 days in group of termites composed of 70 major workers and 30 minor soldiers. In the experiment, groups were offered one of two food pairings (1: P:C 1:3333 vs P:C 1:10; 2: P:C 1:3333 vs PC 1:2) with animal (a) or plant protein (p). The blue bars present the food collected from the diet rich in carbohydrate, and the red bars the food collected from the diet rich in protein. Error bars are 95% confidence intervals. The quantity collected at the group level was divided by the number of individuals still alive when the food was offered, to take into account any difference in mortality between the colonies or treatments.

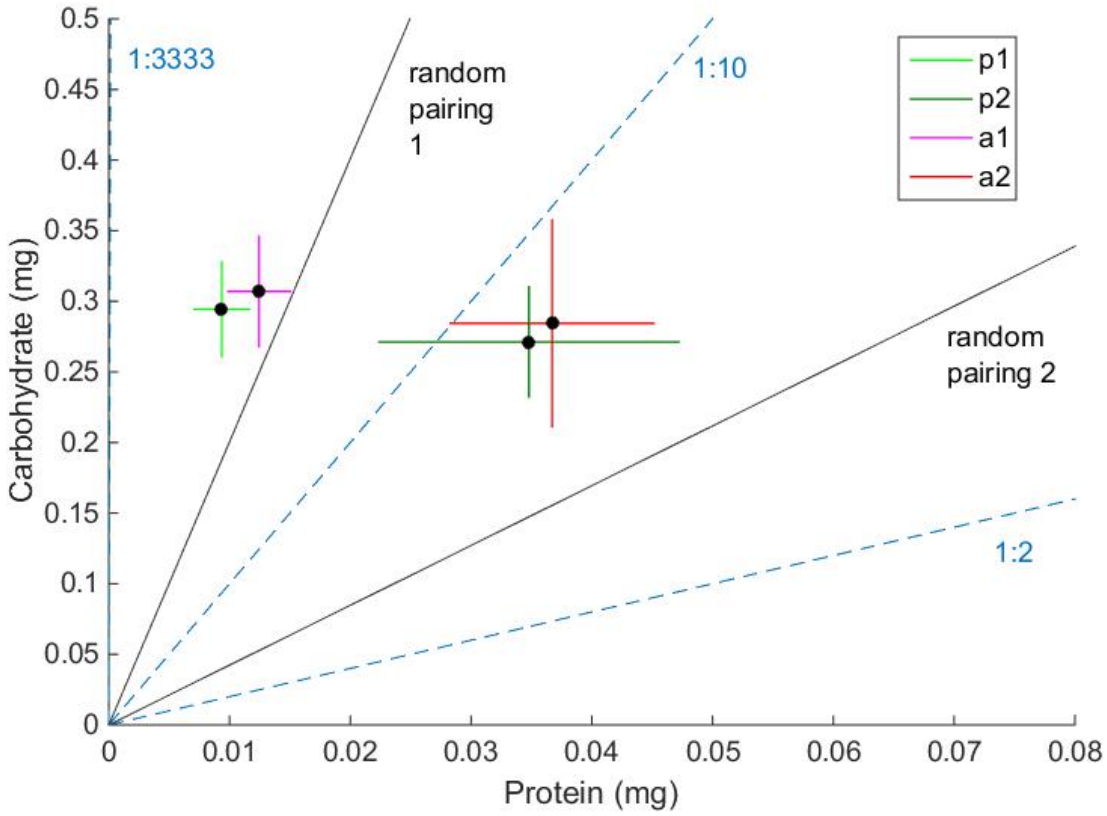



Figure 2: Effect of protein type on protein and carbohydrate collection. Full circles represent the amount of protein and carbohydrate collected. Error bars are 95% confidence intervals. Dashed blue lines represent the P:C ratio of the 3 foods offered, black lines represent the expected ratios where intakes would fall if feeding had occurred randomly between the two foods, for each food pairing. In the experiment, groups were offered one of two food pairings (1: P:C 1:3333 vs P:C 1:10; 2: P:C 1:3333 vs PC 1:2) with animal (a1, a2) or plant protein (p1, p2). Food collection was measured over 12 days in group of termites composed of 70 major workers and 30 minor soldiers. The quantity collected at the group level was divided by the number of individuals still alive when the food was offered, to take into account any difference in mortality between the colonies or treatments.

### *(b) Macronutrient regulation: Effect of group composition*

In this experiment we investigated if group composition changes the nutritional requirements of the colony and/or contribute to the effectiveness of nutritional regulation. We used only plant protein as mortality was lower on those diets. The food consumption pattern was coherent with the previous experiment. Termites collected less of the protein biased foods (glmm, R2=0.30, diet effect $X^2$=52.13 P<0.001; food pairing effect $X^2$=0.39 P=0.530; Figure 3), and they ate the same amount of food in total (sum of the food eaten from the two diets offered, High P + High

C), regardless of the food pairing and the group composition offered (glmm, R2=0.07, food pairing effect X2=0.01 P=0.917, group composition effect X2=6.37 P=0.094). Termites also did not eat the same amount of protein (glmm, R2=0.37, food pairing effect X2=29.96 P<0.001, group composition effect X2=0.61 P=0.895) while the intake of carbohydrate remained comparable (glmm, R2=0.02, food pairing effect X2=0.49 P=0.483, group composition effect X2=0.58 P=0.902). Termites did not maintain the same ratio in the face of two different complementary food pairings (glmm, food pairing effect X2=15.76 P<0.001, group composition effect X2=2.65 P=0.449; Figure 4), meaning that termites did not have the capacity to regulate both protein and carbohydrate collection, regardless of group composition.

However, increasing the number of individuals to 200 termites slightly modified the pattern observed initially with 100 termites, *i.e.* at the individual level the amount food collected per diet was lower for 200 individuals (caste effect $X^2$=9.16 P=0.027).

When the larvae were present mortality was higher than the other groups which had similar mortality rates (caste effect $F_{1,20}$=19.79 P<0.001, food pairing effect $F_{1,34}$=0.33 P=0.857; mean proportion of dead individuals ± CI95 : 0.10±0.02, 0.09±0.04, 0.28±0.03, and 0.07±0.01, for 70W+30S, 140W+60S, 70W+10S+30L or 45W+25MW+30S respectively). By taking a close look, we noticed that only the larvae died, not the workers or the soldiers.

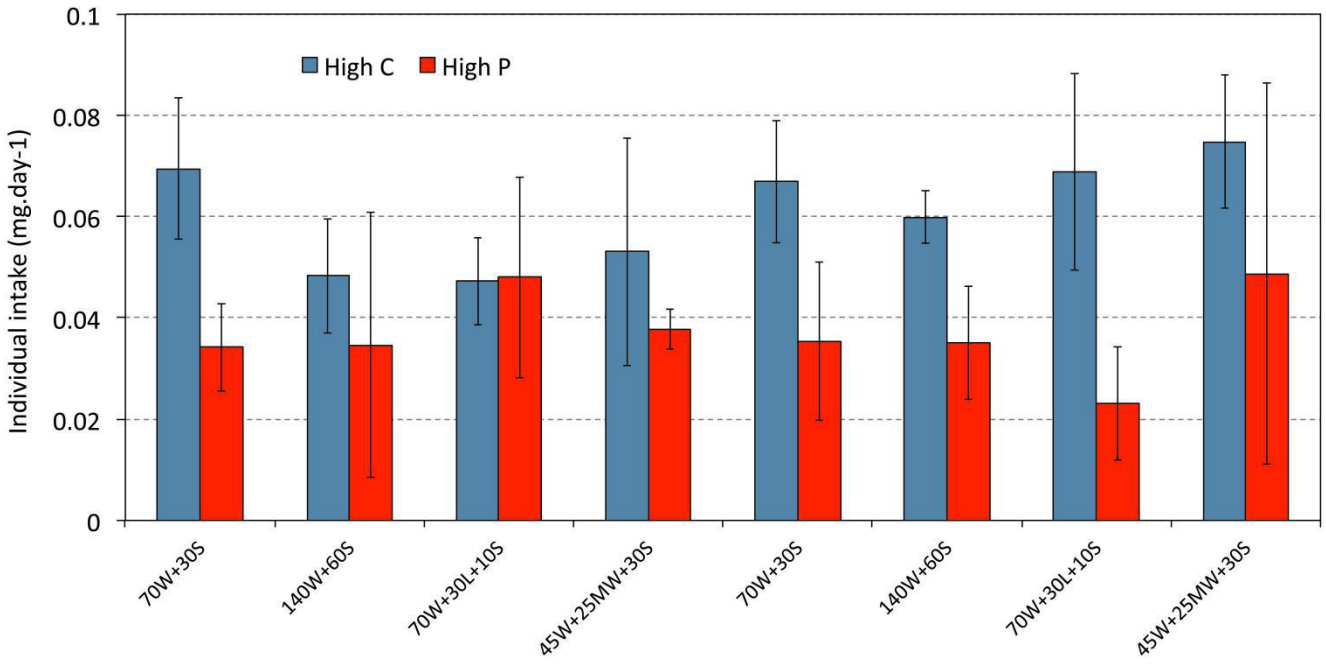



Figure 3: **Effect of group composition on macronutrient intake**. The bars represent the daily intake per individual (mg) for each food pairing. In the experiment, groups were offered one of two food pairings (pairing 1: P:C 1:3333 vs P:C 1:10; pairing 2: P:C 1:3333 vs PC 1:2) with plant protein. Food collection was measured over 12 days in group of termites composed of 70 major workers and 30 minor soldiers (70W+30S), 140 major workers and 60 minor soldiers (140W+60S), 70 major workers, 10 minor soldiers and 30 larvae (70W+10S+30L) or 45 major workers, 25 minor workers and 30 soldiers (45W+25MW+30S). The quantity collected at the group level was divided by the number of individuals still alive when the food was offered, to take into account any difference in mortality between the colonies or treatments. Error bars are 95% confidence intervals.

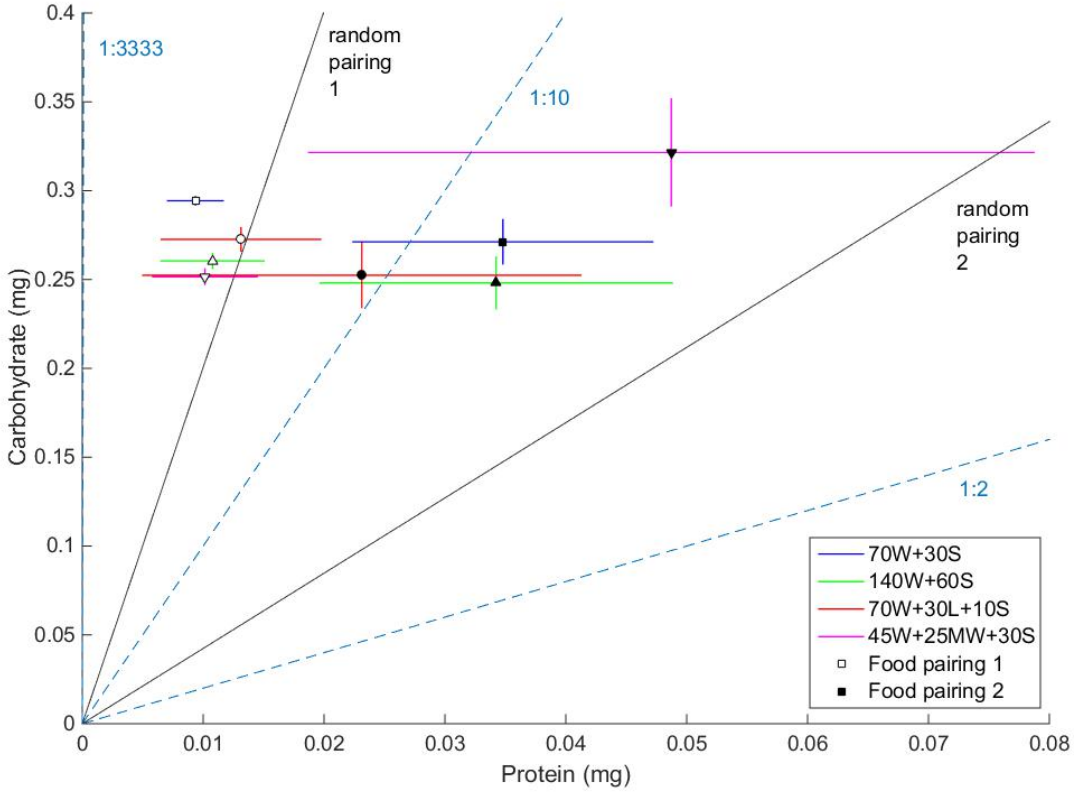



Figure 4: **Effect of group composition on protein and carbohydrate collection .** Food collection was measured over 12 days in group of termites varying in their composition in workers (W),minor workers (MW), minor soldiers (S), Larvae (L). Inverted triangles represent the groups with minor workers, circles the ones with larvae, squares the same group composition we used in the protein type experiment, and triangles the groups where we doubled the number of individuals compared to the protein type experiment. In the experiment, groups were offered one of two food pairings (1: P:C 1:3333 vs P:C 1:10; 2: P:C 1:3333 vs PC 1:2). Open symbols represent the amount of protein and carbohydrate collected for food pairing 1, filled symbols represent the amount of protein and carbohydrate collected for food pairing 2. Dashed blue lines represent 3 foods offered, black lines represent the expected ratio where intakes would fall if feeding had occurred randomly between the two foods, for each food pairing. The quantity collected at the group level was divided by the number of individuals still alive when the food was offered, to take into account any difference in mortality between the colonies or treatments. Error bars represent 95% confidence intervals.

## Discussion

The diet of generalist feeders is comprised of a wide range of nutritionally complementary foods, while specialist feeders consume only a narrow range of foods that closely approximate the required balance of macronutrients. Nutritional heterogeneity has been proposed as explanation for why specialists are less able to regulate macronutrient composition when offered multiple foods (Despland and Noseworthy 2006, Behmer, 2009; Machovsky-Capuska et al., 2016). In this paper we have shown that termites, which are extreme specialists, did not actively select a specific protein: carbohydrate ratio.

Termites appeared to maintain the amount of carbohydrate they collect but the amount of protein varied according to food pairing. Several mechanisms could lead to this pattern. First termites might not be able to detect protein at all, and regulate their carbohydrate intake by preferentially selecting the diets closer to their carbohydrate needs (high C diets). Second, termites might be able to detect protein and avoid high protein diets to some extend, leading to a similar carbohydrate intakes across diet pairs as a by product of the high bias of these diets towards carbohydrate. We could not modify our diets to test this hypothesis, as we could not further lower the amount of cellulose without severely impacting termite survival.

While termites seem unable to regulate their macronutrient intake tightly, they are able to control precisely their intake of micronutrients (Judd et al., 2017). This seemingly contradictory result is easily explained by the fact that termite acquire micronutrients from the soil and not from the wood. Soil varies greatly in its composition of micronutrients, thus termites might behave as food specialists for macronutrients, but as generalists with regards to micronutrients.

Specialists have been shown to not tolerate an excess of nutritionally imbalanced foods as well as generalists (Raubenheimer and Simpson, 2003). In a previous study, we indeed confirmed that termites confined to an imbalanced diet did not over-consume foods rich in protein or lipid and thus suffered a substantial deficit in carbohydrate (Poissonnier et al., 2018). We revealed that termites survived best when they collected a daily amount of carbohydrate comprised between 0.02 and 0.04 mg of per individual (Poissonnier et al., 2018). Interestingly,

here, we have shown that termites keep their intake of carbohydrate around 0.3mg per individual, regardless of the food pairing.

The quantity of protein ingested has been recognized as an important factor for fitness traits such as reproduction and longevity in many organisms (reviewed in Simpson and Raubenheimer, 2012) including termites (Poissonnier et al., 2018), but the quality of protein is also essential (Altaye et al., 2010; Cooper and Schal, 1992; Lee, 2007; Pirk et al., 2010). For instance, in honeybees protein type affected survival, ovarian activity (Pirk et al., 2010), and foraging (Hendriksma and Shafir, 2016). In ants, protein type affected health and led to severe changes in behaviour (Poissonnier et al., 2014). In *Blattella germanica* soybean protein led to better development than casein (Cooper and Schal, 1992). In the African cotton leafworm (Lee, 2007), replacing casein with zein, a low-quality plant protein, altered survival, development, and growth. When offered different food pairings using either Zein or Casein, African cotton leafworms ate more zein than casein in an attempt to compensate for essential amino acids lacking in zein (Lee, 2007). In our experiments, both diets presented all the required essential amino acids but had a distinct amino acid profile. We have shown that whey protein is more lethal than soy and rice protein. Nevertheless, termite food collection pattern was not affected by protein source.

In ants, it was shown that larvae play a key role in nutrient regulation (Dussutour and Simpson, 2008; Dussutour and Simpson, 2009), providing nutritional feedback to workers. In our study, we only found a marginal effect of the presence of larvae and no effect of the addition of minor workers on food collection. Termites are eusocial insects, but contrary to Hymenoptera such as ants, they are hemimetabolous insects where workers moult throughout their lives. Termite larvae are a miniature version of workers and they are active and not legless and grub-like such as larval ants. Larvae and minor workers might therefore share the same nutritional needs as the foragers. Another possibility could be that larvae and workers have different nutritional requirements but regulate nutrient utilization post-ingestively to defend their own intake target. This post-ingestive processing could be done by foragers that could adjust trophallaxis frequency or quality depending on the caste of the receiver (Machida et al., 2001).

In our experiment termites were unable to keep the same intake in carbohydrate per individual when group size was increased two fold. However, termites were able to maintain their total intake when the number of actual foragers was decreased from 70 to 45 (minor workers are not known to initiate foraging), as well as when the number of nutritionally dependant individuals (larvae and soldier) was increased from 30 to 40 and to 55 (minor and soldier). Foragers needed to satisfy their own nutritional requirements in addition to the needs of other colony members. This result reveal that termites, as ants (Cook et al., 2010; Dussutour and Simpson, 2008; Dussutour and Simpson, 2009), achieve nutritional homeostasis collectively. Foragers might have solved this challenge by increasing their nutrient collection at the individual level to collect enough food for the whole colony as described in ants (Bazazi et al., 2016).

Termites were not able to balance both carbohydrate and protein by eating from various food but managed to keep the amount of carbohydrate per individual relatively constant regardless of colony composition and food pairing. This result reflects *Nasutitermes exitiosus* (and other termites) ecological and social lifestyle. Those termites live their entire life feeding on a single type of food that is mostly comprised of carbohydrate. Thus, at the individual level, they only ought to adjust the quantity of food collected to feed the entire colony. Moreover, there is now strong experimental evidence showing that symbiotic microorganisms provide termites with essential nutrients (Douglas, 2009) and might help their host to maintain a certain nutrient homeostasis under nutritionally unbalanced conditions.

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# Chapter 6

# Discussion & Perspectives

*"Your assumptions are your windows on the world. Scrub them off every once in a while, or the light won't come in."*

Isaac Asimov

# Chapter 6 - Discussion and perspectives

My research was set in the field of nutritional ecology, with social insects as a model. The aims of my PhD were first, to investigate the link between social immunity and nutrition in eusocial insects using a modelling approach, and second, to understand the link between nutrition, health and behaviour in the understudied termites using an integrative approach.

## 1. Social immunity and nutrition

In Chapter 3, we used an individual based model to explore whether simple mechanisms of foraging decisions could allow a colony-wide regulation of macronutrient intake and how these mechanisms could be used to potentially serve as a basis for social immunity phenomena. Based on results from other experiments, we know that social insects can detect infected nestmates (Baracchi et al., 2012; Drum and Rothenbuhler, 1985; Rosengaus et al., 1999; Waddington and Rothenbuhler, 1976) or even food sources (Fouks et al., 2011). It is also established that the macronutrient composition of the food that insects ingest influences their immune responses (*e.g.* Lee et al., 2006; Povey et al., 2009; Povey et al., 2014). We investigated whether the modulation of food collection by foragers which detected infected nestmates could lead to a better resistance to a pathogen at the level of the colony. We modelled a pathogen that is transferred horizontally by contacts between colony members. Each individual could be in a pathogen free state, contaminated at low level, or seriously infected. Only seriously infected individuals could infect others, and present impaired foraging (as observed in nature). Individuals could fight the pathogen by increasing

their intake of protein (the "defence IT"), and non-infected individuals had the possibility to change their Intake Target (IT) preventatively when they detected infected nestmates. Seriously infected individuals could revert to the "contaminated at low level" state if they had managed to bring their nutritional state close to the defence IT. We measured how pathogen spread, colony nutritional performance and foraging effort were impacted by immune response duration and the probability to engage in a social immune response (*i.e.* to preventatively switch to a defence IT). We found that the probability to engage in a social immune response was significantly reducing the number of infected individuals and increasing contamination latency, as well as the nutritional performances, when immune response durations were short (while it had little effect for long immune responses). Another interesting finding was that the failure of infected workers to forage led to a lower infection of the colony, as the infected foragers were then isolated from the other workers thus decreasing the risk of an initial contamination of the colony. This effect was however reversed when the pathogen transmission rate was increased, in which case the pathogen could still propagate through the colony while the foragers' pool was increasingly depleted by the rejection of contaminated individuals. This led to a vicious circle where the lack of foragers further degraded the ability for the colony to develop an effective immune response and led to a dramatic collapse of the colony nutritional status.

In conclusion we revealed how collective nutrition might lead to an efficient social immune response, and how its effects varied with the type of immune response displayed by the workers and with infection rate. We could further modify the model to understand the link between nutrition and immunity. For instance, in our simulations the individuals could not die or recover completely from an infection. However some pathogens can kill individuals (*e.g.* Nosema (Higes et al., 2007)), and workers can get rid of certain pathogens (*e.g.* foulbrood and chalkbrood disease (Spivak and Reuter, 2001). We could also further explore the effect of the

rules of compromise on the colony response. In the present model, we used the closest distance rule of compromise (most often used to explore theoretical scenarios of social nutrition, see Lihoreau et al., 2014; Lihoreau et al., 2015), but some species follow different rules: honey bee workers, for example, use an "asymmetrical quadratic" rule of compromise when balancing carbohydrates and essential amino acids (Paoli et al., 2014). An obvious next step would be to test our predictions experimentally. Our paper was inspired by the fungal pathogen Nosema that is a well-known fungal pathogens of bees. Protocols to create experimental infections with Nosema are established (Higes et al., 2007), as well as artificial diets to manipulate the macronutrient ratios of foods (Altaye et al., 2010). Experiments are currently underway at the University of Toulouse (France) by Lihoreau's team (one of the co-authors of the paper) to decipher whether bee foragers would actually modify their food collection if they detect infected individuals, and whether their intake of nutrient affects the colony's resistance to Nosema. Similar phenomenon could be investigated in other systems such as ants or termites, as well as in other pathogens differing in their effects and transmission.

## 2. Termite nutritional ecology

### *Adapting the Nutritional Geometric Framework (NGF) to termites*

The experimental part of my PhD was focused on termite nutritional ecology, by applying the NGF to study how *N.exitiosus* regulated macronutrient intake. A first challenge, which represented a significant part of this PhD, was to design custom-made termite housing and artificial diets that would suit the peculiar and delicate lifestyle of wood-eating termites. We wanted a setup that allowed us to 1) measure food consumption, 2) recover termites that died to measure survival and body contents, and 3) evaluate tunnelling behaviour (as a marker

of foraging activity). Termite die very easily from desiccation, therefore we had to provide them with a humid substrate. With substrate such as plaster of Paris, the termite could not dig their tunnels well, and with a conventional substrate like vermiculite, dead termites were impossible to find and count properly without disturbing the surviving individuals. On those substrates termites also had a tendency to mix food and substrate together, which interfered with food consumption weighing. We opted for a layer of agar in a Petri dish, as it provided the most stable humidity, allowed the termites to dig tunnels that were easily measured by taking a picture of the bottom of the Petri dish, and facilitated the counting of dead bodies. For the artificial diets we designed powdered-based foods to remove any possible effects of differential attraction to foods according to their humidity. Those diets were composed of carbohydrate (cellulose), lipids (sterols), and protein (whey, or a mix of rice and soy protein).

### *NGF experiments*

In Chapter 4 we constrained groups of major workers and minor soldiers to a single diet. We used diets composed of a majority of cellulose as termite diet is mostly composed of carbohydrates. We varied the concentration of the 3 macronutrients from 80 to 99% of cellulose, and from 0% to 16% of lipid or protein. We measured the intake, lifespan, tunnelling activity as well as protein and lipid content of termite that died during the experiment. In short termite did not show any regulation of macronutrient intake, as they ate the same amount of each foods. Carbohydrate intake had the most impact on their lifespan. Survival was reduced when carbohydrate intake was below or above a certain value, around 0.03 mg per individual per day. Longevity was affected to a lesser extent when termites ingested excesses of lipids and proteins. Tunnelling activity shows similar results to lifespan. Diet slightly affected body contents but only in workers. The soldiers, which are fed

secondarily by trophallaxis, seemed to be protected from the deleterious effect of the diet. Their survival depended mostly on the number of workers alive within the nest and to a far lesser extent to the diet. This suggests that the food received by soldiers had a different nutritional quality than the diet offered to the workers. Those conclusions confirm the predictions that extreme food specialists such as termites might fail at avoiding excesses and shortages of macronutrients, and would be impacted by this lack of regulation when fed an imbalanced diet.

The next step to further confirm that termites did not actively regulate their intake in terms of macronutrient ratio was to design a set of experiments where we offered termites a choice between different complementary foods. Moreover, we previously only had tested a single group size, and only 2 castes were present. Both group size and caste are factors that can influence foraging and nutrient collection. In Chapter 5 we addressed these points by giving termites access to one of two possible pairs of artificial diets. The diets within a pair were complementary (see Chapter 1, section 2), and we used 2 different P:C ratios. We did not vary the amount of lipids as they did not have a strong effect on any traits measured in Chapter 4. We also introduced larvae and minor workers in the colonies, as in ants larvae were found to play an important role in foragers' regulation of nutrition (Dussutour and Simpson, 2009). Minor workers were chosen as their role appear similar to nurses in ants, they do not take an important role in foraging, and appear to stay within the nest. As such, they might provide nutritional feedbacks to the foragers. Termites did not select a specific P:C ratio. They collected a similar amount of carbohydrate across all pairings, while the amount of protein was not maintained. Termites preferred foods rich in carbohydrates over foods that are extremely rich in protein compared to what they are likely to find in nature. Our experiments did not however permit to decipher whether those observations resulted from an avoidance of protein-rich foods, a regulation of carbohydrate intake, or a combination of both. The results

from chapter 4 suggest that if there is an active regulation of carbohydrate, it is not a very precise one, as termites ate all diets regardless of their compositions. The variations in composition where a lot smaller than in Chapter 5, hence the difference in results. It would be interesting to find a compound that can not be digested by termites, but that they would ingest, in order to dilute the diet and investigate whether termite modifiy their collection to maintain the same carbohydrate intake as the one found in our experiments.

Surprisingly, termites collected less food per individual when the group size was doubled to 200 individuals. Either the foragers failed at meeting the needs of all individuals, or the individuals might have required less nutrient through an effect of the group size on metabolism or activity. Caste composition did not affect collection, indicating that either the castes share the same nutritional needs, or that the foragers failed at meeting different needs. Termites might lack the sensory abilities to detect animal protein, which is unnatural for termites and could potentially explain the lack of regulation recorded observed in Chapter 4. To address this issue, we used the exact same groups as in Chapter 4, and gave termite access to one of two pairs of P:C ratio as above, but we used plant protein (a mixture of rice and soy protein) instead. Results did not differ between the two protein sources.

Chapter 4 and 5 provide the first complete investigation of how a termite species regulates its macronutrient intake, and how the ingestion of those macronutrient affects their life history traits. A combination of experiments where animals have no-choice (restricted to a single diet, as in Chapter 4) and experiments where animals can freely alternate between diets with complementary composition (as in Chapter 5) is often used in NGF studies. The cross-validation between those two types of experiments is necessary to ensure that a true IT was found. If the ratio and amount of nutrients selected in the choice experiment indeed corresponds to an optimum and is linked to the highest fitness values in the no-choice experiment, then we can be confident that the IT measured is a ‘true’ one. As we discussed in

Chapter 1 and 2, ITs can vary according to a number of factors. If the quantity and ratio of nutrient selected while having a choice differ from the highest fitness values recorded when the animals are fed a single diet, then the reason behind those difference must be found in order to measure the IT. For instance the nutrient intake leading to the best survival and the highest reproductive output might differ. Animals might then compromise between maximising lifespan or maximizing reproduction, as in Drosophila (Lee et al., 2008). Therefore the selection of the fitness traits to be measured is something to consider when designing NGF experiments. In our case, results from both experiments are inconsistent. *N.exitiosus* did not regulate their intake when restricted to a single diet, but they collected similar amounts of carbohydrate when offered the choice between two complementary diets. Further experiments are needed to investigate this phenomenon, for instance by varying nutrient concentration, or by running the choice experiment of Chapter 5 with other nutrients to see if the pattern is similar.

These results also validate important predictions from nutritional geometry (Behmer, 2009a; Raubenheimer et al., 2009; Simpson and Raubenheimer, 2012). There is ample evidence that generalist and specialist feeders use different rules of compromise, generalist feeders being more inclined to over or under eat, probably because their chances to encounter a complementary food is higher. This results in aligned intakes in generalists versus arc shaped ones in specialists (see figure 1 below).

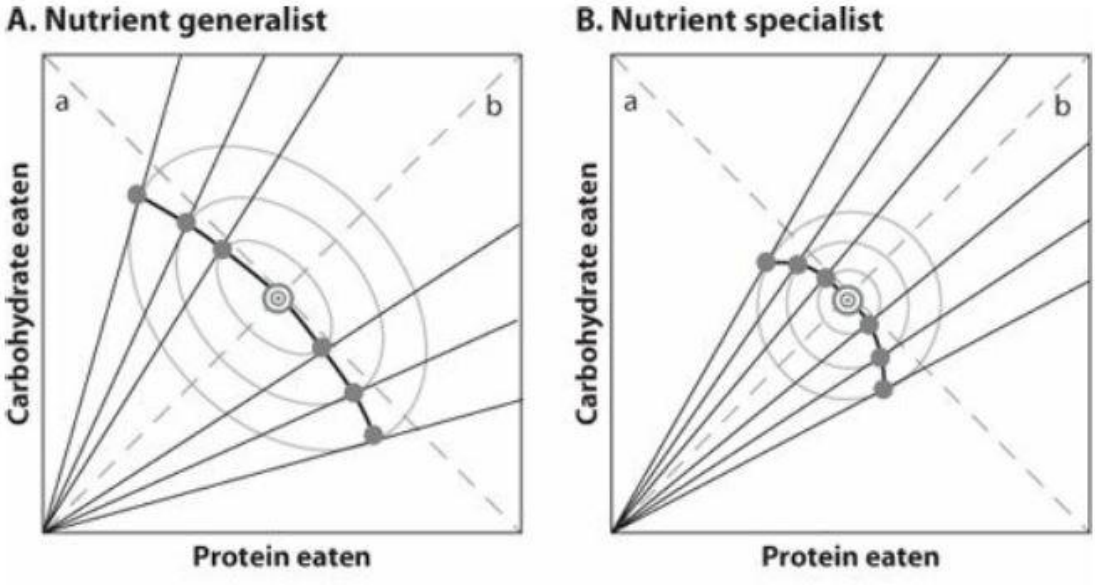


Fig. 1: Schematic summarizing the predicted differences between nutrient generalist and specialist feeders. For illustrative purposes, we have assumed that both the generalist (A) and the specialist (B) have the same intake target (bull's-eye symbol). Compared with the specialist, the generalist is less susceptible to variation in P:C ratio in the diet. The intake array for the generalist is straighter (less arc-shaped) than that for the specialist (from Simpson and Raubenheimer, 2012).

Nutritional geometry theory also suggests that extreme dietary specialists might lose their ability to regulate their intake in terms of nutrient ratio provided that this species 1) specialises on foods that are invariant in their composition of nutrients, 2) does not display strong variations of their IT during their lifetime in terms of the ratio of nutrient (but the quantity needed can vary). Such an organism would only regulate their nutrition by eating a certain amount of food, and would not demonstrate a separate regulation for each nutrient. Termites might validate such predictions, and it would be interesting to look at the genomic basis of this evolutionary change. In a sense, termites did not display a clear "rule of compromise" because they did not defend a specific ratio of macronutrients in their food, but only regulated carbohydrate (or avoided diets highly biased in protein). Any deviation from the expected blend of macronutrients, such as the one that we induced in our artificial diets, had severe consequences on termite survival. The discovery of such a lack of active

macronutrient intake regulation opens interesting questions regarding the evolution of diet within termites. For example, Australian Nasutitermes have originally colonised Australia as arboreal nesting, wood feeding species, but rapidly evolve to a large range of nesting and diets, including grass and soil feeding (Arab et al., 2017). Some of these diets might not be invariant enough in their composition to warrant a lack of macronutrient regulation. Such regulatory mechanisms might have therefore evolved to re-appear in some of the Nasutitermes phylogenic tree.

Measuring macronutrient consumption and its impact on the longevity, body composition and tunnelling activity of termites was a much needed step forward in our understanding of termite nutritional ecology. However, we barely scratched the surface of this complex phenomenon.

## *Microbiota*

Douglas said in her 2014 review on bacterial-insect symbiosis that the "*dissection of the interactive effects of microbiota, immune function, and nutrition is an emerging research priority that will be facilitated by a sure foundation of understanding of microbiota effects on insect nutrition*". We have addressed the question of the interplay between microbiota and nutrition in Chapter 2, but here we will focus on a question that we started investigating using the protocol developed in Chapter 4. Using the same setup and group composition, we collected 4 new colonies of termites and reared groups on diets varying in their P:C:L ratio for about a month. The aim of the study was to provide a first set of data on how macronutrient intake affects the microbiota in termites. We sacrificed workers at several time intervals, and we sent them to Thomas Bourguignon's lab in Okinawa where we extracted gut DNA for 16S

rRNA gene sequencing, to characterise the communities of bacteria. Unfortunately, this experiment is not included in the thesis, as we do not have the sequencing results yet.

## *Nutrition and eusociality in termites*

Termite caste development is another fascinating field that is likely linked with nutrition, and therefore sociality. Eusociality in termite evolved via the sub-social route (Noirot, 1985). The older offsprings start helping in the rearing off their younger siblings, and then enter a phase where their development towards a reproductive adult is slowed down or stopped, via stationary or regressive moults. The mechanisms of this developmental arrest are of prime interest as they constitute the proximate factors by which a first sterile caste was produced in termite colonies (Hunt and Nalepa, 1994). Termite caste development has been the focus of many studies in the past, but the factors influencing caste determination remain largely unknown and understudied. Genotype is thought to have little to no role, caste determination being rather caused by the activation or dis-activation of genes controlling the development of sexual or soldier-like characters (Lo et al., 2009). Environmental factors influencing those genes range from nutrition to aggressive interactions and the use of pheromone and physical interactions that modulate the endocrine activity of other individuals (reviewed by Brent in Gadau and Fewell, 2009). Juvenile hormone controls moulting, and juvenile hormone analogue effects have been extensively studied. A sudden increase in JH titers leads to the development of soldier-like characters in young instars that are still flexible in their development (reviewed in Howard, R. and Haverty, M., 1979). In an attempt to measure the changes of macronutrient intake linked to soldier development, we exposed first instar minor workers of *Nasutitermes exitiosus* to methoprene, a juvenile hormone analogue that induces moulting from worker towards soldiers. This transition to soldier did not occur

frequently enough to measure significant changes in food intake. However, 100% of young nymphs moulted and developed a soldier-like head, with a nasute characteristic of soldiers (*cf* pictures below). This finding is interesting, as nymph are believed to be unable to alter their developmental pathway and moult into soldiers. This transformation was induced artificially, but is not completely unnatural, as a specimen of a similar intercaste was found in a wild colony in another Nasutitermes species (Hojo et al., 2004). This finding highlights the fascinating plasticity of caste development in termites and illustrates that moulting into other castes is possible, but controlled by environmental factors that need to be determined.

Pictures of different termite castes. On the left "normal" castes are presented: 2 soldiers (dark brown heads with a nasute), a major worker and a nymph (white individual with wing buds and red eyes). The picture on the right illustrates the intercaste individual, which presents characteristics of both nymph (wing buds and red eyes) and soldier (nasute).

Nutrition has often been put forward as a potentially major factor in termite caste development, and therefore in the evolution of eusociality. Surprisingly few studies have investigated the effect of nutrition on caste development in termite. Numerous studies have revealed that low quantity and quality of food prolong juvenile development in termite's relatives the cockroaches, where the mechanisms of development are supposedly similar to termites (reviewed in Hunt and Nalepa, 1994). For instance in cockroaches, development into

the adult form can be delayed by food deprivation by almost an order of magnitude. Not only the quantity but also the quality of the food is relevant in this phenomenon. The protein percentage of the food for instance appears essential for development (reviewed in Hunt and Nalepa, 1994). There is an obvious opportunity for NGF studies to shed light on these mechanisms. By using the synthetic diets we developed in Chapter 4 and measuring the development of larvae into different castes according to their intake, we could decipher the effects of (macro)nutrients on caste determination in termites.

Food flow within the colony is an additional area of termite nutrition that is understudied. To whom and under which criteria nutrients are shared must be elucidated in order to build a full understanding of termite nutritional ecology. In termites, workers are in control of what food the other caste members receive. If workers neglect nestmates and do not feed them enough, it could prevent the emergence of reproductive characters in those underfed individuals, as is suggested by Brent in (Gadau and Fewell, 2009). Advances in automated tracking and imagery techniques could help address this question. In ants, Greenwald et al. (2015) used a dual-camera system. One camera measured the food flow (using fluorescent markers mixed to the food), and the other recorded ant trajectories (using miniature barcode identification of each ant). I did some preliminary work to adapt this setup to termites and quantify their food distribution patterns, but results are not yet available due to persistent issues with the setup and tracking software efficiency.

## 3. Conclusion

In conclusion we improved our knowledge of nutritional ecology in social insects, by exploring the role of nutrition in social immunity, and by deciphering how nutrient regulation differed in a specialist feeder compared to the generalist feeders previously studied. We

uncovered that collective nutrition could lead to efficient social immunity responses in insect colonies. Our individual based model confirmed that by altering their food collection to boost the immune systems of their infected nestmates or themselves, foragers can successfully enact a colony-wide response to reduce pathogen spread within the colony. We also designed synthetic diets to study for the first time how social specialist feeders, termites, collect macronutrients and how their intake affects the colony in return. Carbohydrate appeared as the main factor influencing survival and foraging activity, while lipid and protein have a lesser impact. We provide the first evidence that termites do not maintain a strict intake of macronutrient, using an Australian termite species. This result confirms a significant prediction of nutritional ecology theory: animals that become highly specialised in a single type of food that has a stable chemical composition might lose the need and therefore the ability to balance their intake of nutrients. Finally, our experimental protocol can be used to improve our understanding of termite nutritional ecology, by studying the impact of macronutrients on their microbiota, or on their caste development for instance.

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## General Introduction & Discussion

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