Inter-individual variability and division of labour in the ant *Pheidole dentata*

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ABSTRACT

Division of labour is one of the major factors contributing to the outstanding ecological success of ant societies. Ant colonies are functionally single entities composed of thousands of individuals that perform a collection of tasks all directed to the survival of the superorganism. Worker ants perform tasks that include brood care, foraging, nest maintenance, and colony defence. Existing division of labour models assume that the behaviour of individuals is dictated by either factors intrinsic to the individual such as their morphological caste, age, physiological status or by external factors such as spatial location or social interactions. However existing models fail to fully account for the complexity of division of labour patterns observed in social insect societies. Traditionally, in order to account for robustness and resilience, fundamental properties of social insect colonies, behavioural plasticity has been incorporated as a secondary process. I discovered striking levels of inter-individual variation within the worker caste of the ant *Pheidole dentata*. To understand how this variability is generated, I focused on inter-individual foraging behaviour of same age individuals. In a shared social environment, I found that there are two clearly distinct behavioural groups that emerge: the *go* ants which leave the nest and forage, and the *no go* ants which stay in the nest performing brood care. Surprisingly, I found no difference between *go* and *no go* in behavioural capacities, whole-brain biogenic amine levels and receptor expression. Together my results show: (1) that there is abundant inter-individual variability that is relevant for the organization of

division of labour, (2) that inter-individual variability is responsive to the environment, and (3) that biogenic amine systems at a whole-brain scale do not explain the inter-individual variability of same age individuals. As a whole my research leads us to reassess the role of inter-individual variation due to plasticity as an organizing principle for the division of labour in advanced ant societies.

ABRÉGÉ

La répartition des tâches est une des facteurs les plus importants qui expliquent le succès écologique exceptionnel des fourmis. Les colonies de fourmis fonctionnent comme une seule entité composée de milliers d'individus, chacun performant une collection de tâches qui augmentent la survie de ce superorganisme. Les fourmis ouvrières notamment exécutent plusieurs tâches incluant par exemple les soins de la nichée, la recherche de nourriture, le maintien et la défense du nid. Les modèles existants qui expliquent la répartition des tâches prennent pour acquis que le comportement des individus s'explique soit par des facteurs internes à l'individu incluant leur caste morphologique, l'âge, la physiologie ou encore par des facteurs externes comme la location physique ou les interactions sociales. Toutefois, ces modèles n'expliquent pas complètement l'immense complexité des modes de repartition des tâches observées dans les sociétés d'insectes sociaux. Traditionnellement, la plasticité comportementale aurait été incorporée secondairement pour expliquer la nature robuste et la résistance des colonies dinsectes sociaux. J'ai découvert une quantité frappante de variations individuelles chez les fourmis ouvrières de *Pheidole dentata*. Afin de comprendre comment cette variation se produit, j'ai observé la variation des comportements associés a la recherche de la nourriture. Dans le même environnement social, j'ai trouvé qu'il existe deux groupes comportementaux: les fourmis *go* qui sortent du nid pour fourrager, et les fourmis *no go* qui restent dans le nid pour faire des tâches liées au soin de la nichée. Étonnement, chez les fourmis *go* et *no go* il n'y a pas de différence dans les capacités comportementales, les niveaux d'amines

biogènes dans le cerveau ainsi que dans répartition des récepteurs. Dans l'ensemble, mes résultats démontrent que: (1) les variations entre individus existent, qu'elles sont abondantes et pertinentes pour l'organisation et la répartition des tâches; (2) cette variation est une réponse l'environnement; (3) les systèmes d'amines biogènes au niveau du cerveau n'expliquent pas les variations entre les fourmis individuelles ayant le même âge. En général, ma recherche nous mène à réevaluer l'importance de la variation entre les individus dû à la plasticité en tant que principe d'organisation expliquant la répartition des tâches chez les sociétés avancées de fourmis.

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Contribution of Authors

This thesis comprises my original work, three chapters which are in preparation for submission to peer-reviewed journals. Having conceived and performed the majority of the experiments, analysed the data and written the manuscripts, I am the primary author for all. Ehab Abouheif, my supervisor, provided guidance and support throughout the years of work which resulted in this thesis. These manuscripts are a result of the contributions of co-authors, which are stated below:

Chapter 2

Authors: *Ana Sofia Ibarrarán Viniegra, Marc Seid and Ehab Abouheif.*

Ana Sofia Ibarrarán Viniegra designed and performed field collection, experimental design, experiments, analyses, and wrote the manuscript. Marc Seid not only provided support in field collection but also helped with the conceptual development of the project.

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CHAPTER 1
Introduction

The analysis of plasticity of labour roles as a genetic adaptation remains one of the outstanding challenges of insect sociobiology. - *Hölldobler and Wilson, 2008*

1.1 General and Specific Goals

Division of labour is a key feature for the complex organization and ecological success of social insects. Polyphenism in ants produces morphologically distinct castes (e.g., queen, soldiers, and workers), which form one of the most diverse and best differentiated caste systems amongst the social hymenoptera. In addition to the pattern of task allocation associated with morphology, worker ants of the genus *Pheidole* also display a dynamic pattern of division of labour, where through their life they perform different roles within the colonly. The general goal of my PhD research is to address the process underlying the emergence of colony level patterns of colony organization and division of labour. Using the ant species *Pheidole dentata* as a model, we aim to gain insight into how individual behaviour gives rise to colony level patterns of division of labour.

1.2 Eusociality

Eusociality is defined by three fundamental traits: (1) reproductive division of labour (a reproductive and relatively sterile caste), (2) overlapping of two or more generations of adults, and (3) cooperative brood care. Within the 2,600 families of

insects and other arthropods known, only 15 of them have eusocial species. Eusocial insects -honeybees, wasps, termites and, ants- are only 2% of the close to 900,000 species of insects known, yet compose more than half of the global insect biomass (Hölldobler and Wilson [1]). Colonies of eusocial organisms have been around for about 150 million years (Moreau et al. [2], Brady et al. [3]), and have been dominant elements of land ecosystems for at least 50 million years. All of the over 14,000 species of ants are eusocial and therefore they are an ideal model to study of the organization of division of labour.

1.3 Superorganism

William Morton Wheeler first introduced the concept of ant colonies as organisms in 1910 during a lecture at the Marine Biological Laboratory in Woods Hole, Massachusetts (Wheeler [4]). He formally defined organism as "a complex, definitely coordinated and therefore individualized system of activities, which are primarily directed to obtaining and assimilating substances from an environment, to producing other similar systems, known as offspring, and to protecting the system itself and usually also its offspring from disturbances emanating from the environment". Traits fundamental to such an idea are: colonies behave as units, have their own idiosyncrasies, have a growth and reproductive cycle (homoeostasis) and, have differentiated reproductive tissues (males and queens) and non-reproductive tissues (workers). It was only in 1926 when Wheeler formally coined the term superorganism (Wheeler

[5]). In addition to the attributes shared by ant colonies and other organisms, superorganisms have a higher order of biological organization somewhere between individual organisms and ecosystems. Within the concept of superorganism, distinctions have been drawn between those species in which reproductive conflict still exists and those species where reproductive conflict is not present anymore (true superorganisms, where workers are completely sterile). Within social insects ants are the group that has the largest number of species with true superorganisms (Hölldobler and Wilson [1]).

1.4 The evolutionary history of ants

Ants date back to the mid-Cretaceous period, about 125 million years ago. Their phylogenetic relationship with other aculeate Hymenoptera (which include bees, ants, and stinging wasps) is not resolved. However, recent work by Johnson et al [6] supports ants as sharing common ancestor with Apoidea (which includes spheciform wasps and bees). The amazing evolutionary and ecological success of ants is indisputable; they occupy every continent except Antarctica, and thrive in almost all ecosystems (Hölldobler and Wilson [7]). Due to their enormous diversity, ants can function not only as predators and scavengers, but also as cryptic hervibores, tending other insects such as aphids as cattle. At at an ecosystem level, they play an important role in soil-turning.

Eusociality in ants is thought to have evolved just once, a trait that was inherited from their common ancestor and later elaborated. Although all ants are eusocial, there is a wide spectrum of complexity in their organization, specifically in the size

of mature colonies, the degree with which workers are specialized for different tasks, the degree of collaboration and, the complexity of their communication systems. At one end of the spectrum are basal ant species that have small colonies of around a hundred individuals, where workers and queens are morphologically very similar and other than a reproductive division of labour, they are indistinguishable. At the other end of the spectrum are advanced ant societies, which have colony sizes ranging from hundreds to millions of workers, have complex caste systems, patterns of division of labour, and communication systems.

1.5 Colony organization: Historical Perspective

Since the times of Aristotle, we have tried to explain what guides social insect colonies and makes them a cohesive unit. Wheeler ([4]) provides us with a unique historical review. Starting with Aristotle who supposed the colony's activities where directed and regulated by a king (from the greek $\beta\alpha\sigma\iota\lambda\epsilon\upsilon\varsigma$). Later, Swammerdam after realizing the fertile individual is a female he substituted it with queen. And as observed by Solomon, in ant colonies there is "neither guide, overseer, nor ruler". However the idea of a controlling agency prevailed; with authors such as Maeterlinck, a Belgian playwright, poet, and essayist, who used the "spirit of the hive" to describe the force guiding the behaviour of each and every individual within the bee colony. Other authors including Driesch, Eldridge and, Bergson proposed the use of "entelechy" or external factors as the explanatory force organizing the complex activities of the ant colony. Now a days, it is widely accepted that colonies are self-organized, decentralized systems in which behaviour results from the independent decisions and

actions of individual ants.

1.6 Natural Selection vs Emergence

Colony organization is thought to be one of the key traits that have made social insects, especially the ants, so successful. However, whether colony organization is a trait directly selected on, or the result of emergent patterns which arise from individual traits selected on is a key problem in social insect research. Colony organization has been explained as an adaptation that is selected on by natural selection, based on the idea that it optimizes energy use according to the ergonomic principle proposed by Oster and Wilson ([8]). Boneabeau et al [9] introduced self-organization theory to the study of social insects, to explain how collective patterns may emerge from the interaction of individuals with simple behaviours. The authors review a number of instances where self organization can describe collective activity patterns, including: choice between food sources with different profitability (due to either distance or sugar concentration in ants and honeybees, reviewed by Detrain and Denaubourg [10]), thermoregulation in bee hives and building activities in wasps. Self-organization is defined by the authors as "a set of dynamic mechanisms whereby structure appears at the global level, from interactions among lower level components". The authors specify four key elements of self-organization: (1) positive feedback, (2) negative feedback,(3) amplification of fluctuations, and (4) multiple interactions. They also identify three signature features of self-organization: (1) creation of spatio-temporal structures from an initially homogeneous medium, (2) the existence of several stable states (dependence on initial conditions), and (3) the

existence of bifurcation points. Boneabeau et al [9] emphasize that self-organization can be one of the several mechanisms shaping collective behaviour; others include genetic mechanisms, active regulation and pre-patterning. Page and Mitchell [11, 12] and Bonabeau et al. [9] focus on parameters which are part of the different models of self-organization, which can be in turn targets of selection. Therefore, selection can act upon parameters that modulate individual properties, such as the distribution of stimulus response thresholds between individuals, and at the colony level colony size and connectivity between individuals. In the case of social insect colonies natural selection operates on the collective emergent behaviour of social insect colonies, and therefore both processes coexist.

1.7 Division of labour

Division of labour is a stable pattern of variation in the tasks workers perform within a colony (Oster and Wilson [8]). Two general patterns of division of labour are readily recognized in social insects: morphological polyethism and temporal polyethism. Polyphenism in ants produces morphologically distinct castes (e.g. queen, soldiers, and workers), which are one of the most diverse and best differentiated caste systems amongst the social hymenoptera. This polyphenism is under environmental control and thus ant colonies can tune their caste ratios to colony needs and environmental conditions. Patterns of morphological polyethism vary, yet in the case of the ant genus *Pheidole*, the worker caste is composed of at least two subcastes. Large, big-headed soldiers specialized in colony and territory defence and have a role in food storage, while smaller, small-headed, minor workers perform all

the other tasks including brood care, foraging and nest maintenance. In addition to this stable pattern of task allocation associated with morphology division of labour can also be found within subcastes. Within the worker subcaste, ants also display a dynamic pattern of division of labour, where individuals tend to perform different tasks at different times in their life, known as temporal polyethism. One of the first references to this matter might well be that one from Aristotle who in his *History of Animals* describes his observation of bees within the hive having more hair on their bodies than bees which foraged for nectar and pollen, and therefore suggested an age-related division of labour. However, the literature on temporal polyethism has been used interchangeably with age polyethism, which emphasizes the role of age as causal factor defining task performance (Franks et al. [13]). In several instances, the literature on temporal polyethism has been used to refer to the pattern of task performance that correlates with age, where young workers perform tasks within the nest, while older workers perform tasks outside the nest. However, variation in task performance independent of age and morphology has led to alternative approaches, which incorporate the social environment as a force shaping individual behaviour.

1.8 Models of division of labour

Several models aim to explain how division of labour is generated and maintained (summarized in Table 1.1). As classified by Beshers and Fewell ([14]), the existing models address two fundamental aspects of division of labour: (1) decision rules for individual's response to information concerning a task (response threshold, adaptive response thresholds, self-reinforcement and, social inhibition models) and;

(2) how the information is transferred (integrated information transfer, foraging for work, and network task allocation). I will briefly review the existing models.

Fixed response threshold models

Several models have proposed that variation of response thresholds to task-specific stimuli give rise to division of labour amongst workers. This group of models assume that the default state is to not respond to stimulus, unless the response threshold level is reached. This leads to workers with the lowest threshold to respond first and reduce the stimulus level (negative feedback loop). However, if further recruitment to the task is necessary, the response of the first set of workers is not enough to reduce the stimulus, it accumulates leading to individuals with higher thresholds to respond as well. E.O. Wilson first presented this idea in the context of the evolution of temporal castes in ants (Wilson [15]). He proposed that the changes in behavioural response thresholds to stimuli generate an association of age groups and task clusters. Individuals could either have concordant changes in response thresholds giving rise to discrete temporal castes, or if changes are discordant, would result in a continuous temporal caste. Furthermore, he also presents data about worker and soldier ants of *Pheidole dentata* and concludes that there are differences in response threshold to stimuli between castes (workers and soldiers). He then focused on worker ants and concludes they represent a discrete temporal caste with concordant changes in response thresholds, yet points out that it is not an extreme case. Differential response thresholds were proposed to result from caste specific programmed responses of the sensory and nervous system. He later added that

based on experience individuals can modify their responses, however the learning is limited (Hölldobler and Wilson [1]). The concept of differential response thresholds gave rise to mathematical models. The general idea behind this group of models is that an individual has a predetermined response threshold, and based on the need for task performance a task associated stimulus accumulates. If the stimulus reaches the threshold level the individual performs the task, if not the stimulus continues to accumulate (Figure 1.1). Page and Mitchell [11] presented a model based on binary elements with boolean switching functions, connected by a common perceived stimulus. In their model, individuals have two states: "on" or "off" for a particular task. To start, all individuals are in the "off" mode and the stimulus level is set to N (number of individuals). Individuals are randomly assigned a threshold from a discrete uniform distribution and individuals are sampled (either randomly or simultaneously). Depending on their threshold, they are either switched "on" (and the residual stimulus is reduced accordingly) or kept off and the residual stimulus accumulates. The authors use different sampling methods and distributions of thresholds with different means and variances; and test the effect of varying the stimulus level independently of individual responses (residual stimulus). One of the key points they highlight is the effect of their sampling method (random or simultaneous), and what it means in terms of the individuals' connectivity (homogeneous connectivity versus asymmetrical connectivity of individuals). Their model is able to generate homeostasis, an equilibrium at which the number of individuals attending a task is matched to the stimulus level. It shows plasticity in response to changes in stimulus level, and finally, state attractors -states towards which the system tends to evolve- at

which the probability of individuals performing a task remains constant. Bonabeau et al. [16] based on empirical work by Wilson [15] on *Pheidole* workers and soldiers incorporated a second caste with different response threshold distribution to their model. Later, Page and Mitchell [12] incorporated a second task to their model and investigated the effect of interdependence between the thresholds for the two tasks on overall activity levels. Additionally Bonabeau et al. [17] incorporated task succession into their model. Therefore, the stimulus of task B, does not only decrease by the performance of task B, but now the performance of task A increases the demand of task B. The authors also incorporate the idea that the exposure/perception to a stimulus associated with a task depends on the current task performance. Their paper reconciles certain aspects of the Foraging for Work model (summarized bellow) and incorporates constraints that arise from the spatial organization of task in the nest. The authors made the point in their previous paper (Bonabeau et al [16]) that the fixed response threshold model applies only to extremely short time scales, since only at very short time scales can response thresholds be assumed to not change. Therefore, they now also incorporate time into their model, and an individual's probability of encountering a specific task related stimulus varies with age. This means that although response thresholds still remain fixed, the probability of encountering the stimulus does not. Alternatively, incorporating an age-dependent spatial organization of individuals within the nest, they are able to obtain a pattern of temporal polyethism.

Fewell and Bertram [18] empirically addressed changes in foraging behaviour when they manipulated pollen stores in honeybee colonies. Their results indicate

the need to incorporate a dimension of information transfer into the threshold model for it to better represent experimental observations of behavioural response when faced with changing colony needs. Their model, known as Integrated Information Transfer (Figure 1.2), addresses both how workers get information and the variation of task related stimulus thresholds.

Critiques and limitations to response threshold models, include the fact that they do not take into account the spatial organization of the task and individuals. Differential response thresholds between castes have been characterized, however the existence of one stimulus per task has yet to be shown. Another outstanding question is whether the difference lays at the level of the response threshold or at the level of stimulus perception.

Adaptive response thresholds or self-reinforcement model

This model addresses one of the fundamental caveats of the response threshold model; the variability of response thresholds in time. The central idea of this model is that changing thresholds as a result of experience can give rise to division of labour. The novelty incorporated by this model is that behavioural response to stimulus not only depends on the exposure to stimulus *per se*, but also on changing response thresholds (Figure 1.3). An individual's response threshold is updated by previous experience; having successfully performed a task lowers an individual's response threshold, while not performing a task increases it. Experience therefore generates a feedback loop for task performance. Reinforcement is achieved either by using internal reinforcement that increases when the task was performed or by

changes to the response threshold due to "learning". In their model, Theraulaz et al [19] show that when they remove the individuals specialized in a task other individuals take on that task; and therefore conclude that their model can generate flexibility.

The self-reinforcement model on one hand does address the change of response thresholds through time. However, some of the critiques which have been made to the response threshold model still apply such as the fact that it does not take into account the spatial organization of the task and individuals and that the existence of one stimulus per task has not been proven. Furthermore, the question stills remains whether the difference lays at the level of the response threshold or at the level of stimulus perception.

Inhibitor-activator or social inhibition model

Huang and Robinson [20] first proposed the inhibitor- activator model based on empirical work in honeybees. In their model, juvenile hormone (JH) acts as an intrinsic activator, which in a titre dependent manner regulates the timing of behavioural development in an individual. Juvenile hormone, together with a yet unknown extrinsic inhibitor of behavioural development, is transferred between individuals and dictates behavioural development of individuals and whole colonies. The activator-inhibitor model assumes all individuals are actively developing and therefore would naturally progress from in-nest to out-of-nest tasks; inhibition stops them from doing so (Figure 1.4). A key assumption of this model is that both activator and inhibitor titres are correlated with age. The first model based on the inhibitor-activator is

that of Naug and Gadagkar [21] who have individuals that increase the production of both activator and inhibitor as they age. The activator level feeds back onto the individual itself promoting behavioural development, while the inhibitor is transferred to other individuals (authors assume random interactions). For a given individual, the ratio between activator level A (intrinsic) and the level of inhibitor I (extrinsic) determines the tasks it will undertake. The authors empirically derive the A/I ratios of different groups: idle, nurses and, foragers based on work on the eusocial wasp *Ropalidia marginata*. They test their model with different conditions of task demand, through changing brood:adult ratios and age distributions. Task demand manipulations alter interaction rates between adults and therefore the mean age of individuals classified as nurses or foragers. With their model, they are able to generate flexible age polyethism patterns. Beshers et al. [22] apply the idea of social inhibition to behavioural temporal polyethism in honeybees and take the social inhibition model a step further. An individual's state x changes day to day based on a set of rules specified in their model. x depends on the individual's previous state and inhibition by other workers (average x for the group). The authors simulate changes to colony demography and assess the effect on foraging, their results successfully show social inhibition can explain how temporal polyethism is regulated.

Critiques and limitations to the social inhibition model, include the fact that not only has JH been ruled out as the activator molecule since it is not required for behavioural maturation (Sullivan et al. [23]), the existence of the inhibitor has yet to be found. Additionally, the assumption that activator/inhibitor titres are age

dependent allows for little flexibility.

Foraging for work model

This model of task allocation is based on work availability; initially proposed by Tofts and Franks [24]; and later expanded on by Tofts [25]. Simply stated, individuals perform a task which is needed and continue to do so until no longer needed, after which, they move in order to find another task which needs to be performed (Figure 1.5). The model is based on a linear succession of tasks and assumes all individuals are identical (no differences in response thresholds) and are unaware of their own and other's age. An individual performing a specific task, lets say task B, receives work passed on from individuals performing task A ("upstream") and in turn passes work along to individuals performing task C ("downstream"). If an individual performing task B detects an imbalance, not receiving work from A or work not being accepted by C, the individual can move towards the task where it detects a larger imbalance or stays at the current task. In this model, brood care is the first task in the linear array of tasks (spatial correlation of tasks described in Seeley [26]), and there is no previous task an ant performing brood care could move towards. Similarly, at the end of the array of tasks is foraging, where there is no further task an individual could move to. Therefore, the accumulation of workers performing brood care and the incoming work force of newly eclosed workers generates a "push" towards the neighbouring task. The model can give rise to stable states from any initial conditions and is able to do so in a limited number of steps. In terms of a pattern, it produces a weak temporal polyethism when new eclosing ants get incorporated

and old ants are removed. The main two critiques to the model given by Robinson et al [27] are: 1) the assumption that all individuals are identical (evidence of age related physiological differences exists in honeybees) and 2) that it can only produce weak temporal polyethism when strong yet flexible temporal polyethism is the norm. Robinson and co-authors argue temporal polyethism is a byproduct of the developmental process individual ants go through. In a reply to such critique, Franks and Tofts [28] point out that the correlation between physiological change and age does not mean that age causes physiological change, and point out that external changes could drive task allocation resulting in division of labour.

Information centred or network task allocation models

Seeley et al. [29] generated a mathematical model, known as the information centred model, based on how nectar foraging bees respond to changes in resource profitability. A bee's decision to continue exploiting a source or to abandon a source is based on information acquired indirectly through interactions with other nectar foragers. The authors present empirical data on which their model is based. Nectar foragers that are not committed to either nectar source start by following a dancer bee to a nectar source (two sources are available), where they collect nectar and return to the nest. At that point, the individual bee decides whether to abandon the nectar source (if profitability of nectar source is low) and return to being uncommitted and forage based on dance-based recruitment; or continue exploiting that source. If the individual is to continue exploiting the source, it can either unload and exit the nest on a foraging trip to the same source or, dance with the aim of recruiting

more foragers to the source (once again this decision depends on the profitability of the source). Information is transferred between individuals in the "dance-floor" area of the hive. However in the model, a bee will be recruited by the first dancer it encounters, and encounters are random. The model assumes the number of foragers is fixed and they all begin foraging simultaneously for either of the nectar sources available. The central idea behind this model is that each individual is capable of independently assessing the profitability of a source and it is the shared decision rules amongst individuals of what is a profitable source that organizes a concerted colony level response (Figure 1.6). Gordon et al. [30] took the information centred model a step further by looking at the distribution of workers in four different tasks and how that distribution changes when colonies are perturbed. By combining empirical work with *Pogonomyrmex barbatus* ants and models developed to describe the organization of brain processing, they look at changes in activity status within the different tasks. Workers can belong to four different behavioural groups: foragers, patrollers, midden workers and nest maintenance workers. Within each behavioural group individuals can have two states: active and inactive. Based on their behavioural group and activity status, ants are categorized. Individuals can only acquire information through pairwise interactions, and the model assumes all ants within a behavioural group interact before they change their status. Such an assumption is justified by the spatial constraints the nest entrance and immediately adjacent chambers impose on interacting ants. Activity status change is regulated by a negative feedback system; if an active individual interacts with the same number of active and inactive individuals in the behavioural group it remains active. Whereas, if it interacts with

more active than inactive ants it becomes inactive and, vice versa. Ants of different behavioural groups also interact, but only affect each others active/inactive status, not the behavioural group they belong to. The authors are able to achieve global changes in activity level of different behavioural groups in response to perturbations, which do not necessarily affect all behavioural groups directly. Pacala et al. [31] introduced switching between tasks to the model. If two individuals with the same activity status interact their status doesn't change. However if an inactive individual encounters an active one, it is recruited to the behavioural task the active individual is performing. The encounters in their model continue to be random and therefore are dependent on the density of individuals performing one task or the other. The authors also point out individuals are able to manage interaction rates by mechanisms such as group size regulation.

Social interactions have successfully explained recruitment of foragers in *P. barbatus*. Although this group of models can work in the context of recruitment, the extent to which this can be generalized to explain division of labour as a whole remains an open question. Currently, the detailed study of interactions in ants and honeybees is rapidly growing since social interactions as mechanism explaining division of labour is promising.

The models discussed above all aim to explain how division of labour emerges based on different organizing principles, which include: (1) age and physiology, (2) reinforcement (through learning or inhibition), (3) spatial localization, and (4) social

interactions (table 1.1). However, aside from those models which incorporate experience, most models do not explicitly take into account plasticity or inter-individual variation. The general goal of the present work is to contribute to our understanding of division of labour, by assessing how incorporating plasticity and inter- individual variation may extend these models in important ways.

1.9 The ant genus *Pheidole*

The ant genus *Pheidole* is one of the most specious ant genus within the Myrmicinae subfamily, with more than 2,800 species worldwide, and around 600 species in the new world alone (Hölldobler and Wilson [7]). *Pheidole* is one of 300 genera of ants (Bolton [32], Moreau et al. [2] and Moreau [33]), yet represent more than 6% of the entire world ant fauna (Pie and Tscha [34, 35]). Ants of the genus *Pheidole* are true superorganisms, where the queen and thousands of sterile workers coexist with no conflict over reproduction (Hölldobler and Wilson [1]). Additionally this hyper-diverse genus also has the morphological innovation of an additional worker caste, the soldier caste, specialized in defence.

1.10 Division of labour in *P. dentata*

Two *Pheidole* species have been widely used for studies of division of labour in ants; *P. pallidula* and *P.dentata*. For the purpose of my PhD work, I focused on division of labour amongst workers of the ant *Pheidole dentata*. *P. dentata* are distributed from Northern Mexico to mid-east United States. Their habitat varies

from wooded to sandy beaches. Their colouration is quite variable, from golden-brown to darker brown. Coloration appears to be associated with the habitat with light forms commonly found in open grounds and darker forms in forest habitats. In our collection sites around Gainesville, we find colonies living within fallen branches ranging from about 3-15 cm in diameter. The forest form, found in the south, produces monogynous colonies; while the lighter forms associated to the northernmost range are thought to be polygynous. *P. dentata* are easily collected and kept in laboratory conditions. In addition to this, it is a well characterized system where we have extensive background knowledge regarding colony demographics and behaviour (Wilson [36], Calabi and Traniello [37, 38], Burkhardt [39], Seid and Traniello [40]), as well as studies of age-related neurochemical changes (Seid and Traniello [41]) and neuroanatomical changes (Seid et al. [42]). Therefore, *P.dentata* is a well suited model for the study of colony organization. Due to the relatively advanced division of labour in *P. dentata*, it also provides a framework that allows for inferences about the role of plasticity on the evolution of division of labour patterns.

1.11 Biogenic amines from neuromodulation to behaviour

Biogenic amines, as neuromodulators, are known to be involved in the modulation and generation of individual-level behaviours across vertebrates and invertebrates (reviewed in Scheiner et al., [43]). In social insects, biogenic amines are associated with social behaviour (reviewed in Scheiner et al, [43] and Kamhi and Traniello [44]). In the present work I study their role in division of labour. Biogenic amines act as neurotransmitters, neuromodulators and neurohormones; and as such

are known to be involved in in the modulation and generation of behaviours such as learning, memory, reproduction, locomotion, aggression, and social behaviour in a wide variety of species. In hymenoptera, biogenic amines are known to modulate stimuli-specific responses (Scheiner et al. [43]. Unlike classic neurotransimitters -GABA, glutamate and acetylcholine- that act directly on ion channels and have rapid effect on postsynaptic responses; biogenic amines and neuropeptides have an indirect effect on channels that results in modulation of the response elicited by the classic neurotransmitters (reviewed in Bicker and Menzel [45]). Neuromodulators can decrease the threshold required for a stimulus to generate a behavioural response and the underlying action potential (reviewed in Birmingham and Tauck [46]); and do so by modifying neuronal physiology. Neuromodulators can generate changes in cellular traits including membrane resistance, firing rate, bursting properties, adaptation and even the shape of action potentials (reviewed in Birmingham and Tauck [46], Kaczmarek and Levitan [47]). Neuromodulators can also generate changes in gene transcription, protein synthesis, and enzymatic activity (reviewed in Libersat and Pflueger [48]. By modifying neuronal properties, neuromodulators can have an effect on the connectivity of circuits without changing the wiring itself, allowing animals to have immediate changes in responsiveness to their environment (Bicker and Menzel [45], Birmingham and Tauck, [46]). There are five biogenic amines: dopamine, norepinephrin, epinephrin serotonin and histamine. My work will focus on two of them, dopamine (DA) and serotonin (5HT), since previous research suggest these two biogenic amines correlate with temporal polyethism in *Pheidole dentata* (Seid and Traniello [41]). Dopamine is synthesized from tyrosine, which is the precursor

for all catecholamines (dopamine, norepinephrin, epinephrin) as well as octopamine and tyramine in invertebrates (reviewed by Blenau et al., 2001). While serotonin is synthesized from tryptophan and belongs to the tryptamines group along with melatonin and N,N-Dimethyltryptamine.

Biogenic amines act through cell surface receptors members of the G-protein coupled receptors (GPCR) family that make up 1-2% of animal genomes (reviewed in Hauser et al. [49]). The basic structure of such receptors is highly conserved with 7 transmembrane domains (TM), an extracellular amino-terminal and, an intracellular carboxyl-terminus. The binding site of biogenic amines is known as the TM bundle (composed of transmembrane domains 3, 5 and 6). Ligand binding induces a conformational change, which leads to the interaction with G-proteins (reviewed in Scheidner et al. [43], Hauser et al. [49]). Depending on the G-protein subtype, receptor activation effects the second messenger systems: cyclic AMP (cAMP), inositol 1,4,5-triphosphate (IP_3), diacylglycerol (DAG) or Ca_2. Dopamine receptors can be divided into two functional groups depending on their effect on cellular cAMP. Mammals have five subtypes of dopamine receptors. Invertebrates have orthologs for three dopamine receptors. Dopamine receptor 1 (DR1) and DR2 are functionally similar to vertebrate receptors, however insects posses a unique dopamine receptor DR3 which is more similar to an octopamine receptor. DR1 and DR2 belong to the D-1 family of dopamine receptors which are positively coupled to cAMP, while DR3 belongs to the D-2 family of dopamine receptors and is negatively coupled to cAMP. In the case of serotonin receptors in mammals types of receptors have been

described meanwhile in invertebrates, drosophila and honeybee, only the orthologs for serotonin receptor 1 (5HT1), 5HT2 and 5HT7 have been described (reviewed in Tierney [50], Hauser et al., [49], Blenau and Thamm [51]). Based on the type of G-proteins they are coupled with 5HT1 is negatively coupled with cAMP, 5HT7 are positively coupled with cAMP, while 5HT2 increases cellular levels of IP_3 and DAG. Aside from age-related changes in biogenic amine titres with age in *P. dentata* (Seid and Traniello [41]) and the role of biogenic amines in a number of social behaviours in ants (reviewd by Kamhi and Traniello [44]), little is known about their role in the organization of division of labour. Biogenic amines could potentially regulate predetermined responses to stimuli or mediate plastic responses to stimuli in the context of division of labour.

1.12 Closing remarks

My PhD thesis aims to bring us a step closer to addressing one of the central problems of insect sociobiology: "the reconstruction of mass behaviour from a knowledge of behaviour of single colony members " (E.O. Wilson). I present the body of my work in three chapters. Chapter 2 explores the process underlying the pattern of division of labour in *P. dentata*. Chapter 3 focuses on the case study of inter-individual variability in foraging behaviour and behavioural capacities. Chapter 4 explores the dopaminergic and serotoninergic systems in the context of inter-individual variability in foraging behaviour. Finally, I offer some concluding remarks on the significance of this work for the field of insect sociobiology.

References

[1] Hölldobler B, Wilson E (2008) *The superorganism* (Norton, London).

[2] Moreau C, Bell C, Vila R, Archibald SB, Pierce N (2006) Phylogeny of the ants: Diversification in the age of angiosperms. *Science* 312:101–104.

[3] Brady S, Schultz T, Fisher B, Ward P (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants. *PNAS* 103:18172–18177.

[4] Wheeler WM (1911) The ant-colony as an organism. *Journal of Morphology* 22:307–325.

[5] Wheeler WM (1926) Emergent evolution and the social. *Science* 64:433–440.

[6] Johnson B, et al. (2013) Phylogenomics resolves evolutionary relationships among ants, bees, and wasps. *Current Biology* 23:2058 – 2062.

[7] Hölldobler B, Wilson E (1990) *The ants* (Belknap Press).

[8] Oster G, Wilson E (1978) *Caste and Ecology in the Social Insects* (Princeton University Press).

[9] Bonabeau E, Theraulaz G, Deneubourg J, Aron S, Camazine S (1997) Self-organization in social insects. *Trends in Ecology and Evolution* 12:188 – 193.

[10] Detrain C, Deneubourg J (2006) Self-organized structures in a superorganism: do ants "behave" like molecules? *Physics of Life Reviews* 3:162 – 187.

[11] Page R, Mitchell S (1990) Self organization and adaptation in insect societies. *Proceedings of the Biennial Meeting of the Philosphy of Science Association* 2:289–298.

[12] Page R, Mitchell S (1998) Self organization and the evolution of division of labour. *Apidologie* 29:171–190.

[13] Franks N, Tofts C, Sendova-Franks A (1997) Studies of the division of labour: neither physics nor stamp collecting. *Animal Behaviour* 53:219 – 224.

[14] Beshers S, Fewell J (2001) Models of division of labor in social insects. *Annual Review of Entomology* 46:413–440.

[15] Wilson E (1976) Behavioral discretization and the number of castes in an ant species. *Behavioral Ecology and Sociobiology* 1:141–154.

[16] Bonabeau E, Theraulaz G, Deneubourg J (1996) Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proceedings of the Royal Society London B* 263:1565–1569.

[17] Bonabeau E, Theraulaz G, Deneubourg J (1998) Fixed response thresholds and the regulation of division of labor in insect societies. *Bulletin of Mathematical Biology* 60:753–807.

[18] Fewell J, Bertram S (1999) Division of labor in a dynamic environment: response by honeybees (*Apis mellifera*) to graded changes in colony pollen stores. *Behavioral Ecology and Sociobiology* 46:171–179.

[19] Theraulaz G, Bonabeau E, Denuebourg JN (1998) Response threshold reinforcements and division of labour in insect societies. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 265:327–332.

[20] Huang Z, Robinson G (1992) Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proceedings of the National Academy of Sciences* 89:11726.

[21] Naug D, Gadagkar R (1999) Flexible division of labor mediated by social interactions in an insect colony: a simulation model. *Journal of Theoretical Biology* 197:123 – 133.

[22] Beshers S, Huang Z, Oono Y, Robinson G (2001) Social inhibition and the regulation of temporal polyethism in honey bees. *Journal of Theoretical Biology* 213:461 – 479.

[23] Sullivan JP, Jassim O, Robinson GE, Fahrbach SE (2000) Juvenile hormone paces behavioral development in the adult worker honey bee. *Hormones and Behaviour* 37:1–14.

[24] Tofts C, Franks N (1992) Doing the right thing: Ants, honeybees and naked mole-rats. *Trends in Ecology and Evolution* 7:346 – 349.

[25] Tofts C (1993) Algorithms for task allocation in ants. (a study of temporal polyethism: Theory). *Bulletin of Mathematical Biology* 55:891 – 918.

[26] Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology* 11:287–293 10.1007/BF00299306.

[27] Robinson, G.E.and Page R, Huang Z (1994) Temporal polyethism in social insects is a developmental process. *Animal Behaviour* 48:467 – 469.

[28] Franks N, Tofts C (1994) Foraging for work: how tasks allocate workers. *Animal Behaviour* 48:470 – 472.

[29] Seeley, T.D. CS, Sneyd J (1991) Collective decision-making in honey bees: how colonies choose among nectar sources. *Behavioral Ecology and Sociobiology* 28:277–290.

[30] Gordon D, Goodwin B, Trainor L (1992) A parallel distributed model of the behaviour of ant colonies. *Journal of Theoretical Biology* 156:293 – 307.

[31] Pacala S, Gordon D, Godfray H (1996) Effects of social group size on information transfer and task allocation. *Evolutionary Ecology* 10:127–165.

[32] Bolton B (2006) *Bolton's catalogue of ants of the world, 1758-2005* (Harvard University Press).

[33] Moreau C (2008) Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution* 48:224 – 239.

[34] Wilson EO (2003) *Pheidole in the New World: A Dominant, Hyperdiverse Ant Genus* (Harvard University Press, Cambridge, MA).

[35] Pie M, Tscha M (2009) The macroevolutionary dynamics of ant diversification. *Evolution* 63:3023–3030.

[36] Wilson E (1984) The relation between caste ratios and division of labor in the ant genus *Pheidole* (hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* 16:89–98.

[37] Calabi P, Traniello J (1989) Behavioral flexibility in age castes of the ant *Pheidole dentata*. *Journal of Insect Behavior* 2:663–677.

[38] Calabi P, Traniello J (1989) Social organization in the ant *Pheidole dentata*. *Behavioral Ecology and Sociobiology* 24:69–78.

[39] Burkhardt J (1998) Individual flexibility and tempo in the ant, *Pheidole dentata*, the influence of group size. *Journal of Insect Behavior* 11:493–505.

[40] Seid M, Traniello J (2006) Age-related repertoire expansion and division of labor in *Pheidole dentata* (hymenoptera: Formicidae): A new perspective on temporal polyethism and behavioral plasticity in ants. *Behavioral Ecology and Sociobiology* 60:631–644.

[41] Seid M, Traniello J (2005) Age-related changes in biogenic amines in individual brains of the ant *Pheidole dentata*. *Naturwissenschaften* 92:198–201.

[42] Seid, M.A. Harris K, Traniello J (2005) Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. *The Journal of Comparative Neurology* 488:269–277.

[43] Scheiner R, Baumann A, Blenau W (2006) Aminergic control and modulation of honeybee behaviour. *Current Neuropharmacology* 4:259.

[44] Kamhi J, Traniello J (2013) Biogenic amines and collective organization in a superorganism: Neuromodulation of social behavior in ants. *Brain Behavior and Evolution* 82:220–236.

[45] Bicker G, Menzel R (1989) Chemical codes for the control of behaviour in arthropods. *Nature* 337:33–39.

[46] Birmingham T, Tauck D (2003) Neuromodulation in invertebrate sensory systems: from biophysics to behavior. *The Journal of Experimental Biology* 206:3541–3546.

[47] Kaczmarek L, Levitan I (1987) *Neuromodulation: Biochemical control of neuronal excitability* (Oxford University Press).

[48] Libersat F, Pflueger H (2004) Monoamines and the orchestration of behavior. *BioScience* 54:17–25.

[49] Hauser F, Cazzamali G, Williamson M, Blenau W, Grimmelikhuijzen C (2006) A review of neurohormone GPCRs present in the fruitfly *Drosophila melanogaster* and the honey bee *Apis mellifera*. *Progress in Neurobiology* 80:1 – 19.

[50] Tierney A (2001) Structure and function of invertebrate 5-HT receptors: a review. *Comparative Biochemistry and Physiology Part A* 128:791 – 804.

[51] Blenau W, Thamm M (2011) Distribution of serotonin 5-HT and its receptors in the insect brain with focus on the mushroom bodies: Lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arthropod Structure and Development* 40:381 – 394.

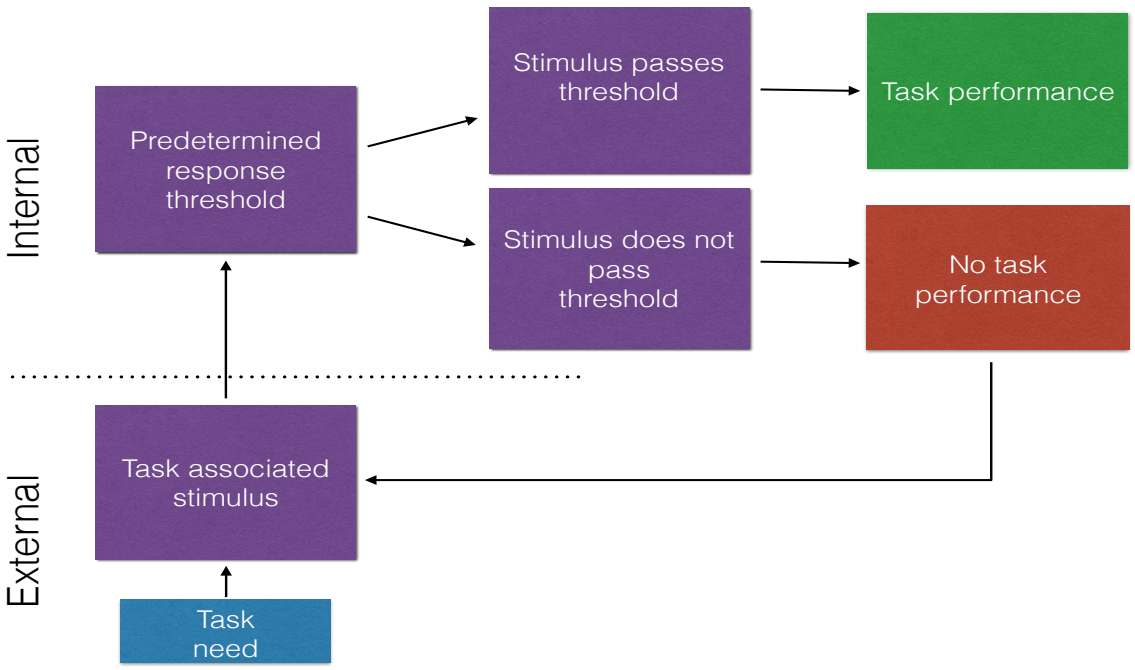

Figure 1.1: **Fixed response threshold model**. An individual has a predetermined response threshold, and based on the need for task performance a task associated stimulus accumulates. If the stimulus reaches the threshold level the individual performs the task, if not the stimulus continues to accumulate.

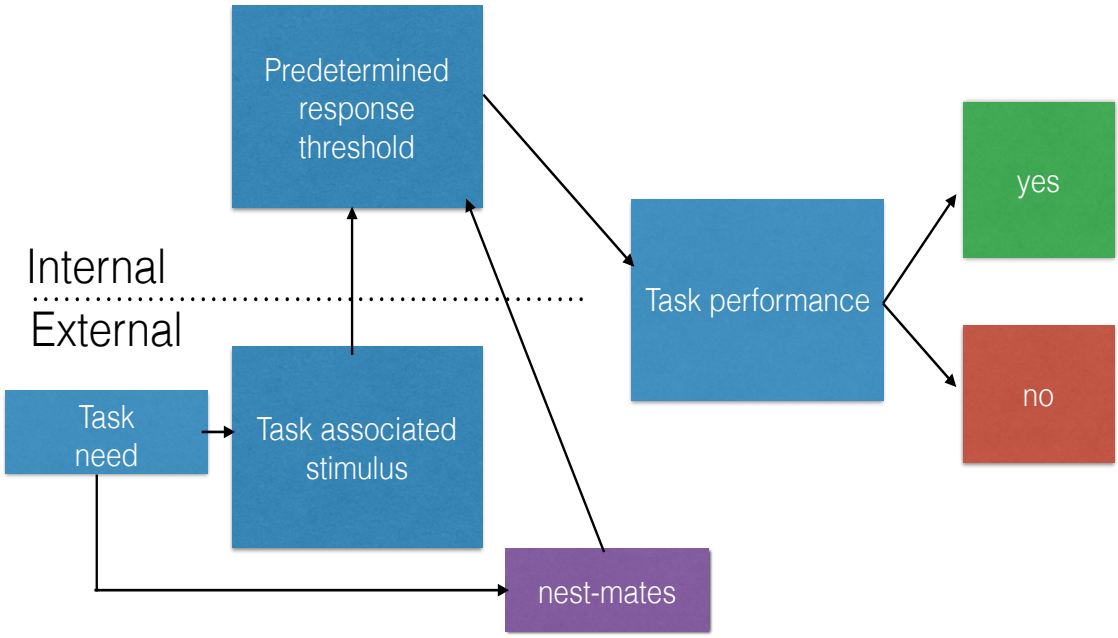

Figure 1.2: **Information transfer model**. An individual has a predetermined response threshold, and based on the need for task performance a task associated stimulus accumulates. If the stimulus reaches the threshold level the individual performs the task, if not the stimulus continues to accumulate. However the individual can also receive information on stimulus levels indirectly through nestmates

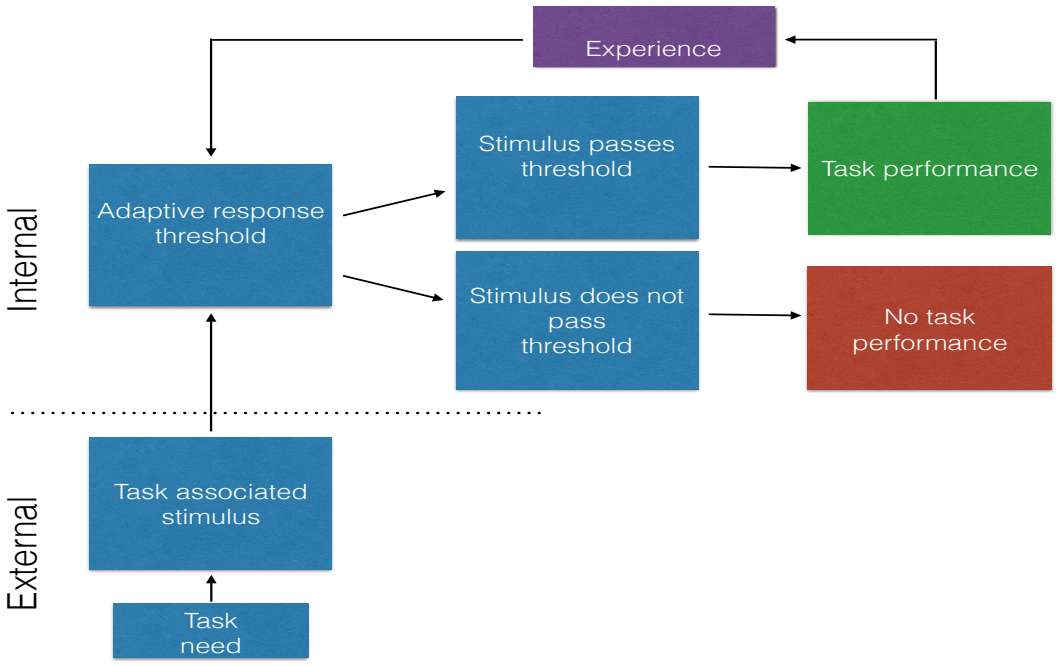

Figure 1.3: **Adaptive response threshold or self-reinforcement model**. An individual has a response threshold, and based on the need for task performance a task associated stimulus accumulates. If the stimulus reaches the threshold level the individual performs the task, if not the stimulus continues to accumulate. However the individual's response threshold can change as a result of experience.

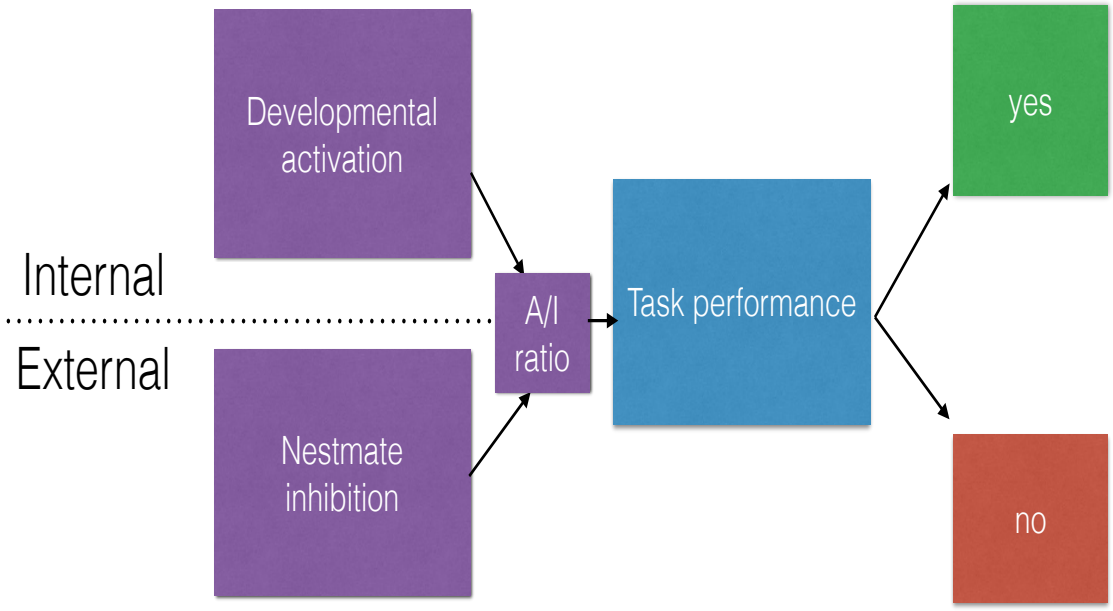

Figure 1.4: **Activator/ Inhibitor or social inhibition model**. As and individual develops it produces an activator molecule which dictates behavioural development. Meanwhile individuals also produce an extrinsic inhibitor of behavioural development that is transferred between individuals. The ratio between the activator and the inhibitor determine the threshold level at which the individual performs the task.

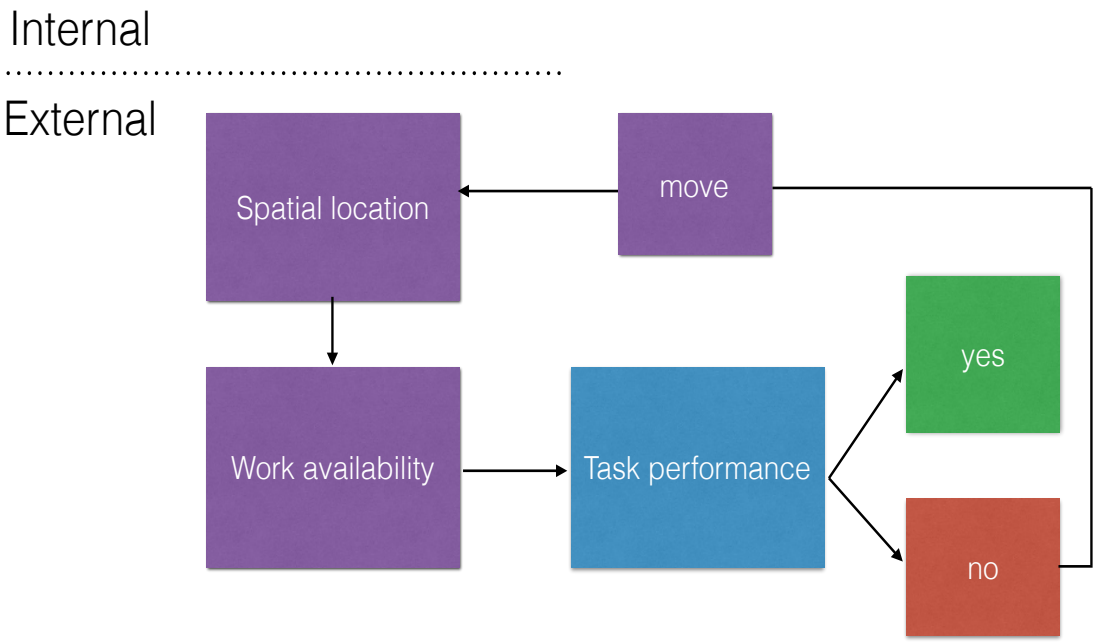

Figure 1.5: **Foraging for work model**. Individuals perform a task which is needed and continue to do so until no longer needed, after which, they move along the spatial sequence of tasks in order to find another task which needs to be performed.

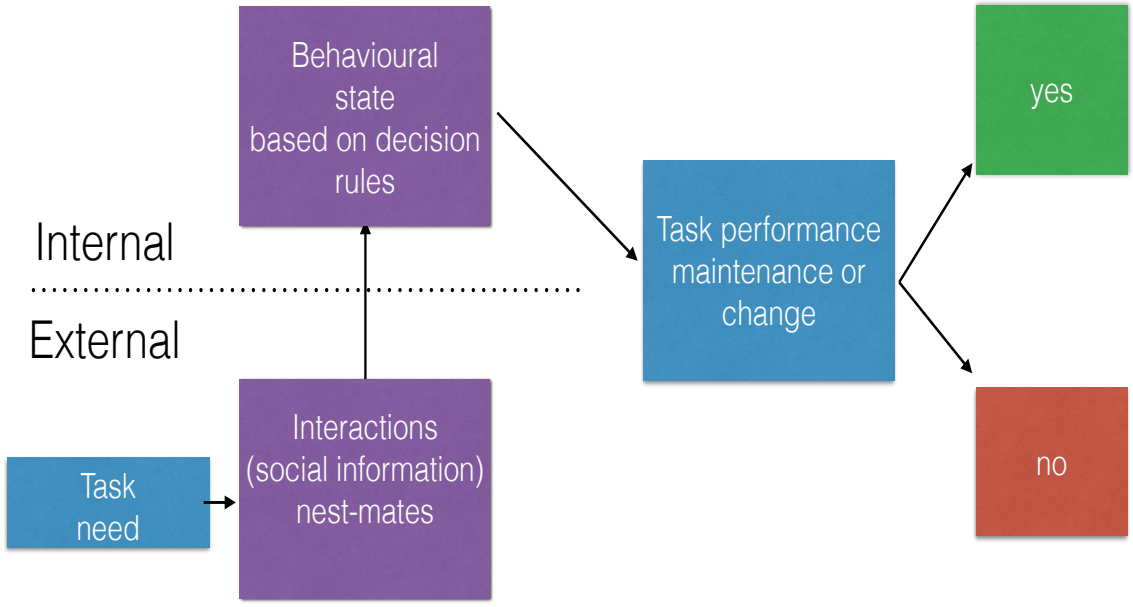

Figure 1.6: **Information centered or network task allocation models**. An individual's behavioural state depends on shared decision rules. Either through direct experience or through social interactions an individual gathers information based on which it decides to continue performing the task or gets recruited to another task.

Model	Assumptions	References	Organizing principles
Fixed response threshold	one stimulus per task, static response thresholds	Wilson [15], Page and Mitchell [11, 12] Bonabeau et al [16, 17], Fewell and Bertram [18] (incorporates info acquisition)	age and physiology
Adaptive response threshold or self reinforcement	experience shapes responsiveness to stimulus	Theraulaz et al [19]	age, physiology, and experience
Inhibitor-activator or social inhibition	ratio of intrinsic activator and extrinsic inhibitor	Huang and Robinson [20], Naug and Gadagkar [21], Beshers and Fewell [22]	age and physiology
Foraging for work	spatial distribution of tasks	Tofts and Franks [24], Tofts [25], Franks and Tofts [28]	spatial location
Network task allocation	threshold in combination with interactions	Seeley [29], Gordon et al [30], Pacala et al [31]	reinforcement

Table 1.1: **Summary of division of labour models**: key assumptions, references and organizing principles

CHAPTER 2
Inter-individual differences and colony level patterns

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2.1 Abstract

Division of labour models traditionally assume that in the case of morphological division of labour all the individuals within a caste have similar sets of behaviours; or that in the case of temporal polyethism all individuals within a particular age group or spatial location behave similarly. In contrast to this assumption, our work shows inter-individual variability in behavioural ontogeny is abundant and appears to be the rule rather than the exception. Bees transition from one set of task to another set of tasks as they age. In *Pheidole dentata*, previous studies of temporal polyethism based on age groups had shown that, unlike bees workers expand their repertoire without abandoning previous tasks. However, our results show that within an age group there is abundant inter-individual variability and same age individuals do not show the expected concerted and concordant addition of tasks to their repertoire. For some behaviours the pattern of repertoire expansion, although different in detail from the one previously described, is reconstructed when we average all the individuals. Therefore, fine-scale inter-individual variability plays an important role in the organization of division of labour within the worker caste of advanced ant societies.

2.2 Introduction

Within-group variation in ants

The first references to individual differences in ants date back the 1930s, and since then, individual differences have been shown in a variety of contexts. Work by Chen [1] showed differences in nest building between individuals of the ant species *Campanotus japonicus*. Barnes [2] described differences in activity levels between individuals in *Aphaenogaster fulva, Lasius flavus, Formica exsectoides* and *Formica fusca*. Work on learning in *Formica incerta* and *Formica subsericea* showed individual differences in maze learning (Schneirla [3], reviewed in Morley [4]). Later work showed division of labour appears within groups of individuals of the same age and size (Lenoir [5]). In the ant *Lasius niger*, Lenoir [6] showed that only 60% of the older workers become foragers while the rest stay in the nest their entire life. Similar results have also been described in other Formicines, *Formica polyctena* and *Formica sanguinea* (reviewed in Lenoir [5]). Corbara and Fresnau [7] found different behavioural profiles among individuals of the same age in *Ectatomma ruidum*. In the ant *Cataglyphis cursor*, Retana and Cerda [8] found only one third of workers had a classical behavioural progression, the rest showed variations. In Ponerines, individual variability was described in *Odontomachus troglodytes* (Dejean and Lachaud [9] and in *Diacamma sp* (Nakata [10]). Variation has been ascribed to individual idiosyncracy (Jeanne [11]) and although within-group variation has been described previously, it is not until recently that it's potential significance for division of labour has been considered (Jeanson and Weidenmüller [12]). However, so far the term inter-individual variability has been used to refer to within-group variation and not

stricktly to variation between individuals. We will use inter-individual variation to refer to the fine-scale variation between individuals. In the present paper, we study significance of inter-individual variability for division of labour; and to understand its role in division of labour we analyse the behavioural progression of worker *P. dentata* individuals as they age.

Division of labour: morphological and temporal polyethism

Division of labour is a key feature for the complex organization and ecological success of social insects. Morphologically distinct castes in ants (queen, soldiers, and workers) form one of the most diverse and best differentiated caste systems amongst the social hymenoptera. The production of distinct morphological castes is polyphenic, meaning that it is mediated by environmental cues including abiotic environmental conditions, such as temperature, humidity, photoperiod and nutrition; and biotic conditions, such as colony composition (reviewed in Wheeler [13]). Thus, ant colonies can tune the ratios of the different morphological castes produced based on colony needs. In the ant genus *Pheidole*, queens are specialized in reproduction, while the sterile worker subcastes undertake all other tasks within the colony. Large, big-headed soldiers are specialized in colony and territory defence and have a role in food storage, while smaller, small-headed, minor workers perform all the other tasks including brood care, foraging and nest maintenance. In addition to the stable pattern of task allocation associated with morphology, worker ants also display division of labour based on the change of roles they perform within the colony during their life time; this pattern in known as temporal polyethism (Wilson [14]). Wilson

in a different study [15] proposed the existence of discrete temporal castes, which are age-independent groups of individuals that emerge within a morphological caste based on the suite of tasks they perform in the colony.

Within temporal polyethism, there are two traits of interest: (1) the sequence of behavioural roles and (2) the organization of this sequence. In terms of task sequence, the shift from performing tasks inside the nest to tasks outside the nest is well established in honeybee workers (Rösch [16, 17, 18], Sakagami [19, 20], Sekiguchi and Sakagami [21], Seeley [22], Kolmes [23], Huang and Robinson[24]). However, while the same pattern is thought to be true for ants (Hölldobler and Wilson [25]), there is little data supporting the existence such pattern (reviewed in Gordon [26]). Variation in the inside-nest to outside-nest sequence has been found in three ant subfamilies: Myrmicines (Beshers and Traniello [27]), Formicines (Corbara et al. [7] and Retana et al. [8]) and Ponerines (Dejean and Lachaud [9] and Nakata [10]). Thus, there is a striking difference in terms of the organization of task sequence in the temporal polyethism patterns between honey bees and ant species. In addition to the inside-nest to outside-nest pattern, honeybees shift from one set of tasks to another set as they age (Seeley [22]). In the ant species *Pheidole dentata* detailed behavioural studies have revealed a pattern of temporal polyethism strikingly different from the one in honeybees. In honeybees, workers shift from one set of tasks to another in the course of their life (Rösch [16, 17, 18]; Sakagami [19, 20], Sekiguchi and Sakagami [21], Seeley [22], Kolmes [23], Huang and Robinson[24]). In contrast, worker ants of the species *Pheidole dentata* show an accumulation of tasks as they age resulting

in behavioural repertoire expansion (Seid ant Traniello [28]). Behavioural repertoire expansion was identified by grouping individuals by age and recording their task performance. Within a whole colony age classes can be classified according to the pigmentation of adult *P. dentata* worker ants (Figure 2.1). Seid and Traniello [28] classified worker ants into four age classes (AC1-AC4), which correspond to the following days post eclosion: AC1 days 1-3 (D1-D3 individuals have a light yellow pigmentation of the head, thorax and abdomen), AC2 (D4-D8 the abdomen develops pigmentation from light to dark brown), AC3 (D9-19 the head develops pigmentation from light to dark brown, followed by the pigmentation of the thorax) and AC4 D20 and older (D20+ individuals develop a darker brown to black pigmentation throughout). Their data was obtained through scan sampling of full colonies for a two hour period during which task performance by age class was recorded. If an individual is observed grooming a larva this behaviour is recorded as a behaviour corresponding to the repertoire of an age class based on the pigmentation of the individual performing the task, (Figure 2.3 A based on [28]) shows the repertoire of tasks performed by each age class. The emerging pattern shows that tasks which are performed by younger age classes are maintained as part of the repertoire of older age classes and new tasks are added on to the repertoire as ants age. Based on the pattern of repertoire expansion, we expected individuals to have relatively concordant age-dependent behavioural progression.

One of the outstanding questions in the field of sociobiology is to understand how division of labour is achieved. Several models have been proposed to explain

how division of labour, specifically temporal polyethism, is generated in social insects. The current models of division of labour (summarized in table 2.1 and reviewed in section 1.8) are based on age, physiology, spatial location or experience as organizing principles. These models implicitly or explicitly assume that depending on the organizing principle used, all individuals belonging to a group are behaviourally homogeneous. However, the existence of variation in so many aspects of individual behaviour (including the onset of behaviours, the timing of behavioural transitions and the final behavioural repertoires) suggests inter-variation is important for division of labour. Our aim is to study the significance of inter-individual variability for division of labour; for this purpose we use temporal polyethism amongst workers of the ant *Pheidole dentata* as a model.

2.3 Materials and methods

Colony collection and care

We collected queenright colonies of *Pheidole dentata* in Gainsville, Florida in spring 2009, 2010, 2012 and 2013. We kept colonies in Fluon-lined plastic boxes (either 27x19x10 cm or 31x22x10 cm depending on colony size) with red cellophane covered test tubes partially filled with water and tight cotton plugs. We fed ants three times a week with a combination of 1 M sucrose, fresh mealworms, fresh waxworms and Whitcomb diet. We maintained colonies in a Conviron environmental chamber (Controlled Environments Ltd., Winnipeg, Manitoba) under a 12L:12D light cycle at 27°C and 70% relative humidity.

Experimental setups for behavioural progression

In order to assess individual behavioural progression, we first set up single cohorts consisting of 70 late pupa (medium dark pigmentation), 30 AC4 ants, 6 white pupa, and 10 second to third instar larvae, 10 late larva and a pile of eggs and microlarva (schematic in Figure 2.2 panel A). We placed individuals in a small clear plastic boxes (14 x 10 x 4 cm) with dental stone bottom which retains humidity. For a nest space, we used a microscope slide (0.75 x 0.25 x 0.3 cm) held in place with modelling clay and covered by red cellophane to create an undisturbed dark nest area for ants. Ants were allowed to acclimate to setups overnight and on the following morning pupa had eclosed (Figure 2.2 panel B). We individually marked the newly eclosed individuals using Markel paints and assembled behavioural progression setups on eclosion day 1 (D1) (Figure 2.2 panel C). In order to establish whether demographic composition had an effect on the overall pattern of behavioural ontogeny, we used two experimental setup compositions. Behavioural progression setups (BP setups) contained 20 individually labelled D1 old individuals, 30 D20+ individuals (corresponding to AC4 based on pigmentation), 3 white pupa, 5 dark gutted larva, 5 late larva, a pile of eggs and microlarva. Alternatively setups containing 20 individually labelled D1 old individuals, 30 non labelled D1 old individuals, 3 white pupa, 5 dark gutted larva, 5 late larva, a pile of eggs and microlarva (PF setups). We allowed ants to acclimate overnight and started behavioural observations on D2.

Behavioural observations

With the aim of obtaining individual level behavioural progression data, we followed focal individuals, individually labelled ants, as they aged over the first 20 days D2 to D21 (which covers the period corresponding from AC1 to early AC4 as classified by Seid and Traneillo [28]). We observed setups under a dissecting microscope for a total of 25 minutes/day for 20 days. Observations were distributed over a period of 1.5 hours, 5 minutes observations inter-spaced by 15 minutes of no observation and were completed between 10:00 and 17:00 hours every day. During each 5 minute period, we performed instantaneous scan sampling (based on Altmann [29]) of behaviours performed by the focal individuals. We collected absence/ presence behavioural data for all focal individuals for 13 different mechanical tasks (detailed behaviours and their definitions are shown in Table 2.2).

Behavioural data representation and comparison between studies

The individual absence/presence behavioural data we collected was used to generate a heatmap representation of their behavioural profile for the 13 tasks observed during the 20 day period. Absence presence data is represented in black and white squares respectively (Figure 2.3 B, data represented as a grid). The repertoire expansion model (Seid and Traniello [28]) implicitly assumes that as individuals age, they consistently add tasks to their repertoire and progress through behavioural ontogeny in a concerted manner (adding tasks in the same order and at the same relative time). To translate the repertoire expansion model and compare it to individual-level data we used the behaviours highlighted in figure 2.3 panel A and represented them as

an absence/presence map Figure 2.3 panel B. The behaviours highlighted in panel A are the ones we used in our study with exiting the nest as an addition (see Table 2.2).

Testing for randomness in our data

In order to test the randomness of our data we used a null model and Shannon's entropy index (Shannon [30], based on Kolmes [31]). First, for the null model we randomly generated 20X13 matrices which correspond to 10000 "individuals". We used different probabilities of task performance (performance:non-performance 50:50, 30:70, 10:90), since the level of activity of the observed individuals is lower than what we would expect from a 50:50 chance of task performance. Second we measured Shannon entropy, a method borrowed from the field of information theory used for quantifying uncertainty in a random variable. In our case the random variable represents the tasks an individual performs. The maximum entropy H_{1MAX} ($H_{1MAX} = \log_2 N$, where N is the total number of different behaviours), corresponds to the highest entropy and represents randomness (the equal probability of any individual performing any task). At the other end of the spectrum, a value of entropy closer to zero represents extreme specialization were individuals can be identified based on the performance of a single or small set of tasks (Kolmes use of marginal entropy according to Gorelick and Bertram [32]). The entropy for each setup h is calculated by

$$h = -\sum_{i=1}^{\#\text{ of behaviours}} p_i \log_2 p_i$$

where p_i is the number of ants performing behaviour i over the total number of behaviours.

Data analyses

We performed all data analyses using Mathematica (Wolfram Research).

2.4 Results

Abundant inter-individual variability in behavioural progression

A representative individual (Figure 2.4) is first observed assisting eclosion of nestmates and grooming larva; on subsequent days it starts carrying pupa and grooming pupa adding these two tasks to its repertoire. Allogrooming, trophallaxis, handling dead and exiting the nest come later. And in accordance to the repertoire expansion model (REM) those tasks which we observe being performed early are observed during the subsequent days. From the 13 tasks we analysed, this individual was not observed carrying or grooming eggs and microlarva, feeding larva or foraging. It does show an increase in number of tasks in it's repertoire over time, however it does not cover the full breadth of tasks studied. A representative subset of individuals, with each individual represented by a heat-map is shown in Figure 2.5. Surprisingly, there is abundant inter-individual variability in behavioural ontogeny, suggesting individuals are not a homogeneous group. Using the numbers across columns and letters across rows as coordinates, we can identify a few individuals are not observed performing any tasks during the whole observation period,

those individuals stay inside the nest and remain idle for long periods of time (individuals *5f* and *7c*). Some other individuals perform tasks inside the nest earlier on, and later perform tasks outside the nest; an example of such case is individual *1f*. Other individuals tend to stay inside the nest performing tasks during the whole observation period; an example of such case is *2d*. Although general trends can be described, the pattern of each individual is unique; except for those individuals which are not observed performing any of the tasks studied.

Abundant inter-individual variability is present regardless of setup composition

In order to assess whether inter-individual variability is a consistent trend and not an artefact resulting from a response to extreme environmental perturbations we compared patterns between setups with different compositions. Behavioural progression setups in which the focal individuals where accompanied by D20+ foragers (Figure 2.6 panels A and B) and precocious forager setups, those in which only individuals of the same age as the focal individuals were present (Figure 2.6 panels C and D). We find a similar rage of inter-individual variability regardless of setup composition (Figure 2.6 panels A, B, C, and D). The general patterns emerging from the average of all individuals in each setup are relatively similar between behavioural progression setups (Figure 2.8 panels A and B) and precocious forager setups (Figure 2.8 panels C and D). However to quantify the degree of similarity between the general pattern resulting from the two different setup compositions we used entropy. We found similar levels of entropy (Figure 2.9 behavioural progression setups represented with squares and precocious forager setups represented with diamonds). We

observe a similar range of variability between setup compositions, even when setups initially have the same composition but each setup changes in an independent way over the 20 day observation period.

The abundant inter-individual variation observed is not due to randomness in behaviour

In order to test whether the behavioural patterns observed are random, that is the probability of any individual performing any behaviour is equal, we used two approaches: first, we take a null-model approach, where we compare our data with the pattern that results from ten thousand randomly generated *individuals*. The average of the 10000 randomly generated individuals (Figure 2.10 panel A) while Figure 2.10 panel B shows the average of the 79 individuals we followed through the 20 days. In the case of the randomly generated individuals (Figure 2.10 panel A) there is no temporal pattern of behavioural performance, all behaviours are performed throughout the 20 day period, which is not consistent with the repertoire expansion model.

In order to analyse patterns quantitatively we compared entropy level between the null model (which corresponds to the maximum entropy, represented by circles) and our data represented by squares and diamonds (Figure 2.9). Both approaches support that individuals are not all equally likely to perform any task, that is their behaviour differs from random.

Inter-individual variability partially recapitulates the pattern produced by REM

Based on the repertoire expansion model (REM, Seid and Traniello [28]) we would expect an age-dependent increase in the number of tasks performed by workers. Although the pattern of repertoire expansion disappears in the inter-individual variability (see Figure 2.5), when the absence/presence data for each ant is transformed into the proportion of ants performing each task over the 20 days period (Figure 2.7, values are represented as a corrected gradient from black, closer to zero, to white, closer to one), the pattern of repertoire expansion re-emerges for some behaviours, but is different for others. Individuals perform allogrooming, trophallaxis, grooming and carrying of larva and pupa as the first tasks added on to their repertoire. Later individuals get engaged in assisting eclosion as well as grooming and carrying eggs and microlarva followed by feeding larva. Handling dead, exiting the nest and foraging are the last tasks added to the repertoire. Therefore, the general trend that emerges from our data recapitulates, to a certain extent, the repertoire expansion model previously described for worker *P. dentata* ants.

2.5 Discussion and Conclusions

We found abundant inter-individual variation in behavioural progression between same age individuals and demonstrated that inter-individual behavioural variation is a significant factor for division of labour. Our results show: (1) abundant inter-individual variability when the behaviour of individuals was followed through the first 20 days of behavioural ontogeny; (2) inter-individual variation is present regardless of demography; (3) the inter-individual variation observed is not due to

randomness in the behaviour and the overall pattern that emerges shows traits consistent with the repertoire expansion pattern previously described in *P. dentata*, and (4) variation is found both in the in-nest to out-of-nest transition and the tasks comprising individual's repertoire by day 20. Together our findings support the biological significance of inter-individual variation.

Inter-individual variability: implications for division of labour

A number of models have been proposed in order to understand how division of labour, specifically temporal polyethism is generated. One element that models have in common is that based on the organizing principle individuals are assigned into groups (based on age, physiology, spatial location, or experience). Studies of colony level ratios of physical castes and age classes of *Pheidole dentata* in different habitats found no correlation between caste/class ratios with ecological variables of the different habitats (Calabi and Traniello [33]) and find similar colony level behavioural profiles between habitats. Those findings support our hypothesis that inter-individual variability has a role in the organization of division of labour.

We previously referred to two sub-patterns of task organization found in temporal polyethism: the spatial transition (inside-outside nest) and the task transition/addition pattern (see section 2.2). We find that not all individuals show the transition from inside to outside, since most individuals do not transition to outside the nest tasks over the period of 20 days, although by D20 the full behavioural repertoire of individuals is thought to be developed (Seid and Traniello [28]). In the case of our data (Figure 2.10 panel B) there is a temporal pattern in the performance

of behaviours, with some behaviours observed throughout the 20 days and others being added later on, in accordance with the repertoire expansion model (Seid and Traniello [28]). However our data suggests that the finding of repertoire expansion based on age classes represents the breadth of the set of tasks individuals of a given age can perform, and does not necessarily translate into individuals actually performing all the tasks in their corresponding age class repertoire. In terms of task addition/ transition our data suggest the addition of tasks to the repertoire since tasks performed during the early days of observation are usually observed later as well.

Recently, individual variability has brought lots of attention. Within-group behavioural variation has been shown to be biologically significant not only in ants (Modlmeier et al., [34]), but also in social spiders (Pruitt and Riechert,[35]). Even broader, the importance of recognizing the implications of inter-individual variability at an ecological level has been emphasized (Bolnick et al. [36]). Social insects, specifically ants have a remarkable ecological success. The outstanding question now is whether inter-individual variability has a role in the generation of division of labour in other ant species and social insects in general.

Future research

Although inter-individual variability is the product of complex non-linear interactions between genes and environment, in order to build predictions and test for the possibility of an environmental influence on the generation of inter-individual

variation, we make predictions under a scenario of complete genetic determination, and a scenario of complete environmental determination, and a scenario of complete stochasticity. We propose three alternative hypotheses. First, inter-individual variability could be genetically determined through differences in matrilines or patrilines. In such case inter-individual variability would be unresponsive to environmental conditions and therefore we would predict the existence of clear groups. Individuals from different groups would have different capacities to perform tasks, this could be due to differences in responsiveness to stimuli (Calabi [37], Seid and Traniello [28]). As a whole, colonies would have limited robustness and resilience due to the lack of behavioural plasticity. Second, inter-individual variability could be generated through a plastic process responsive to environmental cues and tuned to colony needs. Plasticity can be either developmental or physiological/behavioural. In the case of developmental plasticity we would expect to find inter-individual variability among individuals as soon as they emerge as adults. Alternatively, in the case of physiological/behavioural plasticity differences would arise after individuals emerge as adults. If inter-individual variability results from physiological/behavioural plasticity, we would expect to find varying responsiveness to the environment during the early adult stages. However due to plasticity itself, individuals would be continuously adjusting to the environment and therefore not form clear groups. Finally, inter-individual variability could be generated through a stochastic process. In this scenario, all individuals have the same responsiveness to the environment but they differentiate into groups due to chance.

Ant colonies of *Pheidole dentata* in their southern range have low genetic variability. Colonies are monogynous and thought to be singly mated, therefore it is unlikely we would observe this level of inter-individual variation as a result of genetic variability. Our results suggest inter- individual variability is not due to a purely stochastic process, since we find evidence against individual's behaviour being random. However it is possible that stochastic processes initially set individuals out into different trajectories which later are reinforced through experience. We therefore propose inter-individual variability is generated through either a plastic process or a stochastic process followed by reinforcement. Differentiating between the two remains challenging. However, several studies have shown response to diverse environmental stimuli such as demographic and work load manipulations (Lenoir [6], Calabi [38, 37, 39]), which support the hypothesis that inter-individual variation is generated through a plastic process.

2.6 Acknowledgments

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References

[1] Chen S (1937) The leaders and followers among the ants in nest-building. *Physiological Zoology* 10:437–455.

[2] Barnes T (1941) Rhythms of activity in ant colonies. *Journal of General Psychology* 25:249–255.

[3] Schneirla T (1943) The process and mechanism of ant learning: the combination-problem and the successive-presentation problem. *Journal of Comprehensive Psychology* 17:303–328.

[4] Morley D (1946) Division of labour in ants. *Nature* 158:913–914.

[5] Lenoir A (1987) *From Individual to Collective Behavior in Social Insects*, Experientia Supplementum, eds Pasteels JM, Jean-Louis D (Birkhauser Verlag, Basel) Vol. 54, pp 219–240.

[6] Lenoir A (1979) Feeding behaviour in young societies of the ant *Tapinoma erraticum*: Trophallaxis and polyethism. *Insectes Sociaux* 26:19–37 10.1007/BF02283910.

[7] Corbara B, Lachaud JP, Fresneau D (1989) Individual variability, social structure and division of labour in the ponerine ant *Ectatomma ruidum Roger* (hymenoptera, formicidae). *Ethology* 82:89–100.

[8] Retana J, Cerda X (1991) Behavioural variability and development of *Cataglyphis cursor* ant workers (hymenoptera, formicidae). *Ethology* 89:275–286.

[9] Dejean A, Lachaud JP (1991) Polyethism in the ponerine ant *Odontomachus troglodytes*: Interaction of age and interindividual variability. *Sociobiology* 18:177–196.

[10] Nakata K (1995) Age polyethism, idiosyncrasy and behavioural flexibility in the queenless ponerine ant, *Diacamma sp. Journal of Ethology* 13:113–123.

[11] Jeanne R (1991) *The social biology of wasps*, eds Ross K, R.W. M (Cornell University Press), pp 398–425.

[12] Jeanson R, Weidenmüller A (2013) Interindividual variability in social insects proximate causes and ultimate consequences. *Biological Reviews* pp n/a–n/a.

[13] Wheeler D (1991) The developmental basis of worker caste polymorphism in ants. *The American Naturalist* 138:1218–1238.

[14] Wilson E (1976) Behavioral discretization and the number of castes in an ant species. *Behavioral Ecology and Sociobiology* 1:141–154.

[15] Wilson E (1968) The ergonomics of caste in the social insects. *American Naturalist* 102:41–66.

[16] Rösch G (1925) Untersuchungen über die arbeitsteilung im bienenstaat. *Zeitschrift für vergleichende Physiologie* 2:571–631.

[17] Rösch G (1927) Über die bautätigkeit im bienenvolk und das alter der baubienen. *Zeitschrift für vergleichende Physiologie* 6:264–298.

[18] Rösch G (1930) Untersuchungen über die arbeitsteilung im bienenstaat. *Zeitschrift für vergleichende Physiologie* 12:1–71.

[19] Sakagami SF (1953) Untersuchungen uber die arbeitsteilung in einem zwergvolk der honigbiene. beitrage zur biologie des bienenvolkes,*Apis mellifera L. Japanese Journal of Zoology* 2:117185.

[20] Sakagami S (1953) Untersuchungen über die arbeitsteilung in einem zwergvolk der honigbiene: Beiträge zur biologie des bienenvolkes, *Apis mellifera L. Japanese Journal of Zoology* 11:117–185.

[21] Sekiguchi K, Sakagami S (1966) Structure of foraging population and related problems in the honeybee, with considerations on the division of labour in bee colonies. *Hokk Nat Agric Exp Stn Rep* 69.

[22] Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology* 11:287–293 10.1007/BF00299306.

[23] Kolmes SA (1985) A quantitative study of the division of labour among worker honey bees. *Zeitschrift fr Tierpsychologie* 68:287–302.

[24] Robinson, G.E.and Page R, Huang Z (1994) Temporal polyethism in social insects is a developmental process. *Animal Behaviour* 48:467 – 469.

[25] Hölldobler B, Wilson E (1990) *The ants* (Belknap Press).

[26] Gordon D, Chu J, Lillie A, Tissot M, Pinter N (2005) Variation in the transition from inside to outside work in the red harvester ant *Pogonomyrmex barbatus*. *Insectes Sociaux* 52:212–217.

[27] Beshers S, Traniello J (1996) Polyethism and the adaptiveness of worker size variation in the attine ant *Trachymyrmex septentrionalis*. *Journal of Insect Behavior* 9:61–83.

[28] Seid M, Traniello J (2006) Age-related repertoire expansion and division of labor in *Pheidole dentata* (hymenoptera: Formicidae): A new perspective on temporal polyethism and behavioral plasticity in ants. *Behavioral Ecology and Sociobiology* 60:631–644.

[29] Altmann J (1974) Observational study of behavior: Sampling methods. *Behaviour* 49:227–267.

[30] Shannon C (1948) A mathematical theory of communication. *Bell Systems technical journal* 27:379–423, 623–656.

[31] Kolmes SA (1985) An information-theory analysis of task specialization among worker honey bees performing hive duties. *Animal Behaviour* 33:181 – 187.

[32] Gorelick R, Bertram S (2007) Quantifying division of labor: borrowing tools from sociology, sociobiology, information theory, landscape ecology, and biogeography. *Insect Sociaux* 54:105–112.

[33] Calabi P, Traniello J (1989) Social organization in the ant *Pheidole dentata*. *Behavioral Ecology and Sociobiology* 24:69–78.

[34] Modlmeier AP, Liebmann JE, Foitzik S (2012) Diverse societies are more productive: a lesson from ants. *Proceedings of the Royal Society B: Biological Sciences* 279:2142–2150.

[35] Pruitt JN, Riechert SE (2011) How within-group behavioural variation and task efficiency enhance fitness in a social group. *Proceedings of the Royal Society B: Biological Sciences* 278:1209–1215.

[36] Bolnick DI, et al. (2003) The ecology of individuals: Incidence and implications of individual specialization. *The American Naturalist* 161:pp. 1–28.

[37] Calabi P (1988) *Advances in Myrmecology*, ed Trager J (E.J. Brill).

[38] Calabi P (1986) Ph.D. thesis (Boston University).

[39] Calabi P, Traniello J (1989) Behavioral flexibility in age castes of the ant *Pheidole dentata*. *Journal of Insect Behavior* 2:663–677.

[40] Page R, Mitchell S (1990) Self organization and adaptation in insect societies. *Proceedings of the Biennial Meeting of the Philosphy of Science Association* 2:289–298.

[41] Page R, Mitchell S (1998) Self organization and the evolution of division of labour. *Apidologie* 29:171–190.

[42] Bonabeau E, Theraulaz G, Deneubourg J (1996) Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proceedings of the Royal Society London B* 263:1565–1569.

[43] Bonabeau E, Theraulaz G, Deneubourg J (1998) Fixed response thresholds and the regulation of division of labor in insect societies. *Bulletin of Mathematical Biology* 60:753–807.

[44] Fewell J, Bertram S (1999) Division of labor in a dynamic environment: response by honeybees (*Apis mellifera*) to graded changes in colony pollen stores. *Behavioral Ecology and Sociobiology* 46:171–179.

[45] Theraulaz G, Bonabeau E, Denuebourg JN (1998) Response threshold reinforcements and division of labour in insect societies. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 265:327–332.

[46] Huang Z, Robinson G (1992) Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proceedings of the National Academy of Sciences* 89:11726.

[47] Naug D, Gadagkar R (1999) Flexible division of labor mediated by social interactions in an insect colony: a simulation model. *Journal of Theoretical Biology* 197:123 – 133.

[48] Beshers S, Huang Z, Oono Y, Robinson G (2001) Social inhibition and the regulation of temporal polyethism in honey bees. *Journal of Theoretical Biology* 213:461 – 479.

[49] Tofts C, Franks N (1992) Doing the right thing: Ants, honeybees and naked mole-rats. *Trends in Ecology and Evolution* 7:346 – 349.

[50] Tofts C (1993) Algorithms for task allocation in ants. (a study of temporal polyethism: Theory). *Bulletin of Mathematical Biology* 55:891 – 918.

[51] Franks N, Tofts C (1994) Foraging for work: how tasks allocate workers. *Animal Behaviour* 48:470 – 472.

[52] Seeley, T.D. CS, Sneyd J (1991) Collective decision-making in honey bees: how colonies choose among nectar sources. *Behavioral Ecology and Sociobiology* 28:277–290.

[53] Gordon D, Goodwin B, Trainor L (1992) A parallel distributed model of the behaviour of ant colonies. *Journal of Theoretical Biology* 156:293 – 307.

[54] Pacala S, Gordon D, Godfray H (1996) Effects of social group size on information transfer and task allocation. *Evolutionary Ecology* 10:127–165.

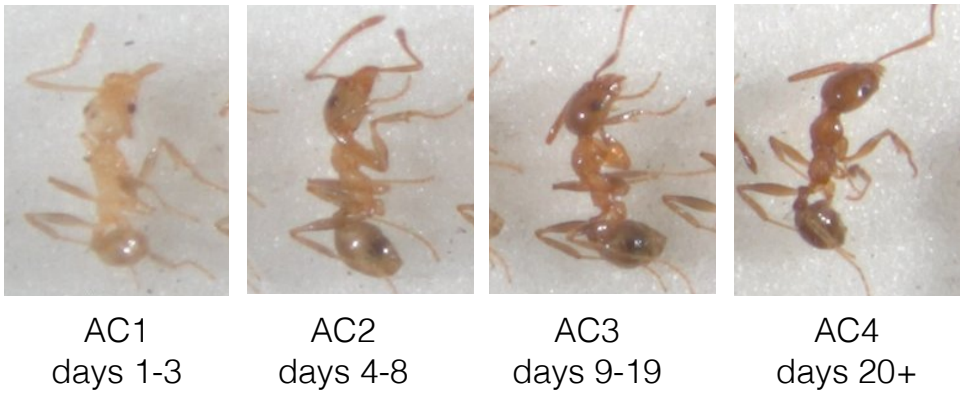

Figure 2.1: **Age classes and cuticular pigmentation in *P. dentata* workers**. Dorsal view of individual worker ants of *P. dentata* classified into age classes (AC) based on cuticular pigmentation. AC1 individuals show light yellow pigmentation throughout the three body segments, this stage lasts for 3 days, AC2 individuals show a darkening of the abdominal segment and this stage lasts for 4 days, AC3 individuals show darkening of the head and this stage lasts for 10 days, finally AC4 individuals develop a dark pigmentation throughout and this pigmentation is maintained for the rest of their life.

Model	Assumptions	References	Organizing principles
Fixed response threshold	one stimuli per task, static response thresholds	Wilson [14], Page and Mitchell [40, 41] Bonabeau et al [42, 43], Fewell and Bertram [44] (incorporates info acquisition)	age and physiology
Adaptive response threshold or self reinforcement	experience shapes responsiveness to stimuli	Theraulaz et al [45]	age, physiology, and experience
Inhibitor-activator or social inhibition	ratio of intrinsic activator and extrinsic inhibitor	Huang and Robinson [46], Naug and Gadagkar [47], Beshers and Fewell [48]	age and physiology
Foraging for work	spatial distribution of tasks	Tofts and Franks [49], Tofts [50], Franks and Tofts [51]	spatial location
Network task allocation	threshold in combination with interactions	Seeley [52], Gordon et al [53], Pacala et al [54]	reinforcement

Table 2.1: **Summary of division of labour models**: key assumptions, references and organizing principles

behaviour	operational definition
allogroom	an individual cleans itself or another individual
trophallaxis	an individual exchanges food with another individual
carry egg or microlarva	an individual transports an egg or a microlarva either by carrying or rolling it around
groom egg or microlarva	an individual cleans an egg or microlarva
assist eclosion	an individual grooms either a late larva as it ecloses into a pupa or a pupa when it ecloses into a young adult
carry pupa	an individual transports a pupa either by carrying or rolling it around.
groom pupa	an individual cleans a pupa
carry larva	individual transports a larva either by carrying or rolling it around.
groom larva	individual cleans a larva
feed larva	an individual either feeds a larva through trophallaxis or feeds a larva solid food such as fragments of dead adult ants
handle dead	an individual carries and/or removes fragments of a dead ant
exit nest	an individual leaves the nest
forage	an individual leaves the nest, finds food and eats food

Table 2.2: ***P. dentata* Ethogram**. Behaviours and operational definitions used for behavioural progression evaluation of individuals

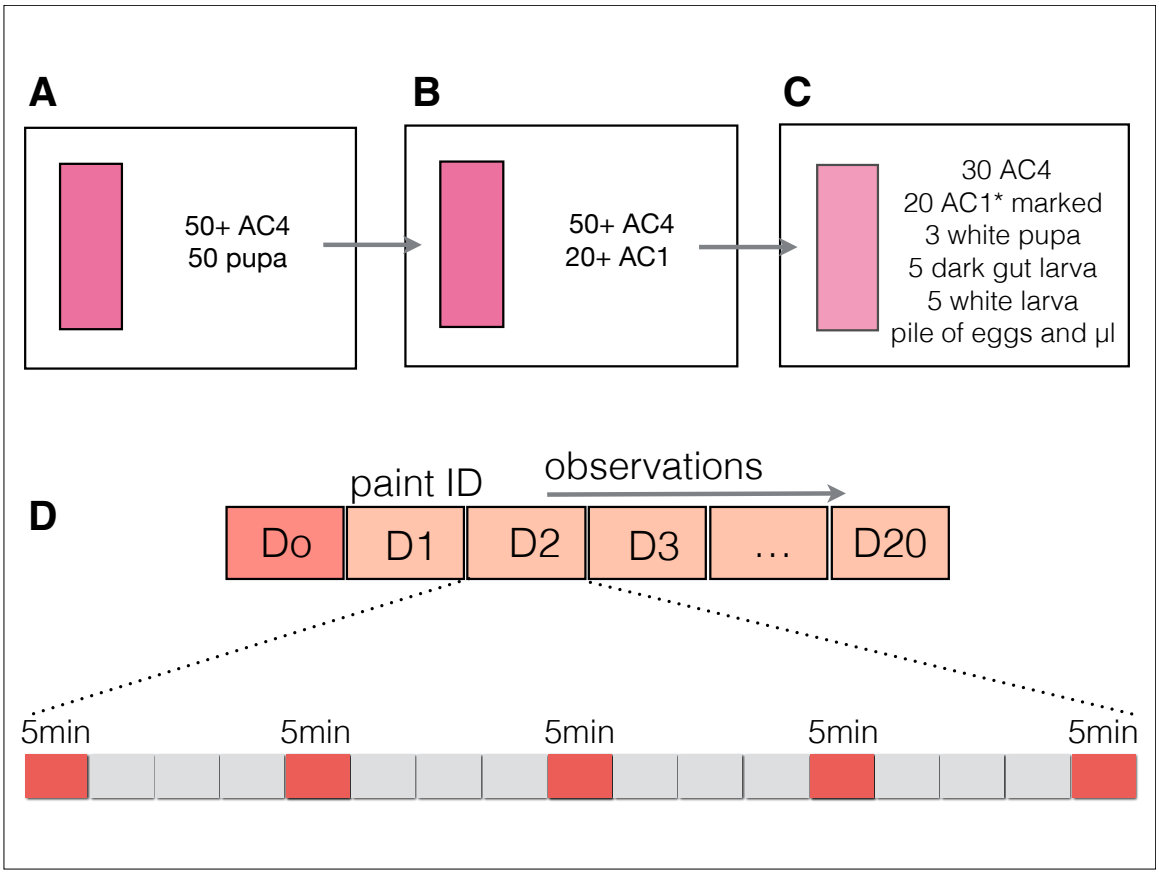

Figure 2.2: **Behavioural setups and observations for long-term behavioural progression**. Schematic representation of setups **A**: single cohort setup **B**: setup composition after eclosion **C**: Behavioural progression setup composition

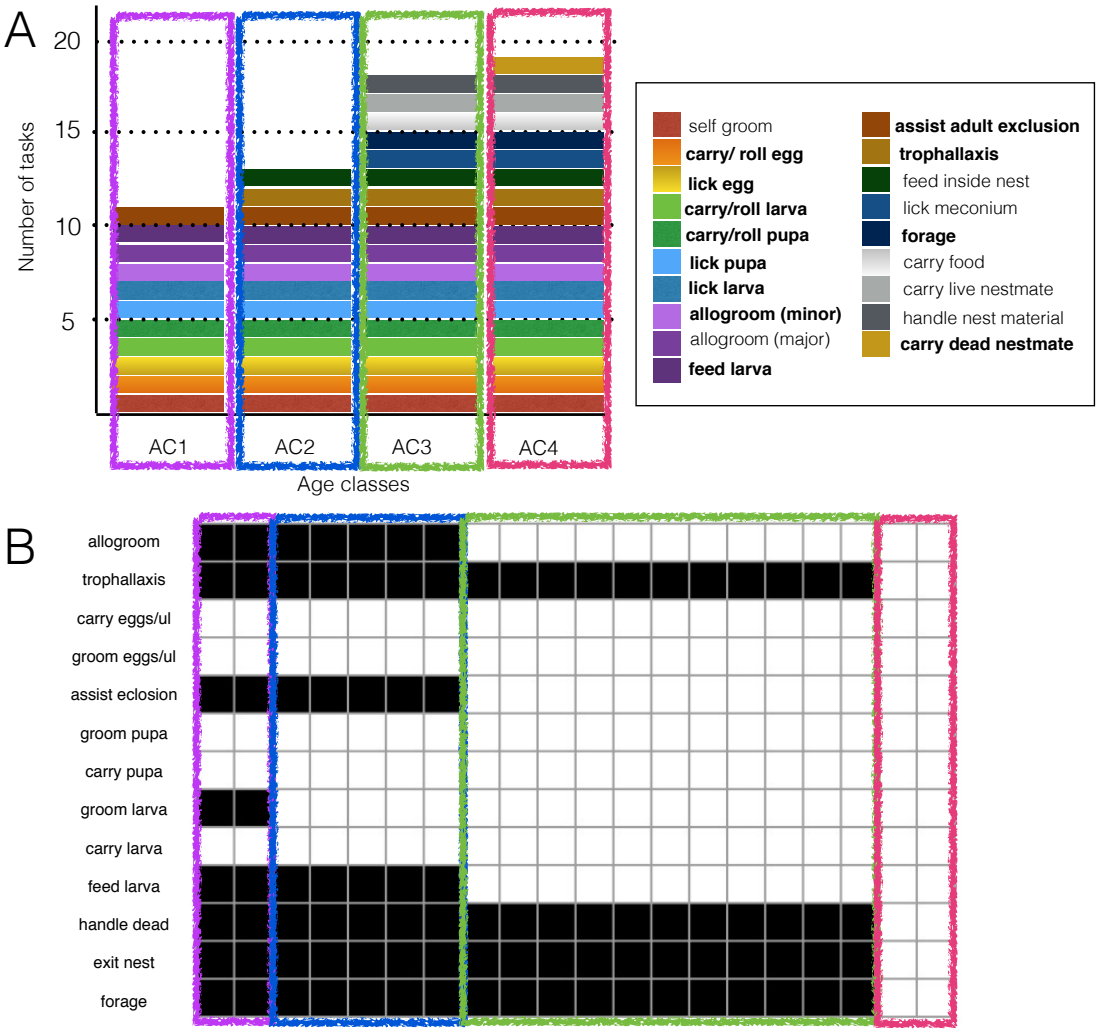

Figure 2.3: **Expectations based on repertoire expansion model**. Based on the repertoire expansion by age classes where each color represents a task performed by a member of each age class regardless of the frequency (modified from [28]) shown in panel A, we would expect the pattern of task absence/presence shown on panel B. The first tasks we would expect to appear in individual's repertoires would be carrying and grooming eggs and microlarva, carrying and grooming pupa and carrying larva. The next task to be added to the repertoire would be grooming larva, followed by allogrooming, assisting the eclosion of a nestmate and feeding larva. Finally the last tasks expected to appear in the repertoire would be directional trophallaxis, exiting the nest along with foraging would be the last tasks to be added to the repertoire.

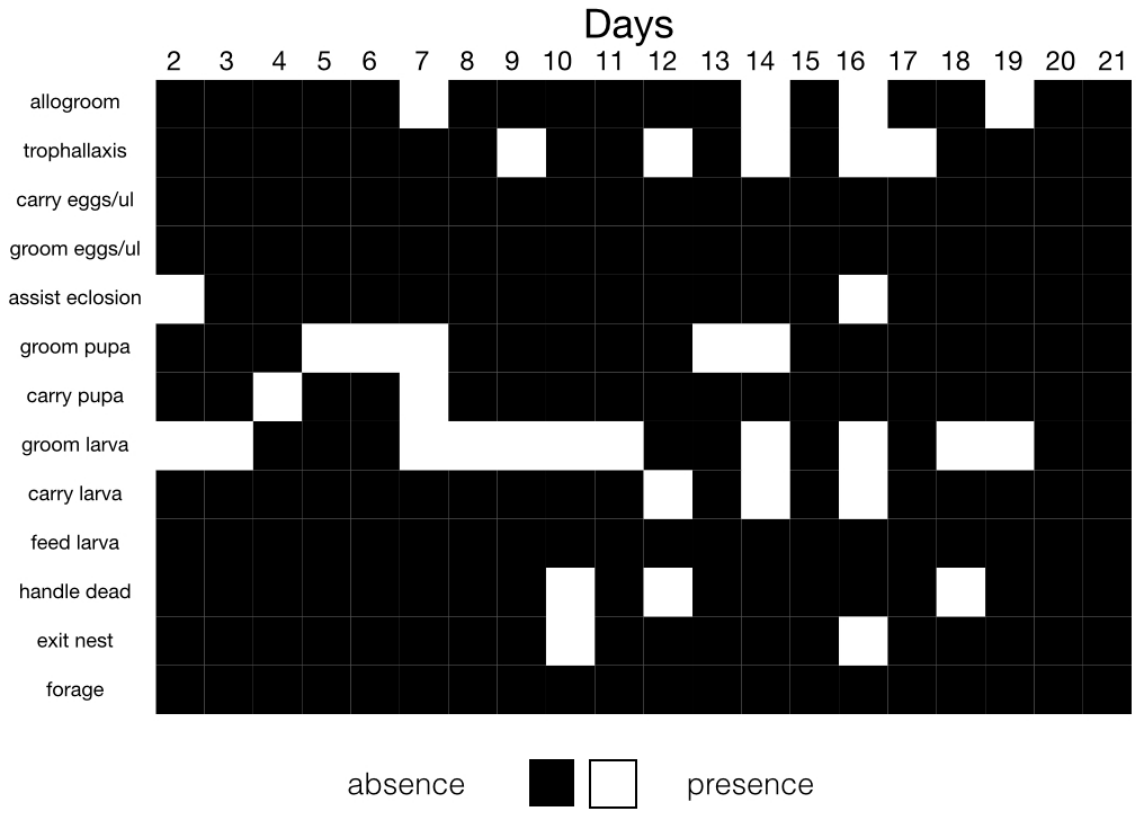

Figure 2.4: **Representative individual in detail: behavioural progression over 20 days**. The behavioural data obtained over the 20 day observation period is represented as a heat map. Days are represented across columns and behaviours as rows. As indicated by the legend, task performance will is represented by a white square, the absence of task performance by a black square. The individual shown first is observed assisting eclosion of nestmates and grooming larva on subsequent days it adds carrying pupa and grooming pupa to its repertoire. Allogrooming, trophallaxis, handling dead and exiting the nest come later. From the 13 tasks we analysed this individual was not observed carrying or grooming eggs and microlarva, feeding larva or foraging. It does show an increase in tasks which comprise it's repertoire over time, however it does not explore the full breadth of tasks studied.

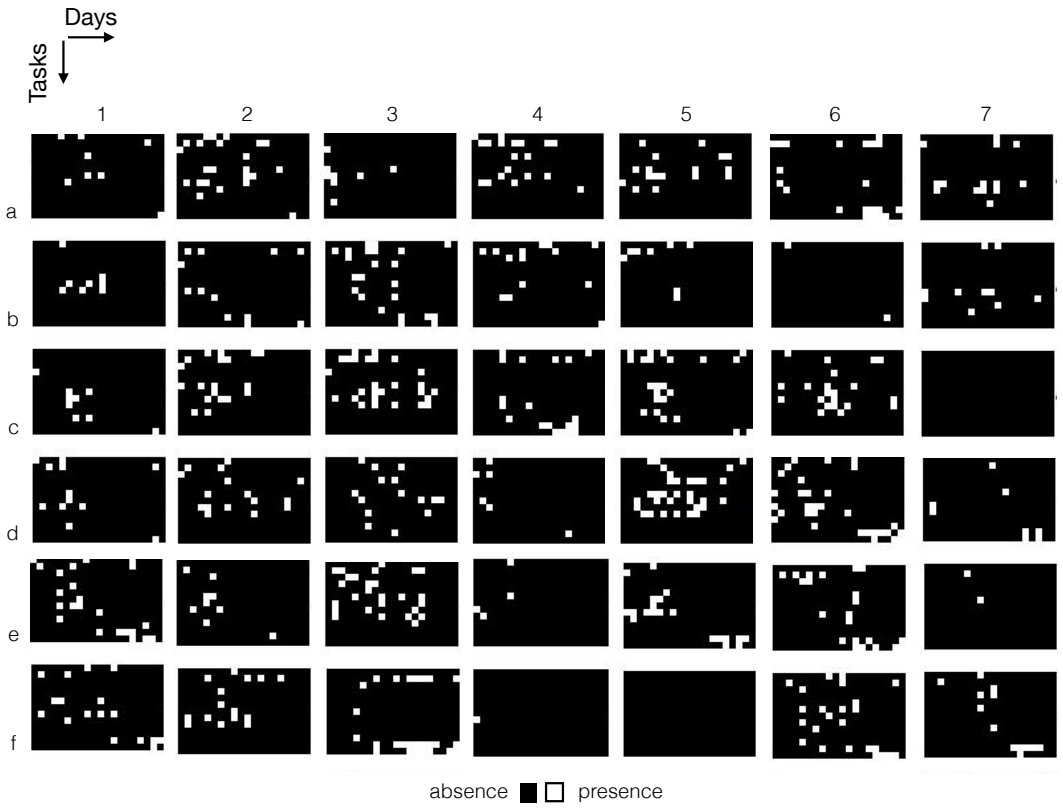

Figure 2.5: **Individual behavioural progression from D2-D21**. Individual behavioural progression from D2-D21 for a subsample of 42 individuals is shown, absence (black) and presence (white) of a behaviour given by day over the 20 day period.

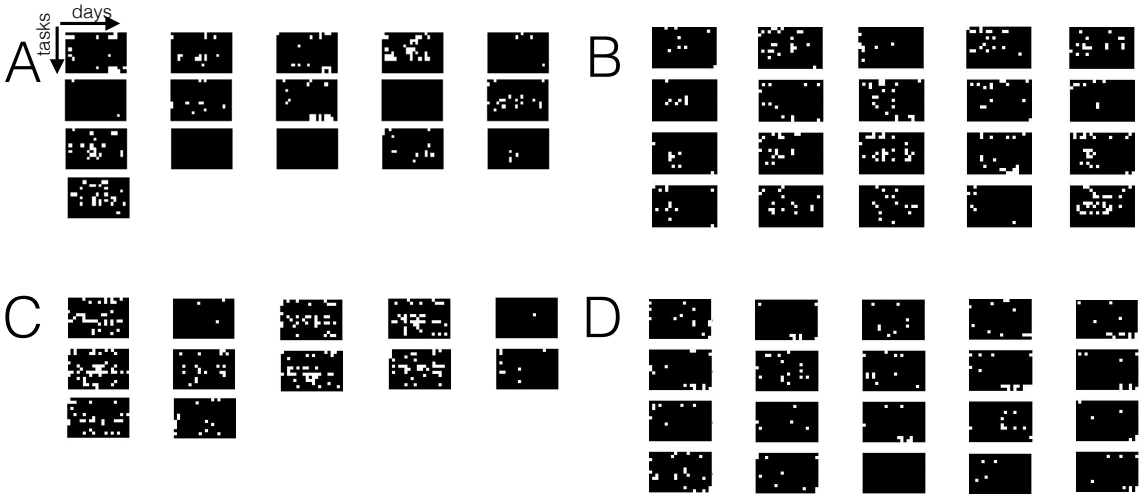

Figure 2.6: **Inter-individual variability and setup composition**. Panels A and B show all the individuals corresponding to two behavioural progression setups. Panels C and D all the individuals corresponding to setups where no D20+ foragers were present. Each square represents an individual with days represented across columns and behaviours represented as rows.

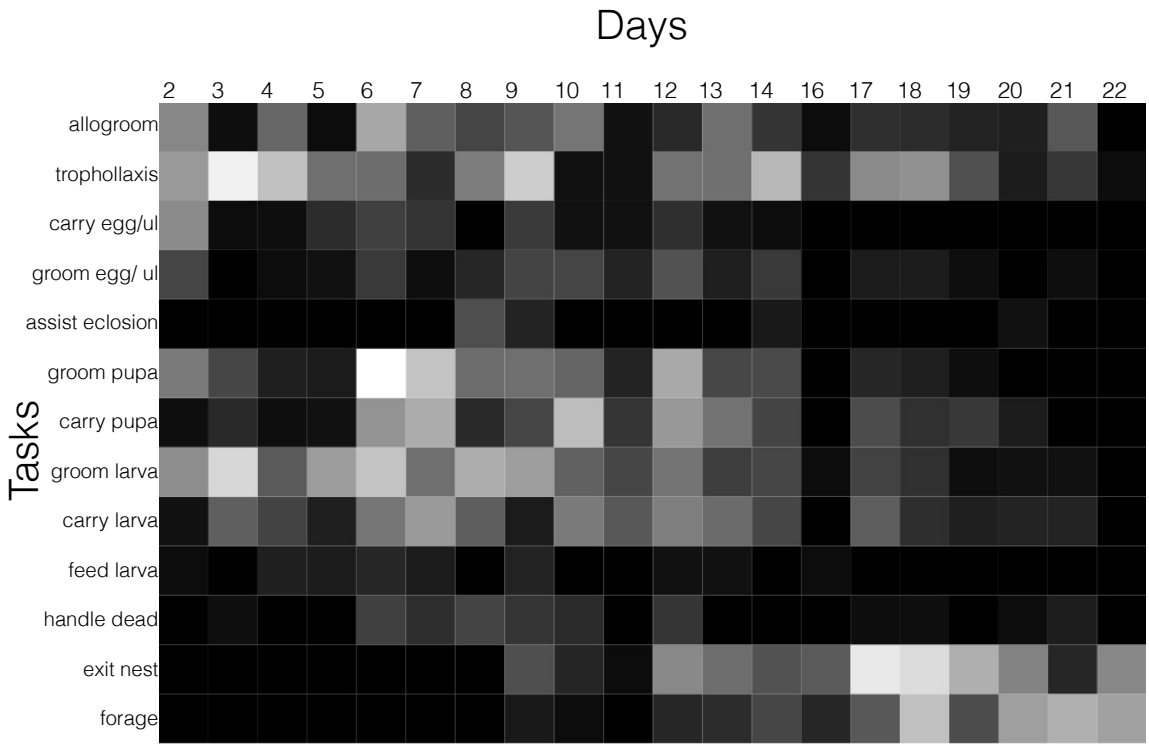

Figure 2.7: **Behavioural progression data from D2-D21**. The proportion of workers performing a task on a given day is shown as a gradient from black (closer to zero) to white (closer to one). N=79 individuals from four different setups. The general trend emerging from all the individuals observed shows allogrooming, trophallaxis, grooming and carrying of larva and pupa as the first behaviours added on to their repertoire. Later individuals get engaged in assisting eclosion as well as grooming and carrying eggs and microlarva followed by feeding larva. Handling dead, exiting the nest and foraging are the last tasks added to the repertoire. The general pattern does show an increase in number of tasks through time, as suggested by the repertoire expansion model.

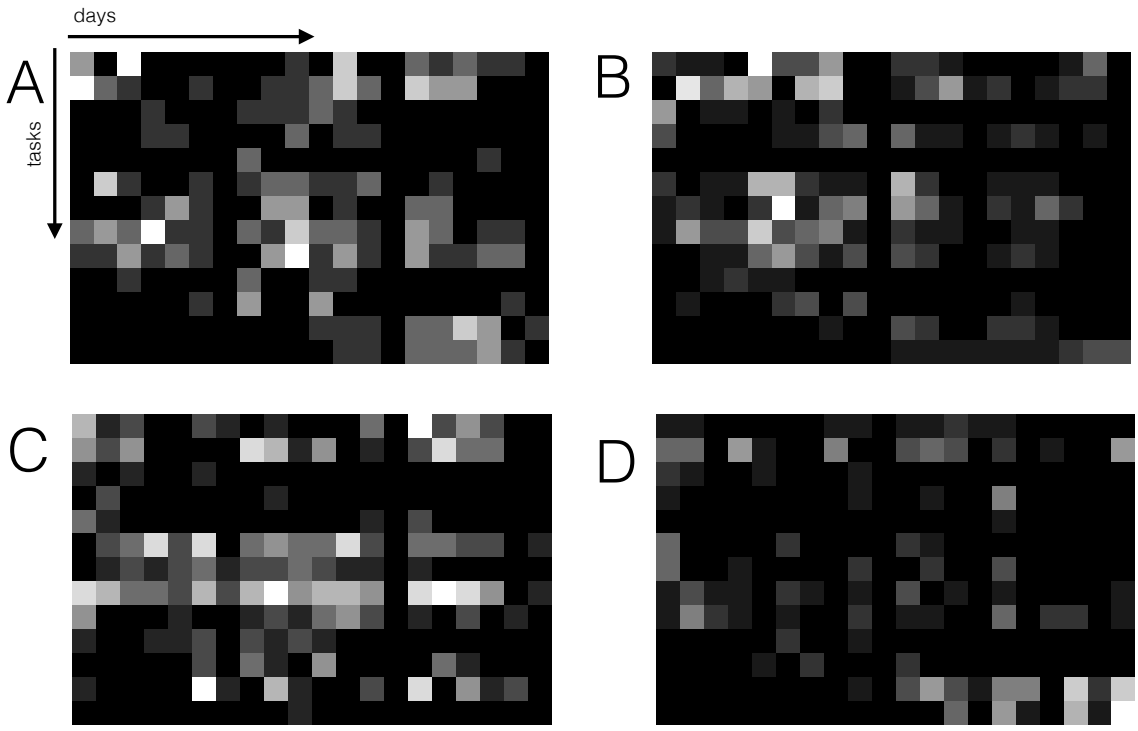

Figure 2.8: **Average pattern and setup composition**. Panels A and B show the average of all individuals corresponding to two behavioural progression setups. Panels C and D show the average of all individuals corresponding to setups where no D20+ foragers were present. In each panel days are represented across columns and behaviours are represented as rows.

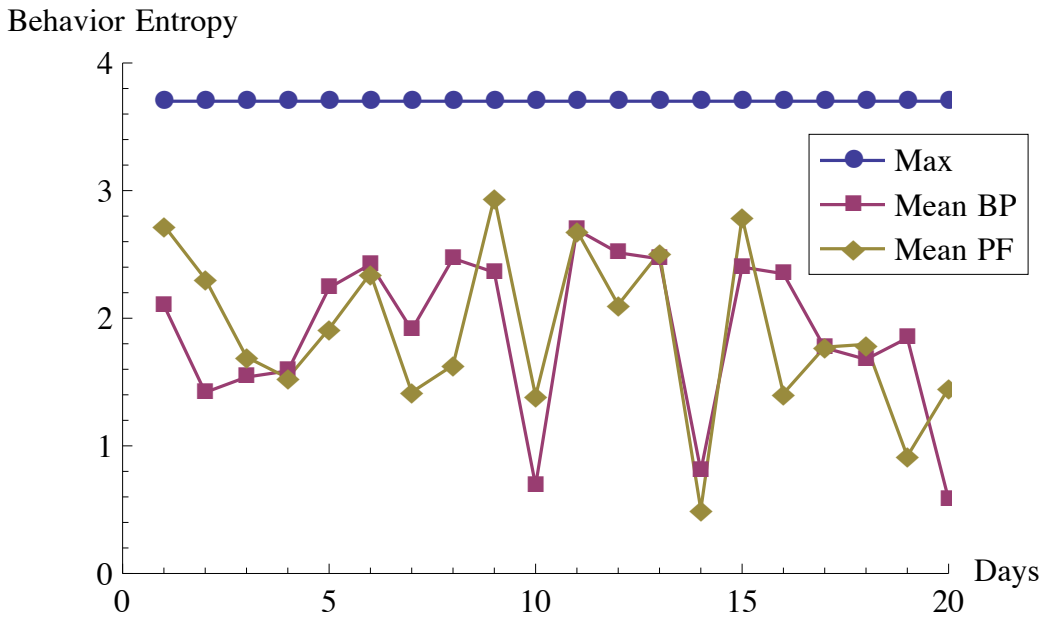

Figure 2.9: **Entropy comparison**. Entropy comparison based on maximum entropy and null model based on 10000 randomly generated individuals (circles), and our data resulting two different setup compositions, setups containing D20+ individuals: BP (squares), and setups lacking D20+ individuals: PF diamonds.

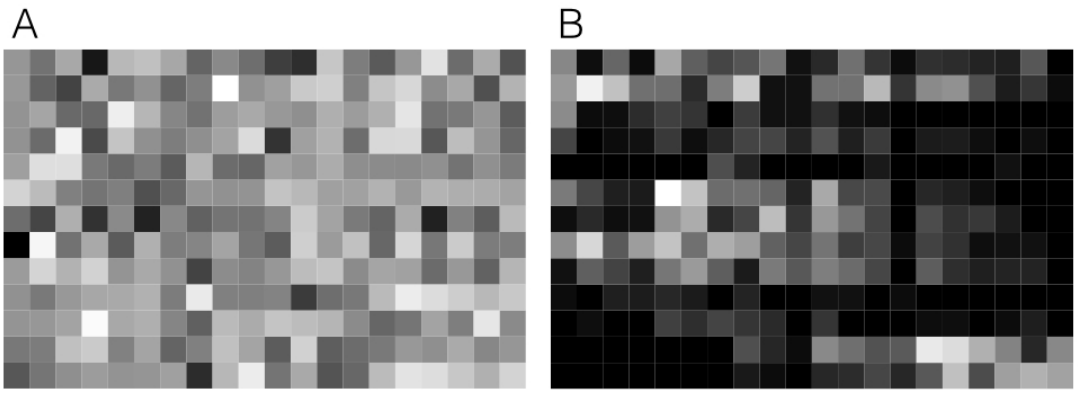

Figure 2.10: **Heatmap representation comparing our data and REM data**.**A**: null based on 10000 randomly generated individuals, **B**: our data resulting from 79 individuals

Connecting statement

In the previous chapter, we described abundant inter-individual variability among worker ants of *Pheidole dentata*. This invites the question of how inter-individual variability is generated. In the following chapter we will focus on behavioural capabilities and inter-individual variability in foraging behaviour.

CHAPTER 3
Go and *no go* ants: inter-individual variability in foraging behaviour

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3.1 Abstract

Division of labour is one of the major factors contributing to the ecological success of ant societies. Ant colonies are comprised of thousands of individuals which perform a myriad of tasks including brood care, foraging, nest maintenance and colony defence. Traditionally, division of labour models assume that the behaviour of individuals is dictated by their caste and behavioural plasticity enables colony robustness and resilience when faced with environmental challenges. Striking levels of fine-scale inter-individual variation within the worker caste in the ant *Pheidole dentata* have been recently described. To understand how this inter-individual variability is generated, we focused on differences in foraging behaviour of same age individuals that share a social environment. We selected foraging behaviour since not only is it one of the behaviours for which fine-scale inter-individual variability was documented, but also due to its importance for fitness. We found that two distinct behavioural groups emerge: *go* ants which leave the nest and forage and *no go* ants which stay in the nest performing brood care. Surprisingly, we found no difference between *go* and *no go* in behavioural capacities. Our results suggests that the differentiation into *go* and *no go* ants is at least in part influenced by the environment.

3.2 Introduction

During E.O. Wilson's early work on caste evolution in ants [1], he defined temporal polyethism as the workers change of role during the course of their lives. This association between age and task has been well established in honeybees, where there is a shift from performing tasks inside the nest to tasks outside the nest with age (Rösch [2, 3, 4], Sakagami [5, 6], Sekiguchi and Sakagami [7], Seeley [8], Kolmes [9], Huang and Robinson[10]). However, the degree to which the age-task association holds is questionable. Wilson himself proposed the existence of temporal castes, those groups of individuals which emerge within a morphological caste based on the suite of tasks they perform in the colony independently of age (Wilson [11]). Additionally, variability appears in several of the studies on behavioural transitions in honeybees (reviewed in Kolmes [9]). In his review, the author summarizes within group variability in the timing of behavioural transitions as well as in the duration of phases during which worker bees engage in the different behaviours (summarized in Figure 3.1). An example of such striking variation is that described by Ribbands [12], who while studying foraging onset and longevity in honeybees, found variation in the onset of foraging within a range of about 20 days. Further variability has been uncovered through the experimental manipulation of either demography or resource availability. Experimental manipulations have revealed variation in three aspects: (1) the timing of behavioural onset and transitions, (2) the duration of different behavioural phases, and (3) the sequence of behaviours. Early studies including Wiltze [13], Nelson [14], Himmer [15], Rösch [4] and Haydak [16] show that the age/task association breaks down in demographically manipulated colonies. Changes in timing

and sequence of behavioural progression have been documented under circumstances of unbalanced age composition (Lindauer [17]). Free [18] described that under abnormal conditions, young workers could accelerate their transition to foraging, or old foragers could revert to nursing duties. Since then, several studies with different degree of manipulations have been done. Winston and Punnet [19] studied the relationship between behavioural ontogeny and colony growth. While the authors confirm the general trend of behavioural progression, they point out considerable variability in the onset of brood care and the onset of foraging of individuals with mean colony age. Winston and Fergusson [20] studied the effect of worker loss on the timing of temporal polyethism, and found the age of foraging onset was significantly different between control colonies and those in which up to two-thirds of all workers had been removed. Responsiveness to demographic manipulations depends on the degree of such manipulations. Kolmes and Winston, [21] found that the removal of between $40 - 50\%$ of either the hive labour or the forager labour, the median age of task performance does not change significantly. However, their data suggest that the variation does differ between control and manipulated colonies (Kolmes and Winston [21]). Other studies have used depletion of certain age or task groups. Demographic manipulations consisting on the removal of older workers simulate conditions that can occur in the wild were predation, nest damage, exposure to dessication and disease can result in dramatic changes affecting the population of older workers differentially. Huang and Robinson [22] used triple-cohorts, composed of young, middle age and old worker bees. They found that the onset of foraging in the middle focal cohort is dependent on interactions with old workers. The removal of old workers accelerates

the onset of foraging while the confinement of old bees in the nest delays the onset of foraging in the same focal group. In the ant species *Pheidole dentata* Calabi [23] showed the early onset of foraging of young workers in demographically manipulated colonies. In the context of our work we refer to those individuals who initiate foraging early as *go* ants and to those same-age individuals who do not accelerate the onset of foraging as *no go* ants. Other studies have focused on the effect of resource manipulations. Kolmes [9] studied responsiveness of behavioural division of labour in colonies facing deprivation of either pollen and nectar or wax. He found differences in the transition age from inside the nest to outside the nest tasks, with both manipulations resulting in a delayed transition. Experimental demographic manipulations have also been performed in primitively eusocial wasps (O'Donnell, [24]) where the key water and nectar foragers were removed to assess replacement. Depending on the resource, recruitment of either workers already engaged in the specific task or from other tasks took place.

Ibarrarán-Viniegra et al. (in preparation, chapter 2) documented abundant inter-individual variability in *P. dentata* workers. The authors proposed inter-individual variability could be generated through a genetically predetermined, environmentally responsive or stochastic process. In the present study we focus on inter-individual variability in foraging behaviour to understand how inter-individual variability is generated. Using demographic manipulations we create a single cohort of same aged individuals and test whether ants of the same age that perform different tasks within the same social context show differences in behavioural capabilities. We predict,

based on their known behaviour in the social context; *no go* ants would out-perform *go* ants in the brood care paradigm. If their behaviour in the social context was determined by a higher sensitivity to brood care they would explore more brood items and focus their exploration on the brood items. Likewise, we predict *go* ants would out-perform *no go* in the foraging paradigm as well as in the exploration paradigm. We would predict *go* ants to more readily find food and spend more time feeding. Additionally we would expect *go* ants to cover more distance and explore more in the exploration assay, since based on the social context observations *go* ants move around more than *no go* ants. We tested whether upon eclosion individuals showed a preference for food or brood. If *no go* and *go* ants are a result of different behavioural capacities, we expected differences could be found as early as eclosion; in which case we predict two clear groups would become evident, one of individuals consistently preferred brood, and another which individuals prefer food.

3.3 Materials and Methods

Colony collection and care

We collected queenright colonies of *Pheidole dentata* in Gainsville, Florida in spring 2009, 2010 and, 2012. We kept colonies in Fluon-lined plastic boxes (either 27x19x10 cm or 31x22x10 cm depending on colony size) with red cellophane covered test tubes partially filled with water and tight cotton plugs. We fed ants three times a week with a combination of 1 M sucrose, fresh mealworms, fresh waxworms and Witcomb diet. We maintained colonies in a Conviron environmental chamber (Controlled Environments Ltd., Winnipeg, Manitoba) under a 12L:12D light cycle

at 27°C and 70% relative humidity.

Go ants: validation setups and observations

To confirm we are able to generate a shift in the onset of foraging by post-eclosion day 5 (D5) we created setups containing 20 D20+ ants, 15 late pupa and 10 larva in a small clear plastic boxes (14 x 10 x 4 cm) with dental stone bottom which retains humidity. A nest-space (0.75 x 0.25 x 0.3 cm) was created with a microscope slide, which was held in place with modelling clay and covered by red cellophane to create an undisturbed dark nest area for ants. Ants were allowed to acclimate overnight, the next day when the pupa had eclosed (D1), we created two setup compositions. Control setups were kept as described above, while experimental setups had all D20+ individuals removed (shown in Figure 3.2). On day five the number of young (D5 old) individuals which exited the nest and ate food (*go* ants) were counted and the proportion of those relative to the total number of young ants present was calculated. Additionally, we calculated the proportion of setups of each type in which *go* ants were observed.

Setups to assess inter-individual variability in foraging behaviour

In order to address inter-individual variability in foraging behaviour we created single cohort setups. First we created setups containing individuals 20 days or older (D20+) and at least 60 dark pupa, from which the next morning we obtained at least 50 individuals that eclosed in the overnight period (D1). Each setup single cohort was composed of 20 D1 labelled focal individuals, 30 D1 non-labelled individuals,

3 white pupa, 5 dark gutted and larva, 5 late larva, a pile of eggs and microlarva (Figure 3.3). We placed ants in small clear plastic boxes (14 x 10 x 4 cm) with dental stone bottom which retains humidity. A nest-space (0.75 x 0.25 x 0.3 cm) was created with a microscope slide that was held in place with modelling clay and covered by red cellophane to create an undisturbed dark nest area for ants. We allowed ants to acclimate an overnight period during which they moved the brood into the nest area.

Observations of early behavioural progression: eclosion to D5

We observed setups under the scope for a total of 25 minutes distributed over a period of about 1.5 hours, 5 minutes of observations inter-spaced by 15 minutes of no observation. All observations were completed between 10:00 and 17:00 hours every day (Figure 3.3). During each 5 minute period, we performed instantaneous scan sampling (based on Altmann [25]) of behaviours performed by the focal individuals. We collected absence/ presence behavioural data for all focal individuals for 13 different mechanical tasks (detailed behaviours and their definitions are shown in Table 3.1). We obtained absence/ presence behavioural data during the first four days post eclosion day (D2-D4). On D5, ants we classified individuals based on their behaviour in two groups: *no go* ants that were never observed leaving the nest and *go* ants that exited the nest and foraged for food. Individuals that exited but did not consume food are not used for the purpose of our study. We used the two behaviourally distinct groups of individuals that emerge within same age individuals in a shared social context to further study inter-individual behavioural variability.

Behavioural capacity tests

Individual ants that were classified into *no go* or *go* categories were assigned a random number for the subsequent behavioural observations. In order to test whether the behavioural differences observed between D5 *no go* and *go* are a result of ants having different behavioural capabilities, we tested individual ants in a set of simple tests. First individuals were placed in an empty 35mm petri dish to evaluate their movement when displaced into a previously unexplored environment (open field test, Figure 3.4, panel A). Their behaviour was video recorded during 5 minutes. Ants were then placed in 35mm petri dish containing a small piece of a mealworm approximately 0.5 cm long (Figure 3.5, panel A). Their behaviour was video recorded for 5 minutes. Finally the individual was placed in a 35mm petri dish containing randomly placed brood, a mix of larva and pupa (Figure 3.6, panel A), and their behaviour was video recorded for 5 minutes. We validated the capacity tests by comparing the foraging behaviour of day 1 old and day 20+ old ants, the methods used were the same as described above and shown in Figure 3.5.

Early preference test

In order to assess whether individuals show early preference for brood or food, we assessed preference in day 1 old ants. Newly eclosed individuals were sampled from single cohort setups and placed in the center of an arena while food was placed on one side and brood on the opposite side (shown in Figure 3.7). The number of instances an ant approached food or brood within a body length distance (approximately $3mm$) were counted as interactions with brood or food. Preference was

determined by the number of interactions with brood or food. Whatever the individual interacted with most was marked as preferred. We recorded no preference in a trial when individuals either stayed in the middle of the arena or interacted the same number of times with each stimuli.

Data analysis

Videos were analysed using Biowatch (https://code.google.com/p/biowatch/ [26] developed in collaboration with Yogesh Girdhar from the Centre for Intelligent Machines at McGill). Our program uses a particle filtering based approached to track multiple moving objects, which in this case is individual ants. As an output Biowatch generates a time labelled list of coordinates for each individual ant. Coordinate files were used to map movement using Mathematica (Wolfram Mathematica) for distance travelled and exploration analysis in the displacement and brood care tasks. We used masks to analyze one setup at a time (Figures 3.4B, 3.5B and 3.6B). The output file was then used in a script that generates the path as shown in Figures 3.4C, 3.5C and 3.6 C). Finally for exploration test specific masks were used. In the case of exploration in the context of displacement the full arena was considered, and based on the antenna length a radius of 2mm around the path was considered as explored (Figure 3.4D). In the case of brood care, a mask was created around the individual brood items and this was used to run BioWatch for brood exploration and number of brood items explored (Figure 3.6D). Foraging behaviour was analyzed using JWatcher [27] to calculate time to find food, lag between finding food and

feeding, number of feeding instances (bouts) and total time spent feeding.

3.4 Results

A higher proportion of *go* ants is produced in response to demographic manipulations.

We first compared the proportion of control and experimental condition setups (Figure 3.2), in which *go* ants are produced. We find *go* ants in 26% of control setups and 80% of experimental setups. We then compared the proportion of *go* ants between control and experimental setups and observe a significant difference in the proportion of *go* ants between control and experimental setups (Mann-Whitney Utest p=0.004 median *no go*= 0, *go*= 0.11, Figure 3.8).

D5 *go* and *no go* possess similar behavioural capacities

We predicted that differences in the capacity to perform tasks are associated with the different behavioural roles individuals have in the social context of the group, *go* and *no go*. We used three behavioural capacity tests to assess whether D5 *no go* and *go* ants differ in their capacity to explore, forage and perform brood care. For each test we evaluated a few parameters. Since the distribution of capacity test parameters is non-normal (Shapiro Wilkins test $p < 0.05$), we used the Mann-Whitney U test to analyse our data.

We first validated the use of capacity tests by comparing two groups of ants that have distinctly different behaviour in the social group, specially in terms of foraging behaviour. Newly eclosed ants (D1) are not observed outside the nest foraging, while ants older than 20 days (D20+) are likely to leave the nest. Within the foraging capacity test we compared the total distance travelled in the foraging

assay, time to find food, feeding delay (time to start eating once food was found), total number of feeding bouts and total time spent feeding. We found statistically significant differences between D1 and D20+ (sample size: D1 n=35, D20+ n=40) in time to find food, feeding delay, number of foraging bouts and total time feeding (Table 3.2). These results indicate that when there are differences in behavioural capabilities we can pick them up.

We then compared D5 old *no go* and *go* ants for the displacement test, we found no statistically significant difference between *no go* and *go* (sample size: *no go* n=42, *go* n=40) in any of the parameters (Figure 3.10 and Table 3.3). In the foraging test, we compared the total distance travelled in the foraging assay, time to find food, feeding delay (time to start eating once food was found), total number of feeding bouts and total time spent feeding. We found no statistically significant difference between *no go* and *go* (sample size: *no go* n=42, *go* n=40) in any of the parameters (Figure 3.11 and Table 3.3). In the broodcare test, we compared the total distance travelled in the broodcare assay, brood care exploration index, the brood exploration index and the number of different brood items explored. We found no statistically significant difference between *no go* and *go* (sample size: *no go* n=39, *go* n=38) in any of the parameters (Figure 3.12 and Table 3.3). Together these results indicate that *no go* and *go* ants are not different in their behavioural capacities.

The difference between *no go* and *go* ants is not due to differences at eclosion.

Since we found no statistically significant differences in behavioural capacities, we asked whether a difference could arise early on setting them on different behavioural trajectories. To test this possibility, we used a preference test to establish whether the difference between D5 old *no go* and*go* ants could be due to differences earlier in their development, potentially from the moment they eclose as adults. We counted the number of interactions with food and brood, and found no clear preference between food and brood in newly eclosed workers (Figure 3.13). At eclosion we find no clear preference for brood care or foraging.

3.5 Discussion

Is there an environmental influence on the generation of inter-individual variability among same age ants performing different tasks within a shared social environment? Our results show: (1) statistically significant difference in behavioural capacity between individuals of different ages (D1 and D20+); (2) we found no difference in behavioural capacities between *go* and *no go* ants (3) no evidence for early preference for food or brood at eclosion. Our data therefore suggests inter-individual variation in foraging behaviour is, at least partially, environmentally influenced.

Although inter-individual variability is the product of complex non-linear interactions between genes and environment, in order to build predictions and test for the possibility of an environmental influence on the generation of inter-individual variation, we make predictions under a scenario of complete genetic determination, and a scenario of complete environmental determination, and a scenario of complete

stochasticity. If inter-individual variation between *no go* and *go* ants was unresponsive to the environment we would have expected to find similar proportions of *go* ants irrespective to demographic composition, and as we described we find a statistically significant difference in the proportion of *go* ants produced in the presence and absence of D20+ individuals. Second, we would expect significantly different behavioural capabilities between the two groups. We would have predicted *no go* ants would out-perform *go* ants in the brood care paradigm and *go* ants would outperform *no go* in the foraging paradigm as well as in the exploration paradigm. Third we would have expected early preference for brood or food if *no go* and *go ants* were generated through a genetically predetermined process or as a result of experience during larval development.

Recently Jeanson and Weidenmüller [28] proposed three scenarios for the relative contribution of different sources of behavioural inter-individual variation in social insects. The sources they propose are: (1) genetic variation, (2) variation generated from experience during larval development up to the point of eclosion and (3) variation generated by experience from eclosion to adulthood. However we propose that aside from genetic variation (which we refer to as predetermination) and experience (which we refer to as plasticity since it is environmentally responsive) yet another possible source of inter-individual variation exists and this is stochasticity. We have discussed how our results suggest that while we can not rule out a genetic influence, there is an environmental influence on the generation of *go* and *no go* ants. In terms of plasticity our results suggest larval experience does not account for the

variation between *no go* and *go* ants. Our results support inter-individual variability could arise from experience during the period between eclosion and adulthood. Individuals could have similar behavioural capacities yet in the social context they get differentially recruited/inhibited to perform a task through social interactions. This would mean individuals are responsive to their environment and a plasticity mediated process underlies inter-individual variation in foraging behaviour. Huang and Robinson [22] proposed demographic manipulations can cause workers to be prepared to perform tasks, even before the actual need arises. This supports an environmentally responsive process, since the preparedness arises as a response to the environmental conditions. Our finding that the production of *go* ants is dependent on demographic composition, suggests social interactions could be key for the generation of inter-individual variability.

Finally, another possible explanation for our finding of similar behavioural capacity is that individuals with similar capacities are sorted into *no go* and *go* through a stochastic process. In this scenario all individuals are indistinguishable and a number of them begin foraging in the absence of environmental induction. Previous work on inter-individual variability in *P. dentata* suggests it is not a purely stochastic process since, Ibarraran- Viniegra et al. (in preparation, chapter 2) found evidence of non-randomess in the behaviour of *P. dentata* worker ants. However, as reviewed by Kilfoil et al [29], stochastic variation could initially set them in different trajectories and experience could later reinforce those trajectories. In the broader context of division of labour in social insects, our results emphasize the need to incorporate inter-individual variation to division of labour models. We propose that inter-individual

variability does not result from division of labour, but rather that plasticity mediated inter-individual variability has a role in generating division of labour.

3.6 Conclusions

Inter-individual variation in behaviour has been shown not only to be abundant among worker ants in the advanced ant species *Pheidole dentata*; but has also been suggested to have a role in the organization of division of labour (Ibarraran-Viniegra et al. in preparation, chapter 2). Understanding what underlies behavioural differences between same age individuals will takes us closer to understanding how inter-individual variability is generated. Our results support environmental responsive has a role in the generation of inter-individual variability. First, we find no difference in behavioural capacities between D5 individuals regardless of their role (*no go* or *go*) in the shared social context. Second, we find no evidence of early preference for brood or food. Together, our results suggest social regulation as the mechanism through which same age individuals with similar behavioural capabilities divide labour in a single cohort experimental setup.

Future Research

Differentiating between the two -stochastic followed by reinforcement and plasticity- is not trivial. One potentially promising avenue to explore is biogenic amines. Biogenic amines are known neuromodulators, neurotransmitters and neurohormones (Roeder [30], reviewed in Scheiner et al., [31]) which are involved in the modulation and generation of individual-level behaviours in both vertebrates and invertebrates.

In honeybees and ants biogenic amines are associated with social behaviour and division of labour (reviewed in Scheiner et al, [31] and Kahmi and Traniello [32]). Therefore investigating whether *no go* and *go* ants differ in terms of the biogenic amine systems could shed light into what gives rise to inter-individual differences not only in foraging behaviour, but potentially in the broader context of division of labour.

3.7 Acknowledgements

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References

[1] Wilson E (1976) Behavioral discretization and the number of castes in an ant species. *Behavioral Ecology and Sociobiology* 1:141–154.

[2] Rösch G (1925) Untersuchungen über die arbeitsteilung im bienenstaat. *Zeitschrift für vergleichende Physiologie* 2:571–631.

[3] Rösch G (1927) Über die bautätigkeit im bienenvolk und das alter der baubienen. *Zeitschrift für vergleichende Physiologie* 6:264–298.

[4] Rösch G (1930) Untersuchungen über die arbeitsteilung im bienenstaat. *Zeitschrift für vergleichende Physiologie* 12:1–71.

[5] Sakagami SF (1953) Untersuchungen uber die arbeitsteilung in einem zwergvolk der honigbiene. beitrage zur biologie des bienenvolkes,*Apis mellifera L. Japanese Journal of Zoology* 2:117185.

[6] Sakagami S (1953) Untersuchungen über die arbeitsteilung in einem zwergvolk der honigbiene: Beiträge zur biologie des bienenvolkes, *Apis mellifera L. Japanese Journal of Zoology* 11:117–185.

[7] Sekiguchi K, Sakagami S (1966) Structure of foraging population and related problems in the honeybee, with considerations on the division of labour in bee colonies. *Hokk Nat Agric Exp Stn Rep* 69.

[8] Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology* 11:287–293 10.1007/BF00299306.

[9] Kolmes SA (1985) A quantitative study of the division of labour among worker honey bees. *Zeitschrift fr Tierpsychologie* 68:287–302.

[10] Robinson, G.E.and Page R, Huang Z (1994) Temporal polyethism in social insects is a developmental process. *Animal Behaviour* 48:467 – 469.

[11] Wilson E (1968) The ergonomics of caste in the social insects. *American Naturalist* 102:41–66.

[12] Ribbands CR (1952) Division of labour in the honeybee community. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 140:32–43.

[13] Wiltze J (1882) Work done by two quarts of bees before 16 days old. *Glean. Bee Cult.* 10:596–597.

[14] Nelson F (1927) Adaptability of young bees under adverse conditions. *American Bee Journal* 67:242–243.

[15] Himmer A (1930) Von der areitsteilung im bienenstaat. *Bienenztg* 45:39–43, 64–67.

[16] Haydak M (1932) Division of labor in the colony. *Wisconsin Beekeeping* 8:36–39.

[17] Lindauer M (1961) *Communication among social bees* (Harvard University Press, Cambridge).

[18] Free J (1963) The flower constancy of honeybees. *Journal of Animal Ecology* 32:119–131.

[19] Winston ML, Punnett EN (1982) Factors determining temporal division of labor in honeybees. *Canadian Journal of Zoology* 60:2947–2952.

[20] Winston ML, Fergusson LA (1985) The effect of worker loss on temporal caste structure in colonies of the honeybee (apis mellifera l.). *Canadian Journal of Zoology* 63:777–780.

[21] Kolmes S, Winston M (1988) Division of labour among worker honey bees in demographically manipulated colonies. *Insectes Sociaux* 35:262–270.

[22] Huang Z, Robinson G (1996) Regulation of honey bee division of labor by colony age demography. *Behavioral Ecology and Sociobiology* 39:147–158.

[23] Calabi P (1986) Ph.D. thesis (Boston University).

[24] O'Donnell S (1998) Effects of experimental forager removals on division of labour in the primitively eusocial wasp *Polistes instabilis* (hymenoptera: Vespidae). *Behaviour* 135:173–193.

[25] Altmann J (1974) Observational study of behavior: Sampling methods. *Behaviour* 49:227–267.

[26] Girdhar Y, Ibarraran S (2014) Biowatch: A multiple target tracker for analyzing animal behavior. (https://code.google.com/p/biowatch).

[27] Daniel T. Blumstein JCD, Evans CS (2000-2012) Jwatcher. (http://www.jwatcher.ucla.edu/).

[28] Jeanson R, Weidenmüller A (2013) Interindividual variability in social insects proximate causes and ultimate consequences. *Biological Reviews* pp n/a–n/a.

[29] Kilfoil N, Lasko P, Abouheif E (2009) Stochastic variation: from single cells to superorganisms. *HFSP Journal* 1.

[30] Roeder T (1994) Biogenic amines and their receptors in insects. *Comparative Biochemistry and Physiology* 107C:1–12.

[31] Scheiner R, Baumann A, Blenau W (2006) Aminergic control and modulation of honeybee behaviour. *Current Neuropharmacology* 4:259.

[32] Kamhi J, Traniello J (2013) Biogenic amines and collective organization in a superorganism: Neuromodulation of social behavior in ants. *Brain Behavior and Evolution* 82:220–236.

[33] Lindauer M (1952) Ein beitrag zur frage der arbeitsteilung im bienenstaat. *Zeitschrift für vergleichende Physiologie* 34:299–345.

[34] Perepelova L (1928) The allocation of duties among worker honeybees. *Symp. Zool. Soc. London* 14:39–59.

Tables and Figures

behaviour	operational definition
allogroom	an individual cleans itself or another individual
trophallaxis	an individual exchanges food with another individual
carry egg or microlarva	an individual transports an egg
	or a microlarva either by carrying or rolling it around
groom egg or microlarva	an individual cleans an egg or microlarva
assist eclosion	an individual grooms either a late larva as it ecloses
	into a pupa or a pupa when it ecloses into a young adult
carry pupa	an individual transports a pupa either by carrying or rolling it around.
groom pupa	an individual cleans a pupa
carry larva	individual transports a larva either by carrying or rolling it around.
groom larva	individual cleans a larva
feed larva	an individual either feeds a larva through trophallaxis or feeds a
	larva solid food such as fragments of dead adult ants
handle dead	an individual carries and/or removes fragments of a dead ant
exit nest	an individual leaves the nest
forage	an individual leaves the nest, finds food and eats food

Table 3.1: **Behaviour codes and operational definitions used for behavioural progression evaluation of individuals**

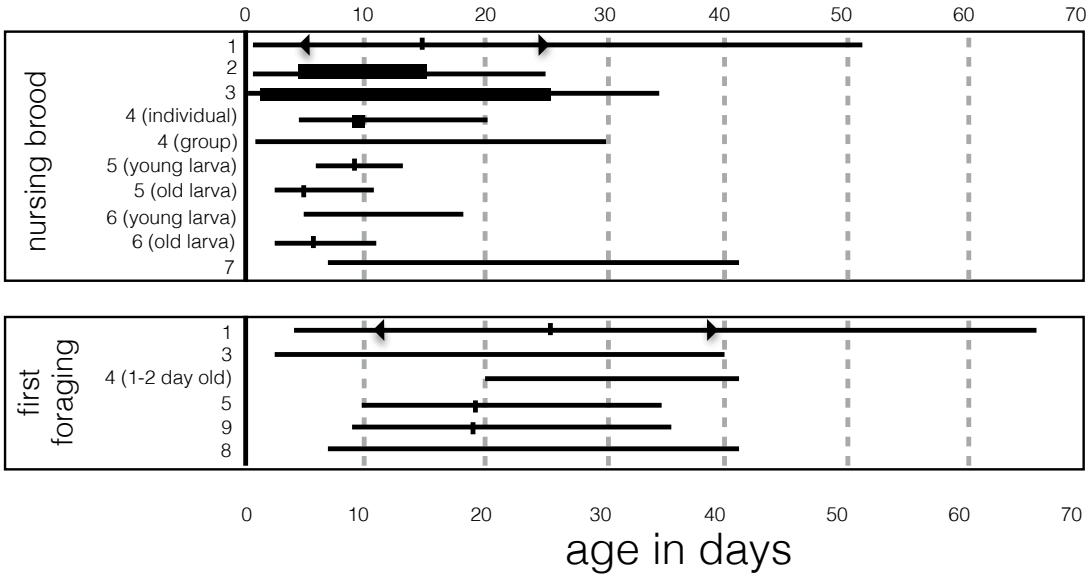

Figure 3.1: **Literature reported variation for the ages at which worker honeybees engage in different behaviours.** Figure modified from Kolmes [9] (**1**) Winston and Punnet [19], (**2**) Seeley [19], 3) Sakagami [5], 4) Lindauer [33] (individual and group), 5) Rösch [2, 3] (young and old), 6)Perepelova [34] (young and old), 7) Himmer [15], 8) Sekiguchi and Sakagami [7], 9) Ribbands [12]. Horizontal lines represent the range with solid blocks representing the days of common occurrence of a behaviour. Triangles represent ± 1 standard deviation and vertical line represents the mean.

capacity test	parameter	p-value	median D1	median D20+
foraging	time to find food	0.004	71.5 sec	10.7 sec
foraging	feeding delay	0.044	94.0 sec	22.2 sec
foraging	total feeding bouts	0.0002	1	2
foraging	total time spent feeding	0.004	1.6 sec	38.9 sec

Table 3.2: **Behavioural capacities comparison between D1 and D20+ ants**. Mann-Whitney U test. Sample size D1 n=35, D20+=40

capacity test	parameter	p-value	median *no go*	median *go*
displacement	Distance	0.19	939.2 mm	1259.6 mm
displacement	Exploration index	0.28	0.53	0.59
foraging	total distance travelled	0.14	628.4 mm	837.3 mm
foraging	time to find food	0.23	14.2 sec	22 sec
foraging	feeding delay	0.32	44.3 sec	10.8 sec
foraging	total feeding bouts	0.23	1	1
foraging	total time spent feeding	0.85	58.6 sec	91 sec
brood care	total distance travelled	0.87	925.7 mm	27.3 mm
brood care	brood care exploration index	0.83	0.29	0.22
brood care	brood exploration index	0.46	0.31	0.23
brood care	number of different brood items explored	0.857	4	3

Table 3.3: **Behavioural capacities comparison between D5 *no go* and *go* ants**. Mann- Whitney U test. Displacement test *no go* n=42, *go* n=40; foraging test *no go* n=42, *go* n=40; brood care test *no go* n=39, *go* n=38.

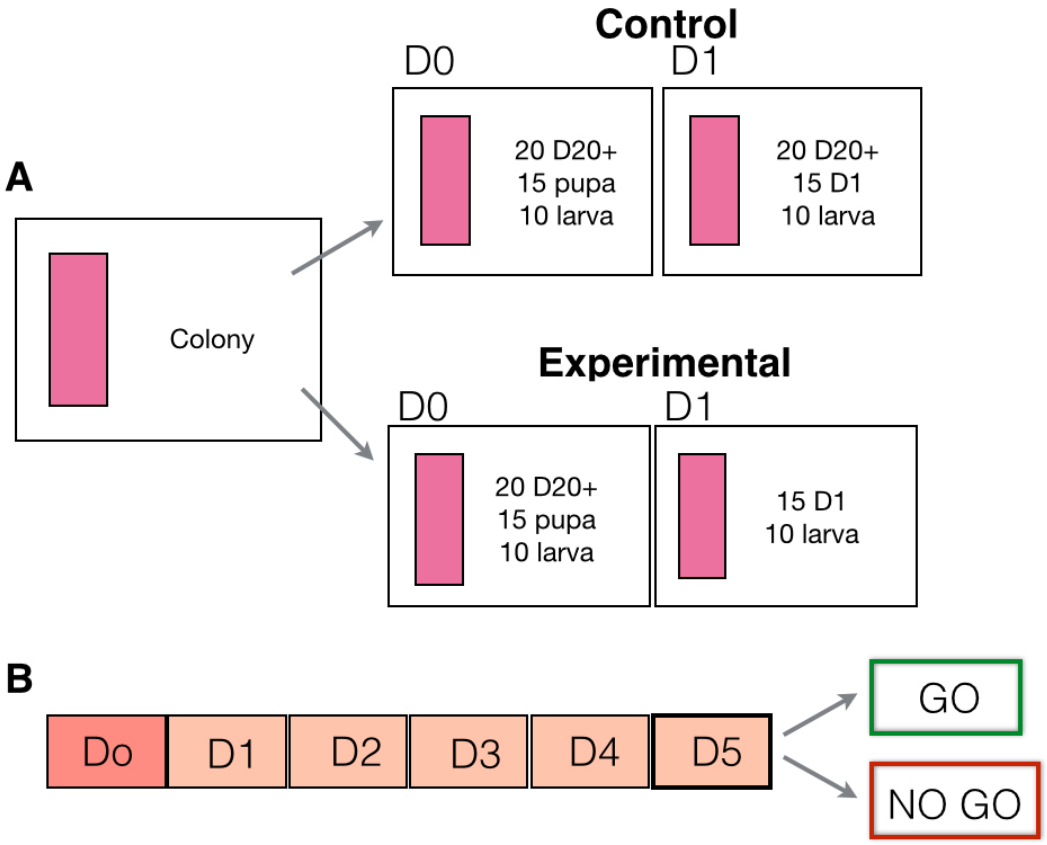

Figure 3.2: **Setups to validate the onset of early foraging** (**A**) Setups used to validate the early onset of foraging (*go* ants). From each colony (n=15) two setup compositions were created: controls and experimental. (**B**) On day five behavioural observations were done and the proportion of D5 old ants foraging was calculated.

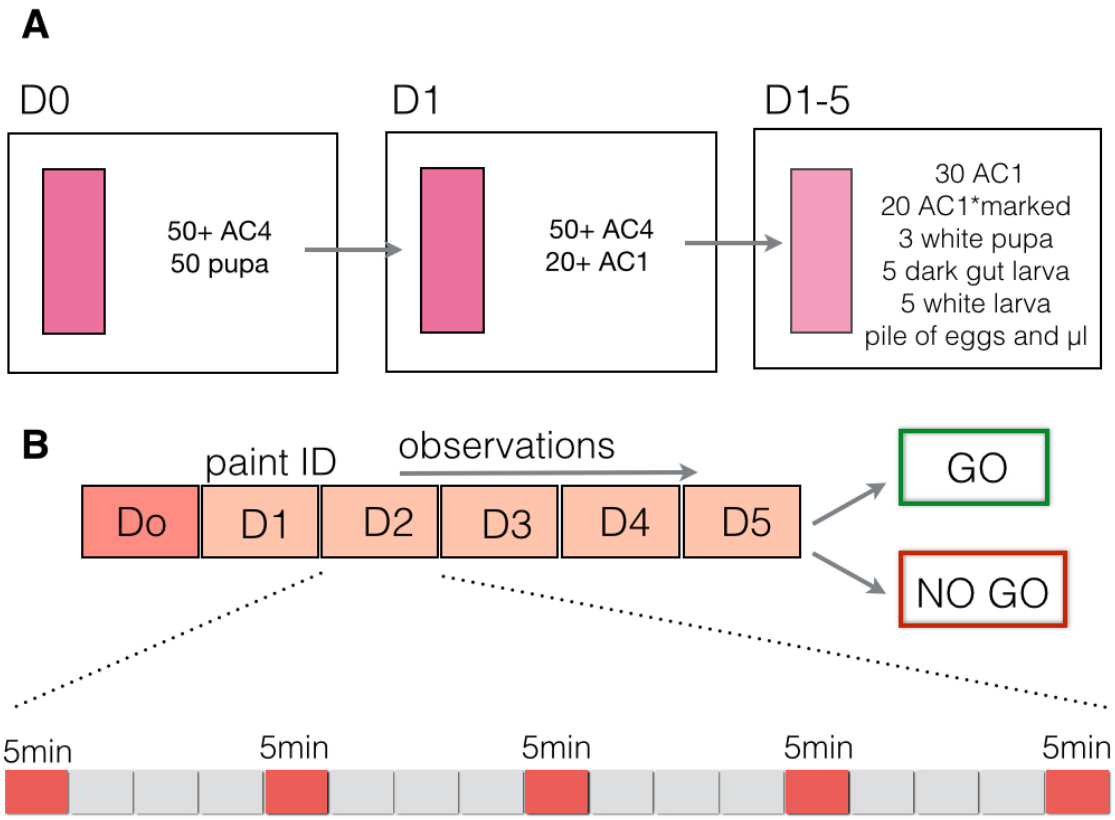

Figure 3.3: **D5 behavioural progression setups.** (**A**) setups used to study behavioural progression during the first 5 days posteclosion. (**B**) Observations were done daily on D5 individuals were grouped based on their foraging behaviour into *no go* and *go* ants which were used for subsequent behavioural analysis.

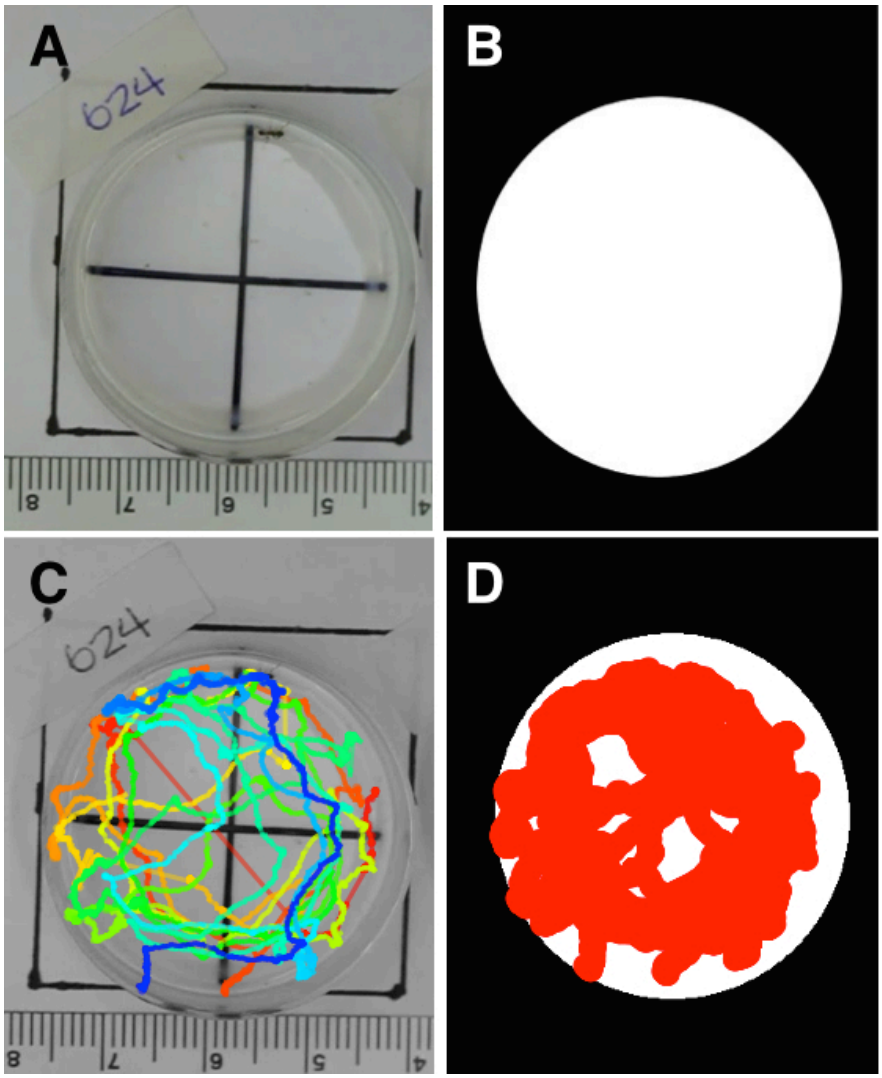

Figure 3.4: **Displacement and exploration capacity test.** (**A**) an individual is assigned a random number and placed into a previously unexplored arena (**B**) Mask created in order to track one individual, since multiple setups are recorded at once (**C**) Path reconstructed from tracking using Biowatch, the color represents time from red to blue used to calculate total distance. (**D**) Creating a $2mm$ radius around the path we calculate proportion of area explored.

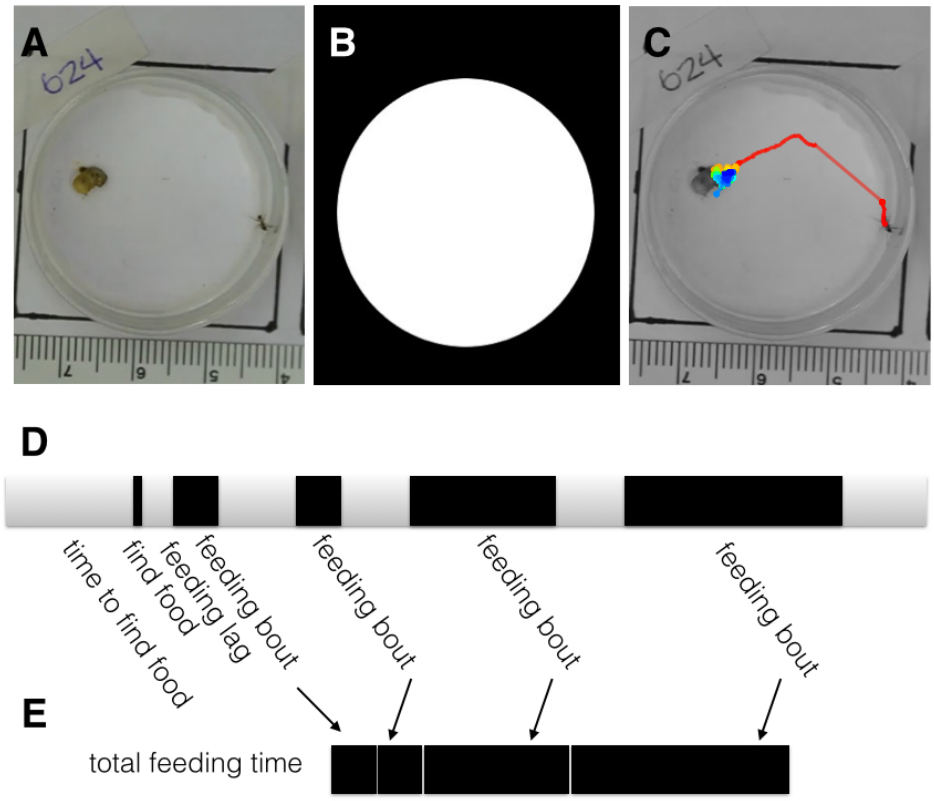

Figure 3.5: **Foraging capacity test.** (**A**) an individual is assigned a random number and placed into a previously unexplored arena containing a $0.5mm$ piece of mealworm. (**B**) Mask created in order to track one individual, since multiple setups are recorded at once. (**C**) Path reconstructed from tracking using Biowatch, the color represents time from red to blue used to calculate total distance. (**D**) Schematic representation indicating the parameters we evaluated: time to find food, feeding lag and feeding bouts. (**E**) Schematic representation for the calculation of total feeding time. Parameter measurement done using JWatcher.

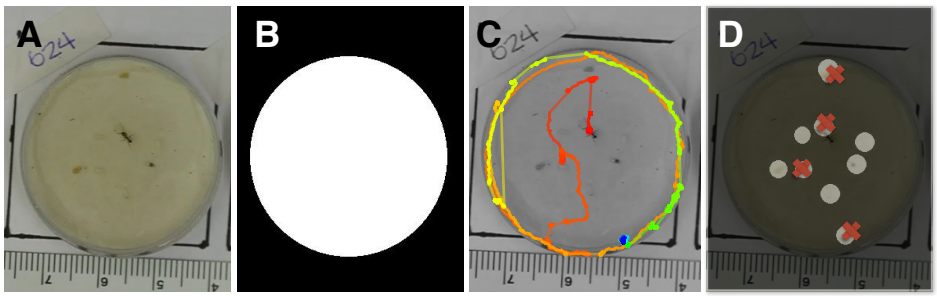

Figure 3.6: **Brood care capacity test.** (**A**) an individual is assigned a random number and placed into a previously unexplored arena containing scattered brood. (**B**) Mask created in order to track one individual, since multiple setups are recorded at once (**C**) Path reconstructed from tracking using Biowatch, the color represents time from red to blue used to calculate total distance. (**D**) Overlay of brood mask used to calculate brood exploration and number of brood items explored, in this case the brood items explored are represented by small red crosses.

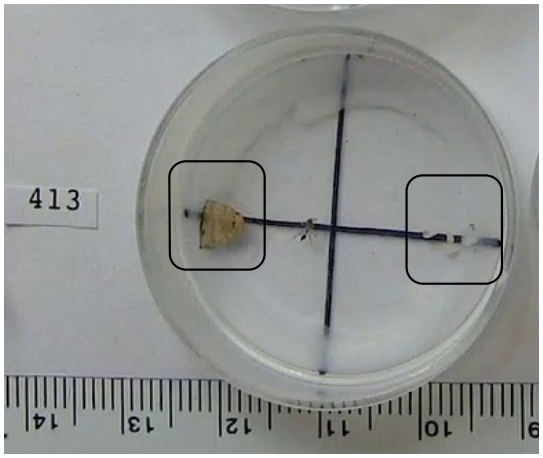

Figure 3.7: **Early preference test.** In order to assess early preference newly eclosed individuals are placed in the centre of the arena where food and brood are placed on either extremes. Behaviour is recorded for 5 minutes to assess preference.

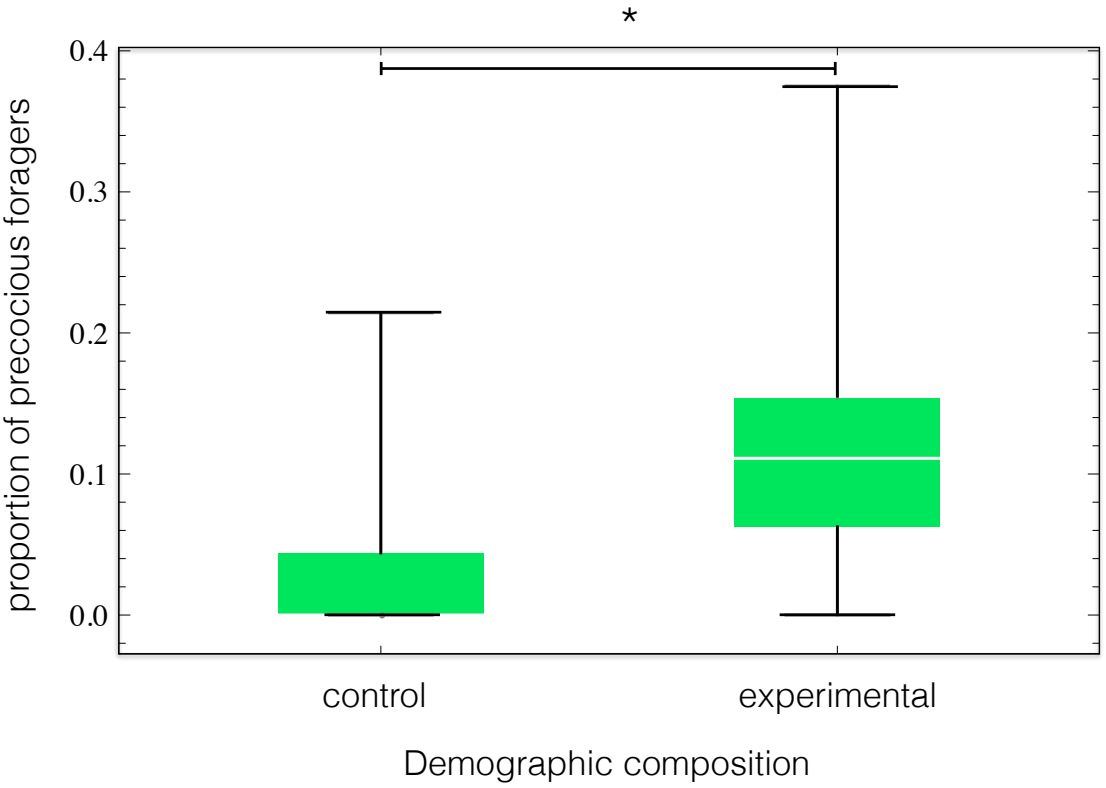

Figure 3.8: **Proportion of *go* ants produced in control and experimental setups.** From 15 colonies paired setups were generated. Based on the behavioural observations performed on D5 the proportion of D5 old ants foraging was calculated from the total number of D5 ants present. Box plots show median values (horizontal lines), and variance. Interquartile ranges indicated by boxes, max and min values indicated by whiskers. Statistical significance $p < 0.05$ indicated by *.

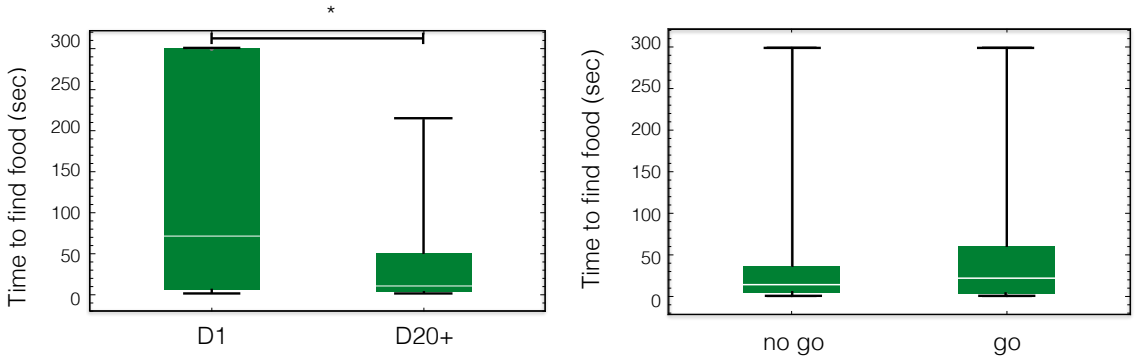

Figure 3.9: **Validation of capacity tests: foraging test. Left**: time to find food in sec comparison between D1 old ants and D20+ ants; **Right**: time to find food in sec comparison between D5 old *no go* and *go* ants. Box plots show median values (horizontal lines), interquartile ranges (boxes), max and min values (whiskers). Statistical significance $p < 0.05$ indicated by *.

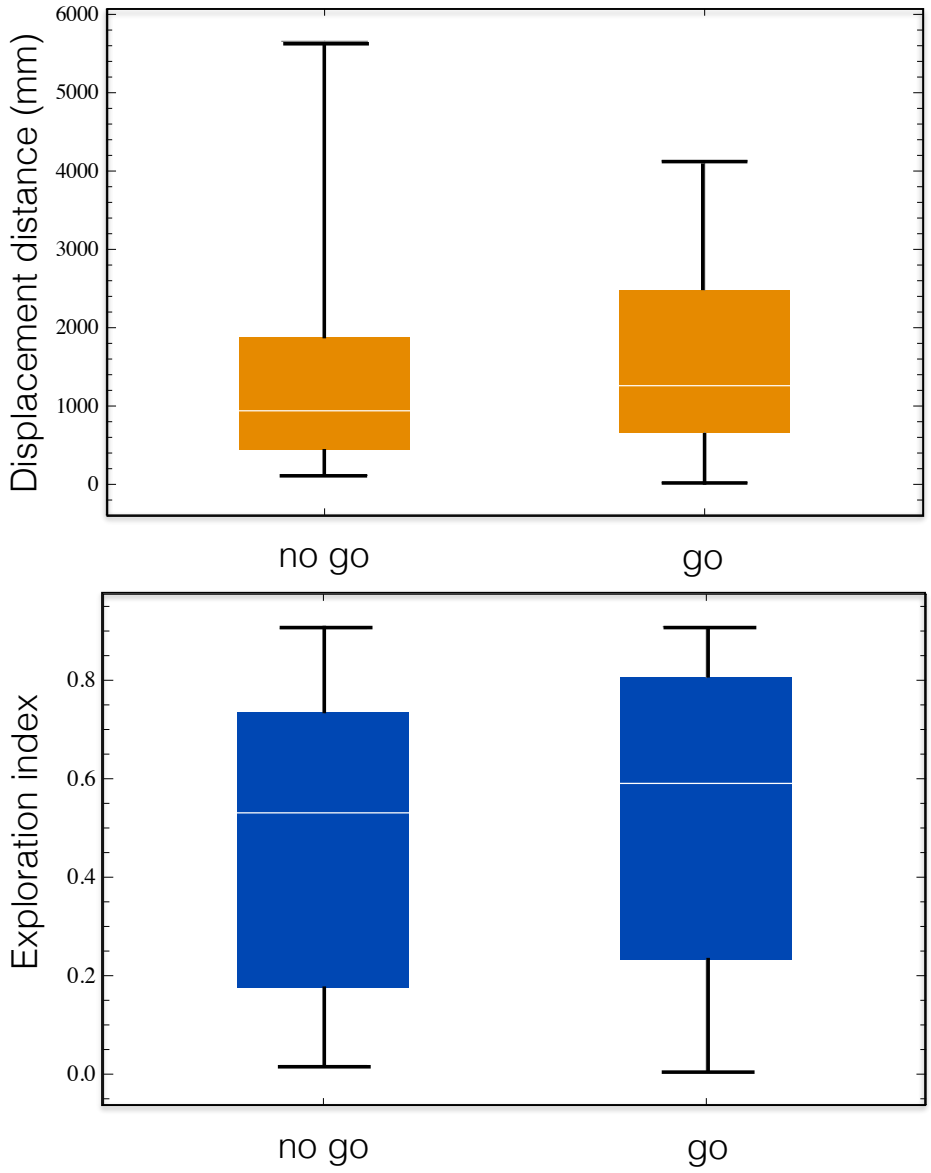

Figure 3.10: **D5 *no go* and *go* ants displacement test: distance travelled and exploration index**. **Top**: total distance travelled when displaced (in mm). **Bottom**: exploration index for *no go* and *go*. Box plots show median values (horizontal lines), interquartile ranges (boxes), max and min values (whiskers).

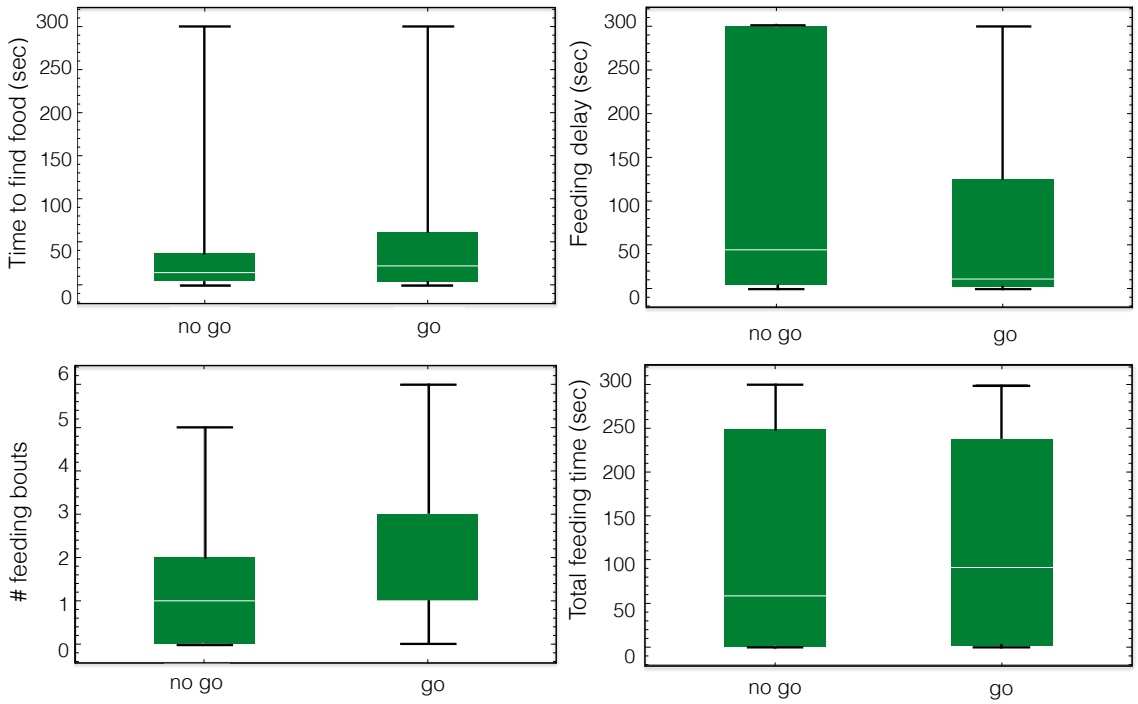

Figure 3.11: **D5 *no go* and *go* foraging test**. **Top** Left: time to find food in sec; Right: feeding delay; **Bottom** Left: number of feeding bouts. Right: total feeding time. Box plots show median values(horizontal lines), interquartile ranges (boxes), max and min values (whiskers).

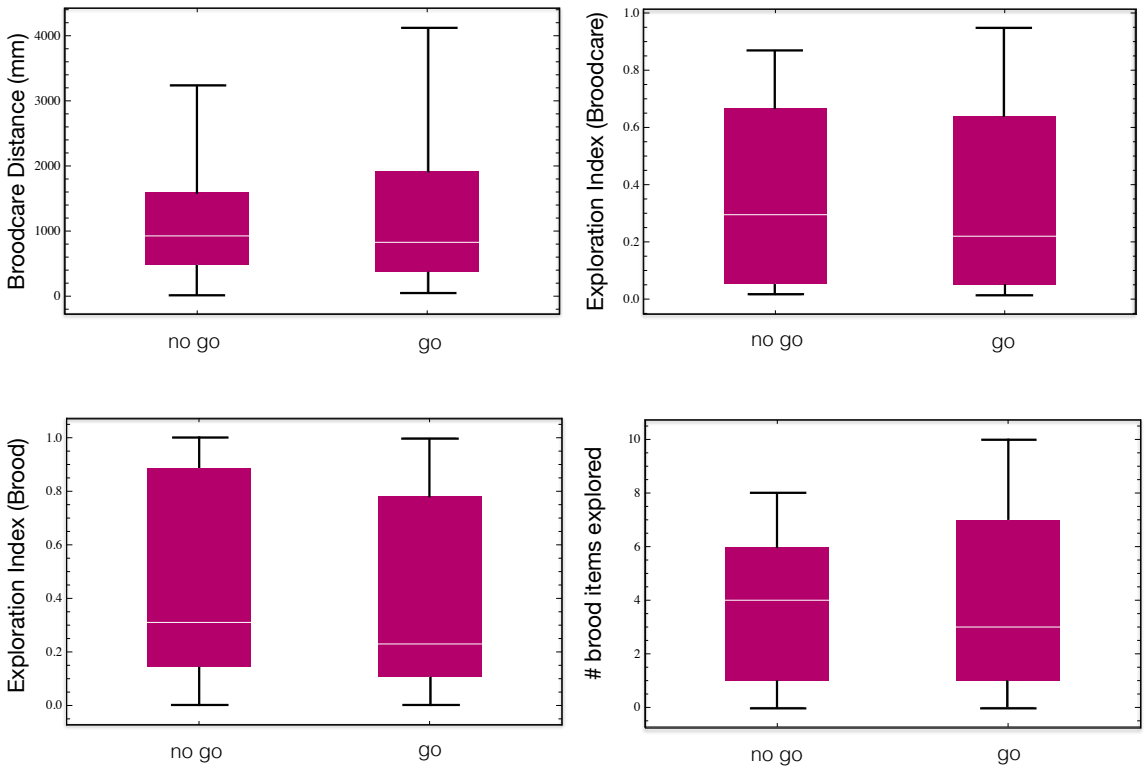

Figure 3.12: **D5 *no go* and *go* broodcare test. Top** Left: total distance travelled in broodcare test; Right: exploration index in test. **Bottom** Left: brood exploration index; Right: number of different brood items explored. Box plots show median values(horizontal lines), interquartile ranges (boxes), max and min values (whiskers).

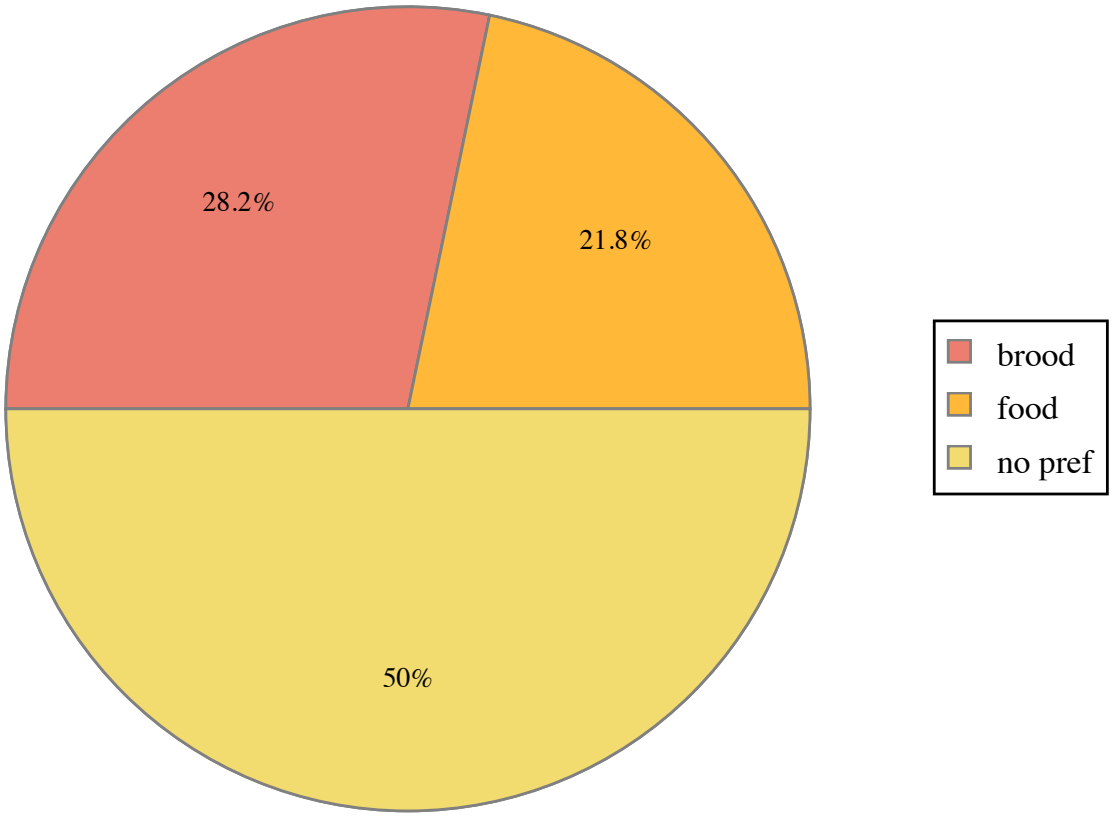

Figure 3.13: **D1 old ants preference for brood or food test.** The proportion of D1 old ants based on preference for brood or food n=46 individuals.

Connecting statement

In the previous chapters, we found that behaviourally distinct day 5 old individuals (*no go* and *go* ants) do not have different behavioural capacities. In order to analyse whether neuromodulation is what gives rise to this behaviourally distinct groups in the following chapter we will focus on the study of the biogenic amine systems: dopamine and serotonin.

CHAPTER 4
Inter-individual differences and biogenic amines from titres to receptors

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4.1 Abstract

Behavioural variation within groups of individuals is ubiquitous across the animal kingdom and within group variation has been shown to be beneficial for the group. In social insects, variation within groups or specialization has long been documented. However, only recently has abundant inter-individual variation been discovered between individual workers of the same age in the ant species *Pheidole dentata* (Ibarrarán-Viniegra et al, in preparation chapter 2). In a shared social environment, two clearly distinct behavioural groups with similar behavioural capacities emerge among individuals of the same age: the *no go* ants which stay in the nest performing brood care and the *go* ants which leave the nest and forage (Ibarrarán-Viniegra et al, in preparation chapter 3). In honeybees early differences in foraging behaviour, equivalent to *no go* and *go* ants, are associated with changes in biogenic amine systems; which suggests biogenic amine systems could modulate this behavioural plasticity to generate inter-individual variability. To test whether biogenic amine systems are involved in differentiating *no go* and *go* ants, we measured titres of dopamine and serotonin and as well as cloned and assayed the expression of receptors for these two biogenic amines. Surprisingly, we find no difference between *no go* and *go* ants in biogenic amine levels and receptor expression. Our results therefore show that the behavioural transition between *no go* and *go* ants is not mediated by dopamine or serotonin and further supports the hypothesis that environmental influence contributes to the generation of inter-individual variability. Unlike in honey bees, where behavioural changes due to demographic manipulations are accompanied by changes in biogenic amine systems, in advanced ant societies

plasticity mediated inter-individual variability is not associated with changes in the dopaminergic or serotoninergic systems.

4.2 Introduction

Biogenic amines are known neuromodulators, neurotransmitters and neurohormones (Roeder [1], reviewed in Scheiner et al., [2]), which are involved in the modulation and generation of individual-level behaviours in both vertebrates and invertebrates. Biogenic amines are involved in movement, motivational state, arousal, and learning in mammals (reviewed in Bicker and Menzel [3] and Scheiner et al. [2]), while in arthropods biogenic amines have been linked to aggression, arousal, learning and memory, locomotion, reproductive state, and the modulation of stimuli specific responses (reviewed in Bicker and Menzel [3], Libersat and Pflueger [4], Scheiner et al. [2]. In hymenoptera, specifically honeybees and ants, biogenic amines are associated with social behaviour and division of labour (reviewed in Scheiner et al, [2] and Kahmi and Traniello [5]).

Biogenic amines as neuromodulators

In honey bees biogenic amines have been shown to modulate sensory responsiveness to gustatory and olfactory stimuli. Octopamine increases sucrose responsiveness, while dopamine decreases it and serotonin has no effect (Scheiner et al.[6] and reviewed in Scheiner et al. [2]). In studies addressing the effect of biogenic amines in learning and memory using classical conditioning, octopamine was shown to increase responsiveness to unconditioned stimuli, while dopamine and serotonin reduced the responsiveness to conditioned stimuli (Mercer and Menzel [7]). Octopamine is known to increase behavioural responsiveness to brood pheromone, which results in increased foraging behaviour (Barron et al. [8] and reviewed in Scheiner et al. [2]).

Previous studies showed octopamine, dopamine and serotonin titres increase in an age-related fashion in bees (Harris et al, [9]; Taylor et al [10], Božič and Woodring [11] Waegner-Hulme et al. [12], Schulz et al., [13]). Octopamine increases as bees age and is associated with foraging (Waegner-Hulme et al. [12], Schulz and Robinson [13]). Octopamine manipulations in young bees can induce the early onset of foraging (Schulz and Robinson [14], Schulz et al [15]), which further supports a causal relationship between foraging and octopamine.

Aside from the age-related differences in titres, differences have been found between groups of bees of similar age that perform different tasks. Dopamine titres increase with age with higher titres in foragers than in individuals of similar age which do not forage. However, within foragers of similar age, titres are higher in dancers compared to followers (Božič and Woodring [11]). Foragers can specialize in pollen or nectar foraging and between these two groups there are differences in titres of dopamine and serotonin (Taylor et al [10], Schulz et al. [13]). Between middle-aged bees there are differences in dopamine titres between food storers and comb builders. Finally, within the old bees of different ages, foragers and soldiers, there are differences in serotonin titres (Schulz et al. [13]).

Biogenic amine receptor expression and age/task related changes in expression are known in bees. The spatial expression of dopamine receptor 2 (Amdop2) changes in an age-related manner in adult honeybees. Within the Kenyon cell layer of the mushroom bodies, two distinct populations exist based on their size and their synaptic targets. The expression domain of the honeybee Amdop2 expands from the large

bodied KC localized in the central part of the calyx to the small bodied KC in the rest of the calyx (Humphries et al, [16]). Throughout development, from larva to adulthood, dopamine receptors Amdop1, Amdop2 and Amdop3 dynamically change expression patterns (Kurshan et al. [17], Humphries et al, [16], Beggs et al. [18]). Age-related changes in dopamine and octopamine receptors were shown quantitatively in subpopulations of honeybee mushroom bodies cell (McQuillan et al. [19]). Beggs et al. [20] showed changes in the dopaminergic system of workers when exposed to queen mandibular pheromone, which include dopamine titres and receptor expression, and subsequently showed dopamine receptor activation by queen mandibular pheromone (Beggs and Mercer [21]).

In ants, developmental changes in biogenic amine titres were described in *Camponotus floridanus* by Punzo and Williams [22]. Age related differences in brain titres of biogenic amines in ants were first described in *Pheidole dentata* workers, a myrmicine, where titres of dopamine and serotonin increase in an age-related fashion (Seid et al.,[23]. A recent study in ants compared titres between control nurses, foragers and reverted nurses of *Formica polyctena* (Wnuk et al. [24]), addressing for the first time age and task related differences in biogenic amine titres. The authors found, differences in octopamine levels with age (nurses significantly differ from foragers and reverted nurses), but not with task. No statistically significant differences were found in dopamine and serotonin titres. In the leaf-cutter ant *Acromyrmex echinatior* Smith et al. [25] found higher levels of both dopamine and serotonin in foragers compared to midden workers. And no differences were found in titres of dopamine, serotonin and octopamine between young workers belonging to different

patrilines. The authors conclude differences exist based on task specialization between foragers and midden workers, which are roughly the same age. In *Pheidole dentata* the onset of foraging is related to an increase in serotonin levels (Seid and Traniello [23], serotonin is also involved in trail following (Muscedere et al. [26]), which in turn is one component of the complex sensory environment foragers face outside the nest. In fire ants, *Solenopsis invicta* nest mate recognition has been linked to octopamine and is dependent on the presence of the queen (Vander Meer et al. [27]). Queen presence has also been shown as a factor of nest mate recognition in *Campanotus fellah* (Boulay et al. Boulay2009). Social bonding and nest mate recognition in ants depend on a signature scent that is maintained through social interactions. In *Campanotus fellah*, social isolation induces an increase in trophallaxis once individuals are reincorporated to the group, which can be reduced by octopamine manipulations, but is independent of serotonin (Boulay et al. [28]). In *Formica japonica* social interactions influence both dopamine and octopamine titres (Wada-Katsumata et al. [29]). Aggression in ants has been linked to serotonin in *Formica rufa* (Kostowski et al. [30]), but in *Formica japonica* the link has been established with octopamine (Aunoma and Watanabe [31]. Even in more basal ants, the queenless ant *Streblognathus peetersi* biogenic amines, specifically dopamine and octopamine are significantly different between hierarchical groups (Cullvier-Hot and Lenoir [32]). In ants, a number of genomes have been sequenced and annotated (Bonasio et al. [33], Smith et al. [34, 35], Suen et al. [36], Wurm et al. [37], Oxley et al. [38]) and therefore biogenic amine receptors have been found through sequence

homology. However beyond that, nothing is known about biogenic amine receptors in ants.

Demographic manipulations have been used to uncouple age and task in honeybees (Waegner-Hulme et al. [12] and Schulz et al., [13]) and ants (Wnuk et al. [24] and Smith et al. [25]. Wnuk et al. compared control nurses, control foragers and reverted nurses of *Formica polyctena* and found significant differences in octopamine levels between nurses and both groups of foragers. Smith et al. [25] compared similarly aged and sized workers of *Acromyrmex echinatior* which were either foragers or midden workers and found significant differences in dopamine and octopamine levels between the groups. Although the exact ages are unknown, it suggests task specialization could be associated with biogenic amine differences. Smith et al. [25] also compared similarly aged young workers from different patrilines and found no significant differences in biogenic amine titres. Together, the results of these studies suggest biogenic amines modulate division of labour. Biogenic amines have therefore been linked with different aspects of social behaviour , making it likely that biogenic amines also underlie inter-individual variability.

Ibarrarán-Viniegra et al. (in preparation, chapter 2), recently documented abundant inter-individual variability in in *Pheidole dentata* ants. The authors propose inter-individual variability could be generated through a predetermined, environmentally responsive or stochastic process. In a follow up study Ibarrarán-Viniegra et al. (in preparation, chapter 3), focused on foraging behaviour as a model to address inter- individual variability. From a single cohort setup of same age individuals sharing a social environment, two clearly distinct behavioural groups emerge: *go* ants

which leave the nest and forage and *no go* ants which stay in the nest performing brood care. The authors found no statistically significant differences in behavioural capacities between 5 day old *no go* and *go* ants. Previous studies suggest biogenic amines are associated with group level behavioural transitions, however the role of biogenic amines in individual behavioural ontogeny is unknown. In the present study we address whether inter-individual variability is modulated by biogenic amines.

4.3 Materials and Methods

Colony collection and care

We collected queenright colonies of *Pheidole dentata* in Gainsville, Florida in spring 2009, 2010 and 2012. We kept colonies in Fluon-lined plastic boxes (either 27x19x10 cm or 31x22x10 cm depending on colony size) with red cellophane covered test tubes partially filled with water and tight cotton plugs. We fed ants three times a week with a combination of 1 M sucrose, fresh mealworms, fresh waxworms and Witcomb diet. We maintained colonies in a Conviron environmental chamber (Controlled Environments Ltd., Winnipeg, Manitoba) under a 12L:12D light cycle at 27°C and 70% relative humidity.

Inducing inter-individual variability in foraging behaviour

In order to analyse inter-individual variability, we focus on foraging behaviour, one of the key behaviours that shows abundant variability (Ibarrarán-Viniegra et al, in preparation chapter 2). We created single cohort experimental setups, where in the absence of old workers some young workers show an early onset of foraging

behaviour. We used early foragers (*go* ants) and *no go* ants to study inter-individual variability in foraging behaviour. First, we created setups containing individuals 20 days or older (D20+) and at least 60 dark pupa, from which the next morning we obtained at least 50 individuals which eclosed in the overnight period (D1). Each single cohort setup was composed of 20 D1 labelled focal individuals, 30 D1 non labelled individuals, 3 white pupa, 5 dark gutted ant larva, 5 late larva, a pile of eggs and microlarva. We placed them in a small clear plastic boxes (14 x 10 x 4 cm) with dental stone bottom which retains humidity. We created a nest-space (0.75 x 0.25 x 0.3 cm) with a microscope slide held in place with modelling clay and covered by red cellophane to create an undisturbed dark nest area for ants. We allowed ants to acclimate overnight, period during which they moved the brood into the nest area.

Biogenic amine titres in individual brains

We sampled individual ant brains for high-performance liquid chromatography (HPLC) measurement validations. We used young ants with callow like pigmentation (AC1: D1-D3) in the immediate proximity of the brood pile. We sampled AC4 (D20+) ants based on pigmentation and location outside the nest. To assess inter-individual variability in foraging behaviour, we measured titres in individual brains of 5 day old *no go* and *go* ants. On day 5, individual ants were classified as either *no go* or *go* ants, based whether individuals were observed inside the nest or outside foraging to assess inter-individual variability in foraging behaviour.

We quantified biogenic amines using high-performance liquid chromatography with electrochemical detection (HPLC-ED). The HPLC-ED system used included the

following components, all manufactured by ESA, Inc (Chelmsford, MA): a model 584 pump, MD-150 ($3x150mm$) reversed-phase analytical column, a $5011A$ dual-channel coulometric analytical cell, and a Coulochem III electrochemical detector. We used settings and mobile phase chemistry in accordance to Muscedere et al. [39], electrode potentials set to 125 and 225 mV for the first and second channels, respectively. Mobile phase chemistry (50 mM citrate/acetate buffer, 1.5 mM sodium dodecyl sulfonate, 0.01% triethylamine, and 24% acetonitrile in MilliQ water). We dissected individual brains in ice cold PBS and homogenized each brain in 55μl of ice cold mobile phase. We centrifuged samples at 10000 rpm for 15 minutes at 4°C, and kept them on ice until injection. We injected 50μl of the sample into the HPLC. We ran standard curves alongside samples for each experiment (2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml and 0.313 ng/ml). We assigned individuals a randomly generated number, in order to keep their behavioural group or age class unknown until data analysis. For each brain we obtained a dopamine and a serotonin titre.

Biogenic amine receptor expression patterns

Receptor cloning and probe synthesis

We isolated total mRNA from *Pheidole dentata* heads using Trizol and oligo-dT methods. We obtained cDNA by reverse transcription and checked its quality in a 1.5% agarose gel. We designed degenerate primers based on sequence alignments for the genes of interest in *D. melanogaster, A.melifera, N. vitripensis, L. humile* and *P. barbatus*. Table 4.1 shows the primer pairs used for PCR and optimal temperatures. The PCR program we used was as follows: 3 minutes at 94 °C, 5 cycles: 30

seconds 94 °C, 1 min 54 °C, 2 min 72 °C, 30 cycles: 30 sec 94 °C, 1 min annealing temperature specific for each gene (see Table 4.1), 2 min at 72 °C, finally 6 min at 72 °C and pause at 4 °C. We purified PCR products of the expected size (Qiagen gel purification kit) and subsequently ligated into the pGEM®-T vector (Promega). We transformed ligation products into $DH5\alpha$ cells and selected white colonies. We performed PCR using M13F and M13R primers to check for presence of the expected length fragment. We amplified colonies containing the expected size insert in bacterial culture and the extracted plasmid DNA was sequenced in Genome Quebec. We obtained the orientation of the insert using the primer sequences and the polymerase binding sites as reference. We then generated antisense probes labelled with DIG.

Immunofluorescent dopamine staining in whole mounts

We sampled old ants (D20+) from whole colonies. We cooled ants on ice and dissected their brains in ice cold phosphate buffer (PBS) with 0.1% Triton $X-100$ (PBT 0.1%). We put brains in ice-cold fixative (0.6% glutaraldehyde in PBS) for 1 h at room temperature (RT). We washed brains in PBT 0.1% three times for 10 minutes and incubated in 0.1% sodium borohydride in PBS for 20 minutes at RT. Sodium borohyride treatment reduces autofluorescence produced by glutaraldehyde fixation (Eldred [40]). We washed brains 2 times for 10 min in PBS, 2 times for 10 min in PBT 0.2% and blocked in 1 : 10 NGS in blocking solution (400μl PAT (PBS, 1% Triton X-100, 1% bovine serum albumina) and 500μl PBT 0.2% for either 2 hours at RT or overnight at 4°C. We incubated brains in 1 : 1000 dilution of polyclonal rabbit antibody specific for dopamine (MobiTec) in blocking solution for at

least 96 hours. We washed brains 3 times for 20 min with PBT 0.2% and incubated in blocking solution for 2 hrs. We used a goat anti-rabbit secondary flourescent antibody (Cy2, Invitrogen) and incubated in 1:1000 for 2h at RT. We washed brains were washed multiple times in PBT 0.2% incubated in 30% glycerol in PBS, then for at least 10 minutes in 50% DAPI in PBS and whole mounted in 80% glycerol.

Observations of early behavioural progression and samples for biogenic amine receptors

We observed setups under the scope for a total of 25 minutes distributed over a period of about 1.5 hours, 5 minutes observations inter-spaced by 15 minutes of no observation between 10:00 and 17:00 hours every day. During each 5 minute period, we performed instantaneous scan sampling (based on Altmann [41]) of behaviours performed by the focal individuals. We collected presence/ absence behavioural data for all focal individuals for 13 different mechanical tasks (detailed behaviours and their definitions are shown in Table 3.1). We obtained presence/ absence behavioural data during the first four days post eclosion (D2-D5). D5 ants were classified based on their behaviour in two groups: *no go* ants that were never observed leaving the nest and *go* ants that exited the nest and foraged for food. For the further study of inter-individual behavioural variability we only used *no go* and *go* ants; we excluded individuals which exited the nest but did not consume food from our study.

Whole mount *in situ* hybridization on ant brains

We cooled ants on ice and dissected their brains in ice cold phosphate buffer (PBS) with 0.1% Triton X−100 (PBT 0.1%). We replaced PBT 0.1% with 325μl

PBT 0.1%, 75μl of 16% formaldehyde and 500μl heptane. We mixed samples by hand 30 to 45 secs and removed the upper phase along with most of the aqueous phase, leaving enough to cover the tissue. We fixed samples for 20min in 610μl fresh PBT 0.1%, 150μl formaldehyde (37%) and 40μl DMSO on a rocking table at room temperature. We washed samples 2 times for 5 min in 100% methanol and stored them in 100% methanol at -30° C (based on Wulbeck and Helfrich Forster). We rehydrated samples in a MeOH:PBT 0.1% series (90:10, 70:30, 50:50, 30:70, 90:10). Washed 3 times 10 min in PBT 0.1%, 3 times 10 min in PBT 0.2% and 2 times 20 min in PBT 1%. We quickly rinsed samples in 50% hybridization solution (dextran based HybB: 50 ml Formamide 100%, 10 ml Salts $10X$, 10ml 50% Dextrane Sulfate, 1ml of $10mg/ml$ Yeast ARNt and 29ml H_2O. $10X$ Salt solution: 17.5g NaCl, 1.21g Tris-base 0.71g $NaH_2PO_4(H_2O)$, 0.71 g Na_2HPO_4, 0.2g Ficoll 400, 0.2 g PVP, 10 ml 0.5M EDTA in total volume of 100 ml, $pH = 6.8$ plus 0.2g BSA post-sterilization.) in PBT 1% and and pre-hybridized them in prewarmed HybB for an hour at 60° C. We prepared probes 1:100 in HybB; incubating them 2 min at 95° C, 1 min in ice and heated to 60°C, we incubated samples at 60°C overnight. We wash the probe out with HybB and transfered samples to PBT 0.2% in a gradual manner (3:1, 1:1, 1:3 HybB:PBT 0.2%) at 60° C. Subsequently, samples are washed with PBT 0.2% at RT and blocked in PAT. We incubated our samples for 2 hours at RT in anti-DIG antibody (1:2000 in PAT). We used NBT/BCIP colorimetric reaction. Samples were finally mounted in 80% glycerol.

Image acquisition and analysis

Zeiss microscope and Axiovision software were used for image acquisition.

Relative quantification of receptor expression by qPCR

Primer design

We designed primers for qPCR using Primer3 [42, 43] for our five target genes and the two reference genes. We used Rp49 and Rps8, which have been shown to be good reference genes and have steady expression during development in honeybees (Lorenço et al. [44]). Primer sequences are shown in table 4.2.

Assay validation and standard curves: RNA extraction, quantification, and quality assessment

To validate our assay, we first ran standard curves on an RNA sample from a pool of head capsules that included individuals from several colonies and of all age classes. We removed head capsules in ice cold PBT 0.1%. We extracted RNA extraction using Trizol following instructions provided by manufacturer. We quantified samples using Nanodrop (Thermo Scientific), additionally quantity and quality assessment was evaluated using Bioanalyzer. The results for the standard curves can be found on table 4.3. The first Ct value, specific for each gene, corresponds to the amplification cycle at which the fluorescence crosses the threshold. The slope of the standard curve is related to the amplification efficiency, a slope of -3.33 corresponds to an efficiency of 100%. A range between 90% and 110% efficiency is optimal. Finally, the melting curve status indicates whether we detected one single amplicon,

which reflects specificity of the amplification. Melting curves show the specific amplification of one single amplicon (Figures 4.6, 4.7, 4.8).

Sampling for quantitative PCR of biogenic amine receptors in individual brains

We made setups containing late pupa and fully pigmented ants. We used those individuals that eclosed overnight for day 1 old samples and the accompanying old individuals for day 20 or older (D20) samples. We sampled day 5 old *no go* and *go* ants from the behavioural setups described above to assess inter-individual variability in foraging behaviour. On day 5 based on the behavioural observations of the previous days, we classified individuals as either *no go* or *go* ants.

Single brain RNA extractions

We extracted single brain RNA on based on Bertucci [45]. Briefly, we dissected each brain in 0.1% PBT and immediately transferred it to a RNase DNase free tube with 50μl Trizol and homogenized it with a RNase-out pretreated pestel. We added 10μl of chloroform and shook the tube vigorously for 20 seconds and incubated it for 10 minutes at RT. We centrifuged samples at 12000g for 15 minutes at 4 °C and transferred the top aqueous phase to a new tube. We added 25μl of water, 0.5μl of glycogen (Invitrogen) and 50μl isopropanol, after mixing gently we incubated samples for 10 minutes at RT. We centrifuged at 12000g for 15 minutes at 4°C and discarded the supernatant. We washed the pellets with 50μl of 75% ice cold ethanol and centrifuged as above. We discarded the supernatant and air dried the RNA pellets for 10 minutes. Finally, we resuspended pellets 10μl of RNase free water and

quantified samples using Nanodrop (Thermo Scientific).

Reverse Transcription: RNA to cDNA

We submitted total RNA samples to the Genomics Centre at the Institute for Research in Immunology and Cancer (IRIC) of the University of Montreal. RNA sample quality and quantity was assessed using Bioanalyzer (Agilent Technologies, Inc., CA). 50-100ng of total RNA was reverse transcribed with random primers in a final volume of 20μl, using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) as described by the manufacturer. Before use, RT samples were diluted 1:5.

qPCR

We determined gene expression level using SYBR green assays. For each assay, we performed a standard curve to ensure that the efficacy of the assay is between 90% and 110%. For SYBR green assays, we performed a melt curve to ensure only a single product was amplified. We performed SYBR green qPCR reactions using 1 ng of cDNA samples, the Fast SYBR Green Master Mix (Applied Biosystems) and 2μM of each primer. We used the The Viia$^{\text{TM}}$7 (Life Technologies) to detect the amplification level. We used the following program: an initial step of 3 minutes at 95°, followed by 40 cycles of : 5 sec at 95° and 30 sec at 60°. We ran all reactions in triplicate and used the average Ct values for quantification. We determined the relative quantification of target genes using the $\Delta\Delta CT$ method. Briefly, we normalized the Ct (threshold cycle) values of target genes to an endogenous control gene, in this case Rsp8 and

Rp49 (ΔCT = Ct target calibrator: $\Delta\Delta CT = \Delta CtSample - \Delta CtCalibrator$). We calculated relative expression (RQ)using the Sequence Detection System (SDS) 2.2.2 software (Applied Biosystems) and the formula is RQ = $2^{-\Delta\Delta CT}$. Significance is determined by RQ values smaller than 0.5 or greater than 2.

4.4 Results

Biogenic amine titres are not significantly different between *no go* and *go* ants

Seid and Traniello [23] previously showed that titres of dopamine and serotonin increase with age in *P. dentata* worker ants. As expected, we found significant differences in both dopamine (DA) titre (ANOVA: FRatio=1256.56, p< 0.0001, AC1 n=38, AC4 n=32) and serotonin (5HT) titer (ANOVA: FRatio=1103.13, p< 0.0001, AC1 n=38, AC4 n=32) between unmanipulated AC1 and AC4 ants (Figure 4.1). Dopamine titres of day 5 old *no go* (Figure 4.2) had a mean of 52.8856 pg/brain (S.D.=4.87561 pg/brain), and *go* ants had a mean of 53.8099 pg/brain (S.D.= 6.18991 pg/brain). In contrast to AC1 and AC4, we found no statistically significant differences between *no go* and *go* ants (*no go* n=21, *go* n=28, ANOVA: FRatio=0.319111, p=0.575 Serotonin titres of *no go* ants had a mean of 30.4938 pg/brain (S.D.=2.12159 pg/brain) and *go* ants had a mean of 30.8463 pg/brain (S.D.= 3.32533 pg/brain). Like dopamine, we found no statistically significant difference between *no go* and *go* (ANOVA: FRatio=0.180272, p-value=0.673 *no go* n=21, *go* n=28).

The spatial distribution of dopaminergic cells is conserved between *A. mellifera* and *P. dentata*

First, to validate our whole mount brain *in situ* hybridizations, we cloned tyrosine hydroxylase, the rate limiting enzyme in the biosynthesis of dopamine and compared the expression pattern with that of immunohistochemistry for dopamine. Seven clusters of dopaminergic cells have been identified per hemisphere, based on DA immunoreactivity (Schürman et al. [46]). Using Schürman's localization description the clusters identified are: 1) on the rim of lateral calyx, 2) medial to lateral calyx, 3) frontal and caudal pars intercerebelis, 4) medial frontal proto-deutocerebrum border and 5) lateral frontal proto-deutocerebrum border 6) lateral protocerebrum and 7) lateral proto-deutocerebrum border. We find expression of dopamine and tyrosine hydroxylase in six of the seven clusters through both dopamine immunohistochemsitry and tyrosine hydroxylase (TH) *in situ* hybridization. This result suggests a conserved spatial pattern of dopaminergic cells relative to *A. mellifera* and the ant *Pheidole dentata*, as shown in Figure 4.3.

Partially conserved spatial expression of dopamine and serotonin receptors between *A. mellifera* and *P. dentata*

Since the titres of dopamine and serotonin did not differ between D5 old *no go* and *go* ants we hypothesized differences in receptor expression could underlie the observed behavioural differences. We successfully cloned three putative dopamine receptors (DR1, DR2, DR3) and performed *in situ* hybridization in *P. dentata* ant brains to obtain a general spatial distribution for each one of the receptors. Representative samples are shown in Figure 4.4. We find expression of DR1 in the intrinsic

and lateral cells to the mushroom body calyces of the brain in both *no go* and *go* ants (Figure 4.4 panels A and B respectively). As expected, when we use the sense probe we find no specific label in any of the areas (Figure 4.4 panel C). We find the expression of DR1 in the intrinsic and lateral cells to the mushroom body calyces is conserved relative to honeybee, however we do not find clear expression in the posterior lateral edge of the brain. Schematic representation of Amdop1 expression based on Blenau et al. [47], Kurshan et al. [17] (Figure 4.4 panel D).

We observe expression of DR2 in cell somas within the mushroom bodies and the posterior lateral edge of the brain in both *no go* and *go* ants (Figure 4.4 panels E and F respectively). As expected, when we use the sense probe, we find no specific label in the area (Figure 4.4 panel G). We find the expression of DR2 in the cell somas within the mushroom bodies is conserved relative to honeybee; however the expression in the posterior lateral edge is not described for honeybees. Schematic representation of Amdop2 expression based on Humphries et al. [48] and Kurshan et al. [17] (Figure 4.4 panel H).

For DR3 we find expression in the intrinsic and lateral cells to the mushroom body calyces, in cells neighbouring the optic lobe in both *no go* and *go* ants (Figure 4.4 panels I and J respectively). As expected, when we use the sense probe, we find no specific label in the area (Figure 4.4 panel K). We find the expression of DR3 in these areas is conserved relative to honeybee, however we do not see clear expression adjacent to the antennal lobe. Schematic representation of Amdop3 expression based on Beggs et al. [18] (Figure 4.4 panel L).

We successfully cloned two putative serotonin receptors (5HT1 and 5HT2) and performed *in situ* hybridization in *P. dentata* ant brains Figure 4.5 shows representative samples. The expression pattern of 5HT1 in *no go* and *go* ants is in the Kenyon cell layer of the mushroom bodies as well as within the basal ring region of the mushroom body cup (4.5 panel A and B respectively). No label is observed in the areas when we test the sense probe (4.5 panel C). We find the expression of 5HT1 within the basal ring region of the MB is conserved relative to honeybee, however the expression in the Kenyon cell soma layer and in the frontal and lateral protocerebrum has not been described in honeybees. Schematic represenation based on review by Blenau and Thamm [49] (Figure 4.4 panel D), it is important to note the pattern described by the authors is based on immunohistochemistry which might explain inconsistencies in the detailed pattern when comparing the localization of proteins and mRNA. We find expression of 5HT2 in the intrinsic and lateral cells of the mushroom body calyces, as well as in the posterior lateral edge of the brain in both *no go* and *go* ants (4.5 panel E and F respectively). We see no labelling of these areas when we use the control sense probe (4.5 panel C). In honeybees, the Am5HT2 receptor is known to be expressed in the brain (Thamm et al. [50]), however the spatial distribution within the brain has not been described. Yet the expression pattern found in ants is relatively consistent with the serotonin binding pattern reviewed by Thamm et al. [50] (4.5 panel H.

Expression levels of dopamine and serotonin receptors do not significantly differ between *no go* and *go* D5 ants

Since dopamine and serotonin titres differ significantly between young and old ants, with old ants having higher titres, we expected receptor expression would differ

between them. We first compared one day old (D1) and 20 plus day old (D20+) individuals for DR1, DR2 and DR3 (Figure 4.9). We found a statistically significant difference for DR2 between D1 and D20 (Figure 4.9 panel C). Statistical significance is determined when RQ values are lower than 0.5 (50% reduction) or higher than 2.0 (two-fold difference) relative to the calibrator sample, in this case D1-1. DR1 and DR3 showed no significant difference in relative expression levels (Figure 4.9 panels A and E). Expansion of the spatial expression of Amdop2 in honeybee (Humphries et al. [16] and Kurshan et al. [17]) suggest changes in expression with age. We found no statistically significant difference in the expression of any of the dopamine receptors between D5 *no go* ((samples N1, N2, N3) and *go* (samples F1, F2, F3) ants (Figure 4.10 panels B, D and F, calibrator sample N1).

We found no statistically significant difference in the expression level of serotonin receptors 5HT1 and 5HT2 between one day old (D1) and more than 20 day old (D20) individuals (Figure 4.11). We also found no statistically significant difference in the expression of serotonin receptors between 5 day old *no go* (samples N1, N2, N3) and *go* (samples F1, F2, F3) for 5HT1 and 5HT2 (Figure 4.12).

4.5 Discussion

Biogenic amine titres are not associated with task differences amongst same age workers

We tested whether the aminergic systems dopamine and serotonin are involved in the generation of inter-individual variability in behaviour between same age ants in the ant *Pheidole dentata*. We find: (1) the titres of these two biogenic amines show age-related changes but found no evidence of task- related differences between *no go*

and *go* day 5 old ants; (2) We find similar spatial expression patterns for dopamine (DR1, DR2 and DR3) as well as the serotonin receptors (5HT1 and 5HT2) between *no go* and *go* day 5 old ants; and (3) We find similar levels of expression of the dopamine and serotonin receptors between *no go* and *go* day 5 old ants. However, unlike honeybees, we do find a reduction in the expression level of DR2 with age. Together, our results suggest that at the level of whole brain titres or receptor expression, biogenic amines are not correlated with inter-individual variability in foraging behaviour between same age individuals.

Biogenic amine titres in worker *Pheidole dentata* ants are age-related and not task-related

Based on honeybees (Waegner-Hulme et al. [12]), we expected a significant difference in biogenic amine titres between *no go* and *go* ants. However, we show that dopamine or serotonin titres in same age ants that perform different tasks are not different. We therefore conclude that in the worker ants of *Pheidole dentata* biogenic amine titres in the brain differ with age but not with task among individuals of the same age. Our results suggest that the biogenic amines dopamine and serotonin are not associated with behavioural differences in same age individuals. We do not rule out biogenic amines are modulators of social behaviours, however we find no evidence of biogenic amines having a role in division of labour.

The expression pattern and quantitative expression of biogenic amine does not differ between same age workers performing different tasks

Biogenic amine receptors are G-protein coupled receptors and therefore depending on the type of G-protein they are coupled with they can have different cellular effects on key second messenger pathways (cAMP, IP_3 and DAG). Therefore the same titre of ligand could have a different effect depending on receptor expression. Based on the literature, age-related changes in receptor expression have been documented in honeybees. Dopamine receptors Amdop1, Amdop2 and Amdop3 show changes in expression pattern through development and with age (Kurshan et al. [17], Humphries et al. [48] and Beggs et al. [18]). Age-related changes in dopamine and octopamine receptors were shown quantitatively in subpopulations of honeybee mushrooom body cells (McQuillan et al. [19]). The spatial expression pattern of AmDop2 receptor has also been described to change with age (Humphries et al. [48]). However, task related differences in spatial or level of receptor expression in same age honeybees has not been documented. We find no significant differences in the quantitative expression of receptors between D5 old *no go* and *go* ants. However we do find differences in expression of the dopamine receptor DR2 with age. The expression domain of dopamine receptor 2 in honeybees expands with age (Humphries et al. [48]), which would suggest a quantitative increase with age. This receptor in honeybee has been linked to the modulation of motor behaviour (Mustard et al. [51]). RNAi down regulation of AmDop2 resulted in a reduction of walking behaviour, which translated into an increase in grooming and stopped behaviours. In the case of *Pheidole dentata* workers, D1 old ants showed significantly higher expression of DR2 compared to D20 old ants. Young ants spend more time grooming or stopped, therefore in

order to fully understand the meaning of an increased expression of DR2 in young ants further studies addressing the relative abundance and function of the different receptor types would be necessary to further understand this difference between honeybees and *P. dentata* ants. Together, our findings of similar biogenic amine titres and similar receptor spatial distribution and expression levels support our conclusion that biogenic amine changes, in titres and receptors, are age dependent and not task dependent amongst worker *Pheidole dentata* ants.

4.6 Future directions and Conclusions

One important consideration is whether differences between *no go* and *go* ants exist at finer time scales, for example within the first hour after an individual foraged for the first time compared to individuals who have never foraged and once the individual is set on that trajectory the difference disappears. It is possible that in the scale of days, titres and expression levels are similar because the critical time window is not captured and therefore we do not find a statistically significant difference.

In the present study we cloned receptors based on sequence similarity, however further research is necessary to characterize the dopamine and serotonin receptors in ants through pharmacology, heterologous expression and domain annotation. Additionally, one other thing to consider is the existence of receptor isoforms. For this study, primers were designed based on the longest isoform reported in either drosophila or honeybee, however isoforms can also lead to important differences in cellular responses. In vertebrates serotonin, receptor 1 has two isoforms, isoform a (auto-receptor, expressed in the soma) and isoform b (synaptic auto-receptor) and

differences in receptor isoform expression are associated with copying styles and behavioural flexibility (Koolhaas et al. [52]).

This study is the first to compare the dopaminergic and serotoninergic systems of known age ants that perform different roles in the same social context with the aim of understanding whether these biogenic amine systems are involved in the generation of inter-individual differences in behaviour. Our results show there are no significant differences in titres or expression of receptors of the biogenic amines dopamine and serotonin between same age individuals performing different roles in the same social context. Titres are, as expected, significantly different with age but not with task. This suggests biogenic amines can be involved in the development as ants age however they are not associated with the task ants of the same age perform. In support of biogenic amines having an age-related role in behavioural ontogeny, we find differences in expression of one of the dopamine receptors (DR2) with age. Which suggests that not only do titres of biogenic amines change, but also the relative abundance or each receptor type could change as individuals age. We have laid the foundation on which to build further systemic level research of biogenic amines in ants.

Our results as a whole have important implications for division of labour since a number of the existing models aiming to explain temporal polyethism are based on differential response thesholds between individuals performing different tasks. Biogenic amines, in their role as neuromodulators, have been proposed to have the potential to generate differential response thresholds to stimuli. Our results suggest that if any, differences in biogenic amines occur at the scale of brain regions and are not evident when whole brain titres or receptor expression is compared. However

the fact that differences have been found in honeybees, suggest biogenic amines may have evolved different roles in the division of labour in honeybees and ants.

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References

[1] Roeder T (1994) Biogenic amines and their receptors in insects. *Comparative Biochemistry and Physiology* 107C:1–12.

[2] Scheiner R, Baumann A, Blenau W (2006) Aminergic control and modulation of honeybee behaviour. *Current Neuropharmacology* 4:259.

[3] Bicker G, Menzel R (1989) Chemical codes for the control of behaviour in arthropods. *Nature* 337:33–39.

[4] Libersat F, Pflueger H (2004) Monoamines and the orchestration of behavior. *BioScience* 54:17–25.

[5] Kamhi J, Traniello J (2013) Biogenic amines and collective organization in a superorganism: Neuromodulation of social behavior in ants. *Brain Behavior and Evolution* 82:220–236.

[6] Scheiner R, Plückhahn S, Öney B, Blenau W, Erber J (2002) Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. *Behavioural brain research* 136:545–553.

[7] Mercer A, Menzel R (1982) The effects of biogenic amines on conditioned and unconditioned responses to olfactory stimuli in the honeybee *Apis mellifera*. *Journal of comparative physiology* 145:363–368.

[8] Barron A, Schulz D, Robinson G (2002) Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *Journal of Comparative Physiology A* 188:603–610.

[9] Harris JW, Woodring J (1992) Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (*Apis mellifera*) brain. *Journal of Insect Physiology* 38:29 – 35.

[10] Taylor D, Robinson G, Logan B, Laverty R, Mercer A (1992) Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. *Journal of Comparative Physiology A* 170:715–721.

[11] Božič J, Woodring J (1998) Variations of brain biogenic amines in mature honeybees and induction of recruitment behavior. *Comparative Biochemistry and Physiology, Part A* 120:737–744.

[12] Wagener-Hulme C, Kuehn J, Schulz D, Robinson G (1999) Biogenic amines and division of labor in honey bee colonies. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 184:471–479.

[13] Schulz D, Robinson G (1999) Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 184:481–488.

[14] Schulz D, Robinson G (2001) Octopamine influences division of labor in honey bee colonies. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 187:53–61.

[15] Schulz DJ, Elekonich MM, Robinson GE (2003) Biogenic amines in the antennal lobes and the initiation and maintenance of foraging behavior in honey bees. *Journal of Neurobiology* 54:406–416.

[16] Humphries M, et al. (2003) Invertebrate d2 type dopamine receptor exhibits age-based plasticity of expression in the mushroom bodies of the honeybee brain. *J Neurobiol* 55:315–30.

[17] Kurshan P, Hamilton I, Mustard J, Mercer A (2003) Developmental changes in expression patterns of two dopamine receptor genes in mushroom bodies of the honeybee, *Apis mellifera*. *J Comp Neurol* 466:91–103.

[18] Beggs K, Hamilton I, Kurshan P, Mustard J, Mercer A (2005) Characterization of a d2-like dopamine receptor (amdop3) in honey bee, *Apis mellifera*. *Insect Biochem Mol Biol* 35:873–82.

[19] McQuillan H, Nakagawa S, Mercer A (2012) Mushroom bodies of the honeybee brain show cell population-specific plasticity in expression of amine-receptor genes. *Learning and Memory* 19:151–158.

[20] Beggs K, et al. (2007) Queen pheromone modulates brain dopamine function in worker honey bees. *Proc Natl Acad Sci U S A*.

[21] Beggs K, Mercer A (2009) Dopamine receptor activation by honey bee queen pheromone. *Curr Biol*.

[22] Punzo F, Williams D (1994) Free amino acids and biogenic amines in the brain of the carpenter ant, *Campanotus floridanus* (buckley) (hymenoptera, formicidae). *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* 107:387 – 392.

[23] Seid M, Traniello J (2005) Age-related changes in biogenic amines in individual brains of the ant *Pheidole dentata*. *Naturwissenschaften* 92:198–201.

[24] Wnuk A, Wiater M, Godzinska E (2011) Effect of past and present behavioural specialization on brain levels of biogenic amines in workers of the red wood ant *Formica polyctena*. *Physiological Entomology* 36:54–61.

[25] Smith A, Muscedere M, Seid M, Traniello J, Hughes W (2013) Biogenic amines are associated with worker task but not patriline in the leaf-cutting ant *Acromyrmex echinatior*. *Journal of Comparative Physiology A* 199:1117–1127.

[26] Muscedere M, Johnson N, Gillis B, Kamhi J, Traniello J (2012) Serotonin modulates worker responsiveness to trail pheromone in the ant *Pheidole dentata*. *Journal of Comparative Physiology A* 198:219–227.

[27] Vander Meer R, Preston C, Hefetz A (2008) Queen regulates biogenic amine level and nestmate recognition in workers of the fire ant, *Solenopsis invicta*. *Naturwissenschaften* 95:1155–1158.

[28] Boulay R, Soroker V, Godzinska E, Hefetz A, Lenoir A (2000) Octopamine reverses the isolation-induced increase in trophallaxis in the carpenter ant *Camponotus fellah*. *Journal of Experimental Biology* 203:513–520.

[29] Wada-Katsumata A, Yamaoka R, Aonuma H (2011) Social interactions influence dopamine and octopamine homeostasis in the brain of the ant *Formica japonica*. *Journal of Experimental Biology* 214:1707–1713.

[30] Kostowski W, Tarchalska-Krynska B, Markowska L (1975) Aggressive behavior and brain serotonin and catecholamines in ants (*Formica rufa*). *Pharmacology Biochemistry and Behavior* 3:717 – 719.

[31] Aonuma H, Watanabe T (2012) Octopaminergic system in the brain controls aggressive motivation in the ant, *Formica japonica*. *Acta Biologica Hungarica* 63:63–68.

[32] Cuvillier-Hot V, Lenoir A (2006) Biogenic amine levels, reproduction and social dominance in the queenless ant *Streblognathus peetersi*. *Naturwissenschaften* 93:149–153.

[33] Bonasio R, et al. (2010) Genomic comparison of the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Science* 329:1068–1071.

[34] Smith CD, et al. (2011) Draft genome of the globally widespread and invasive argentine ant (*Linepithema humile*). *Proceedings of the National Academy of Sciences* 108:5673–5678.

[35] Smith CR, et al. (2011) Draft genome of the red harvester ant *Pogonomyrmex barbatus*. *Proceedings of the National Academy of Sciences* 108:5667–5672.

[36] Suen G, et al. (2011) The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. *PLoS Genetics* 7:e1002007.

[37] Wurm Y, et al. (2011) The genome of the fire ant *Solenopsis invicta*. *Proceedings of the National Academy of Sciences* 108:5679–5684.

[38] Oxley PR, et al. (2014) The genome of the clonal raider ant *Cerapachys biroi*. *Current Biology* 24:451 – 458.

[39] Muscedere M, Johnson N, Gillis B, Kamhi J, Traniello J (2011) Serotonin modulates worker responsiveness to trail pheromone in the ant *Pheidole dentata*. *Journal of Comparative Physiology A* pp 1–9.

[40] Eldred W, Zucker C, Karten H, Yazulla S (1983) Comparison of fixation and penetration enhancement techniques for use in ultrastructural immunocytochemistry.

[41] Altmann J (1974) Observational study of behavior: Sampling methods. *Behaviour* 49:227–267.

[42] Koressaar T, Remm M (2007) Enhancements and modifications of primer design program primer3. *Bioinformatics* 23:1289–91.

[43] Untergrasser A, et al. (2012) Primer3 new capabilities and interfaces. *Nucleic Acids Research* 40:e115.

[44] Lourenço A, Mackert A, Cristino A, Simoes Z (2008) Validation of reference genes for gene expression studies in the honey bee, *Apis mellifera*, by quantitative real-time rt-pcr. *Apidologie* 39:372–385.

[45] Bertucci L, Noor M (2001) Single fly total rna preparations for rt-pcr. *Drosophila Inf. Serv.* 84:166–168.

[46] Schürmann F, Elekes K, Geffard M (1989) Dopamine-like immunoreactivity in the bee brain. *Cell and Tissue Research* 256:399–410.

[47] Blenau W, Erber J, Baumann A (1998) Characterization of a dopamine d1 receptor from *Apis mellifera*: cloning, functional expression, pharmacology, and mrna localization in the brain. *J Neurochem* 70:15–23.

[48] Humphries M, et al. (2003) Invertebrate d2 type dopamine receptor exhibits age-based plasticity of expression in the mushroom bodies of the honeybee brain. *Journal of neurobiology* 55:315–330.

[49] Blenau W, Thamm M (2011) Distribution of serotonin 5-HT and its receptors in the insect brain with focus on the mushroom bodies: Lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arthropod Structure and Development* 40:381 – 394.

[50] Thamm M, et al. (2013) Function and distribution of 5-ht2 receptors in the honeybee *Apis mellifera*. *PLoS ONE* 8:e82407.

[51] Mustard JA, Pham PM, Smith BH (2010) Modulation of motor behavior by dopamine and the d1-like dopamine receptor amdop2 in the honey bee. *Journal of Insect Physiology* 56:422 – 430.

[52] Koolhaas J, de Boer S, Coppens C, B. B (2010) Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology* 31.

gene	annealing temp	forward primer	reverse primer
DR1	61°C	ATGACMTTTGCCGGGGTSAACGATCTC	TGAAGGCCGAGTTRCTGTAGCCGAGCCA
DR2	59-60°C	TGTGCGTCATYAGCYTRFAYCGTTAYTGGGC	CGAAARTCTCTGCTCCAGCARGCRTATAT
DR3	58.8°C	AAGCACACGAACAATAGAAGAGTRTGG	TCCCATCATCTTGCAMGTRAAGAASGGCA
TH	54-55.8°C	ACCGARGAGGAAGTDGTGCT	GAGTCVCGRCCVTCMAAGAA
5HT1	57.5°C	TGGTCGCCTGYCTHGTWA	ATCCTTSTGAATGCCTGCC
5HT2	56-58°C	CATAATGCACATGTGYTTCATC	ACGAAYGTGAARAACACCAG

Table 4.1: **Primers and temperatures used for PCR amplification** Target genes DR1: dopamine receptor 1, DR2: dopamine receptor 2, DR3: dopamine receptor 3, 5HT1: serotonin receptor 1 and 5HT2: serotonin receptor; and reference genes (Rp49: ribosomal protein 49 and Rps8: ribosomal protein s8)

Gene code	Gene name	Forward primer	Reverse primer
DR1	dopamine receptor 1	GCCACACAACGACGATGCCA	ATCAAGGATCCCCTCAGGTACGG
DR2	dopamine receptor 2	TCGCGCCGCCGTATTACAGA	TGAGTTCGAGCTCGCCCGAG
DR3	dopamine receptor 3	AGACACCTAACACGATCGCC	GGGAGAAGAGTTCGGCCAAG
5HT1	serotonin receptor 1	CATCCTCAGGCTCGTGACG	CGTGGTTCTCGTGATGGTGA
5HT2	serotonin receptor 2	ACTGGCCATGCTGGTCTCCA	GGCCGCGGCATGATATTCGA
Rp49	ribosomal protein 49	GGGCCAGTACTTGATGCCTA	CCAGTTGGTAGCATGTGACG
Rps8	ribosomal protein s8	GAGCCTGCCTTGGAAGAAC	TCCAGGCCTGCTTGCTATAC

Table 4.2: **Primers used for qPCR for target genes and reference genes** (DR1: dopamine receptor 1, DR2: dopamine receptor 2, DR3: dopamine receptor 3, 5HT1: serotonin receptor 1 and 5HT2: serotonin receptor 2) and reference genes (Rp49: ribosomal protein 49 and Rps8: ribosomal protein s8)

Gene code	gene name	first Ct value	efficiency
DR1	dopamine receptor 1	25	107%
DR2	dopamine receptor 2	22	101%
DR3	dopamine receptor 3	26	109%
5HT1	serotonin receptor 1	25	105%
5HT2	serotonin receptor 2	28.7	129%
Rp49	ribosomal protein 49	24	95%
Rps8	ribosomal protein s8	21	95%

Table 4.3: **Standard curve results for qPCR primer pairs**

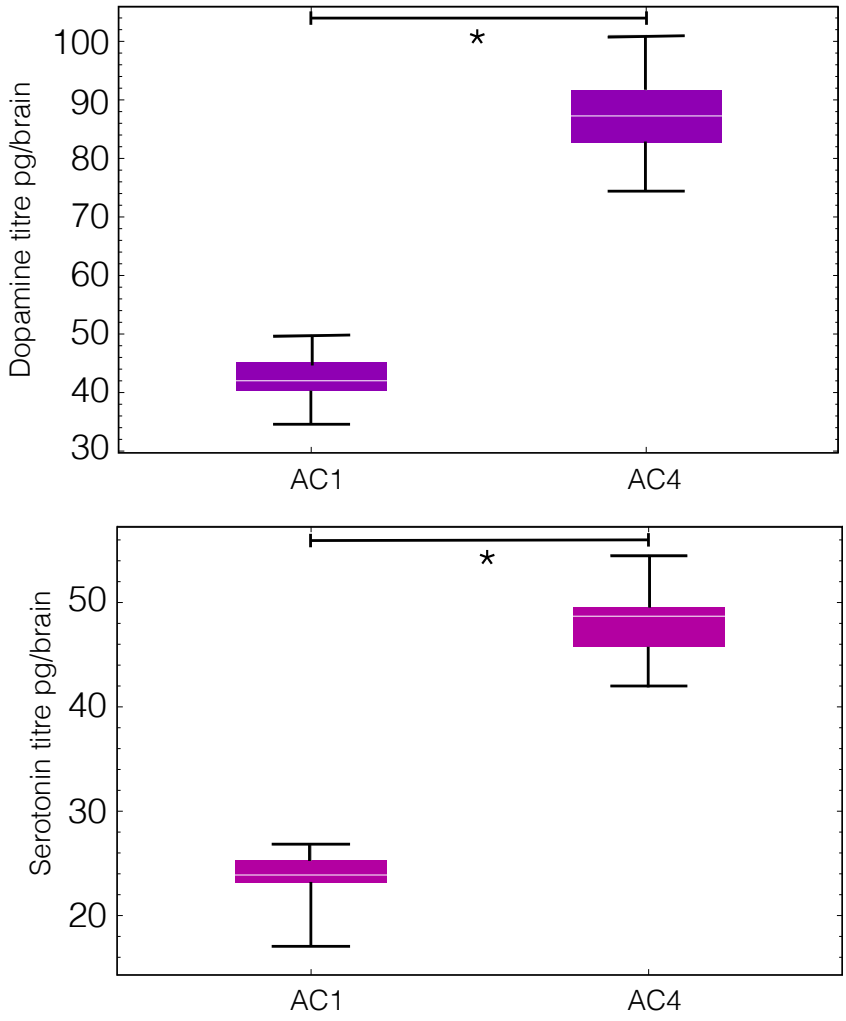

Figure 4.1: **Biogenic amine titres in pg/brain of AC1 and AC4 workers of *P. dentata***. X axis: age class AC1 and AC4 (AC1: n= 38 and AC4: n=32). Y axis: biogenic amine titre in pg/brain. Box plots show median values(horizontal lines), interquartile ranges (boxes), max and min values (whiskers). Statistical significance $p < 0.05$ indicated by *.

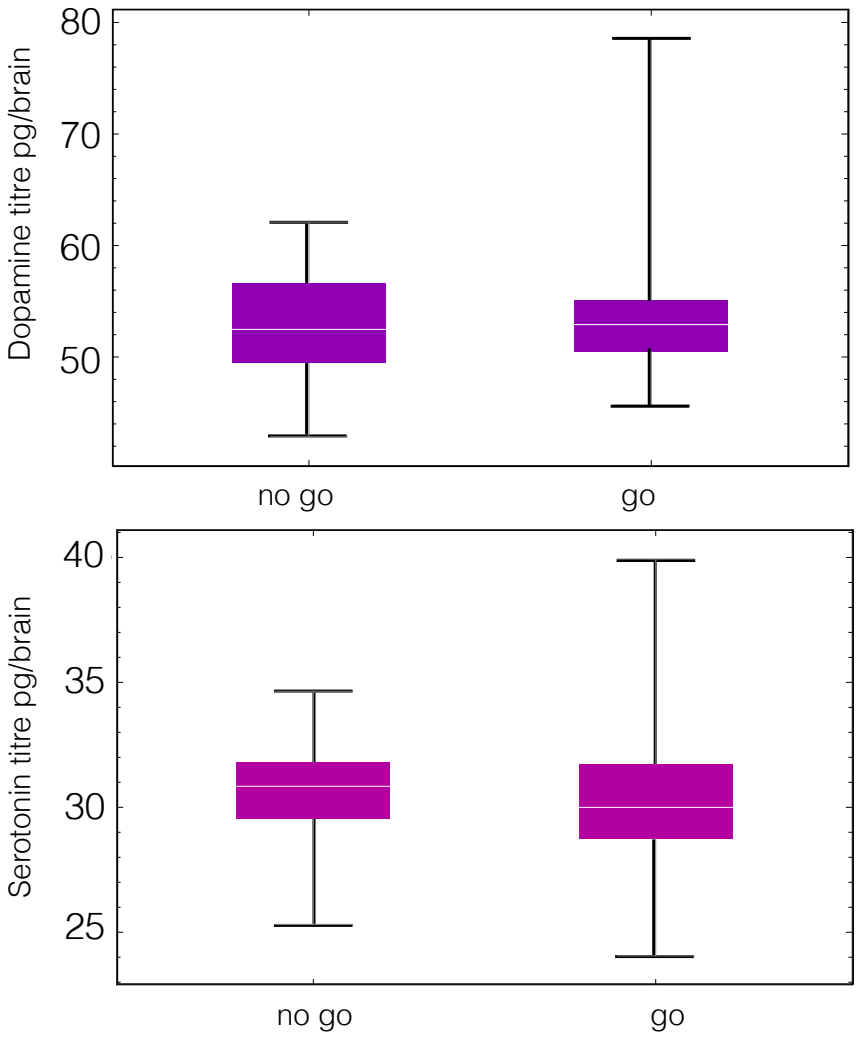

Figure 4.2: **Biogenic amine titres in D5 old *no go* and *go* worker ants of *P. dentata***. X axis behavioural groups: *No go* ants n= 21 and *go* ants n=28. Y axis: biogenic amine titre in pg/brain. Box plots show median values(horizontal lines), interquartile ranges (boxes), max and min values (whiskers).

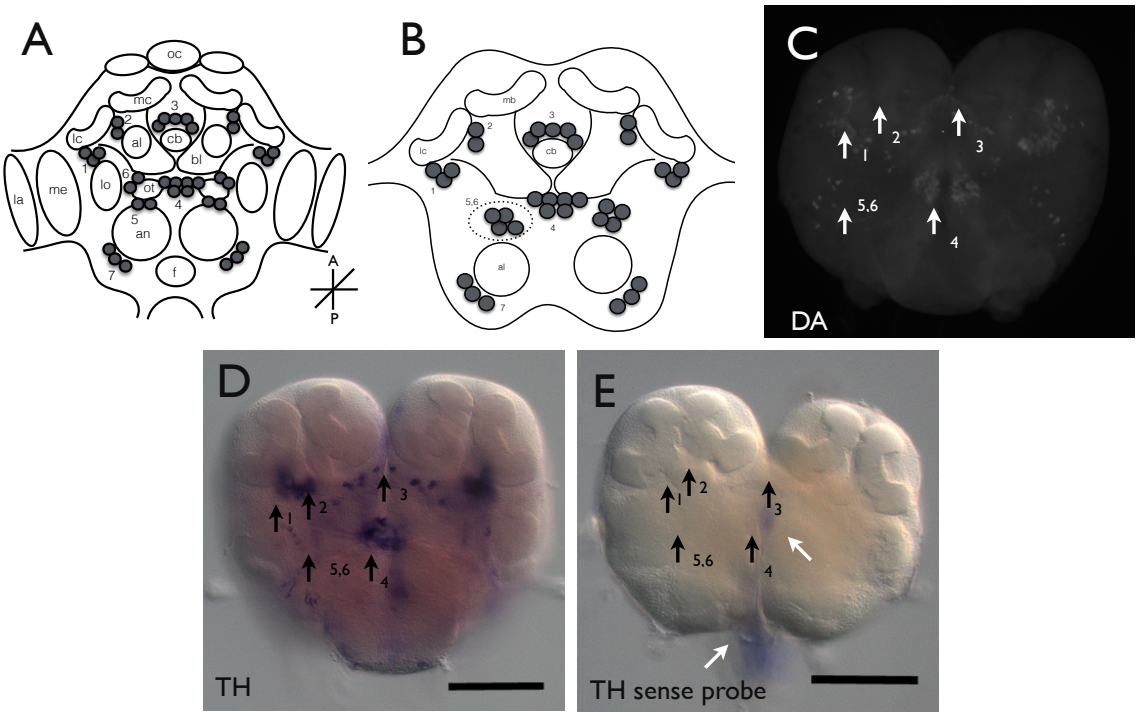

Figure 4.3: **Spatial distribution of dopaminergic cell clusters across species**. Schematic representation of spatial distribution of dopaminergic cell clusters in **A** honeybee, **B** the ant *H. saltator*, **C** dopamine immunohistochemistry *P.dentata* white arrows indicate clusters of DA immunoreactivity (based on on Schürman et al. [46]), **D** *P. dentata* tyrosine hydroxylase (TH) *in situ* hybridization, black arrows indicate clusters of probe binding, **E** *in situ* hybridization with sense probe for TH, black arrows indicate where clusters would be expected, white slanted arrows indicate unspecific signal. Scale bar indicates 100 micrometers. Magnification used 20X. Orientation: anterior is up and posterior down.

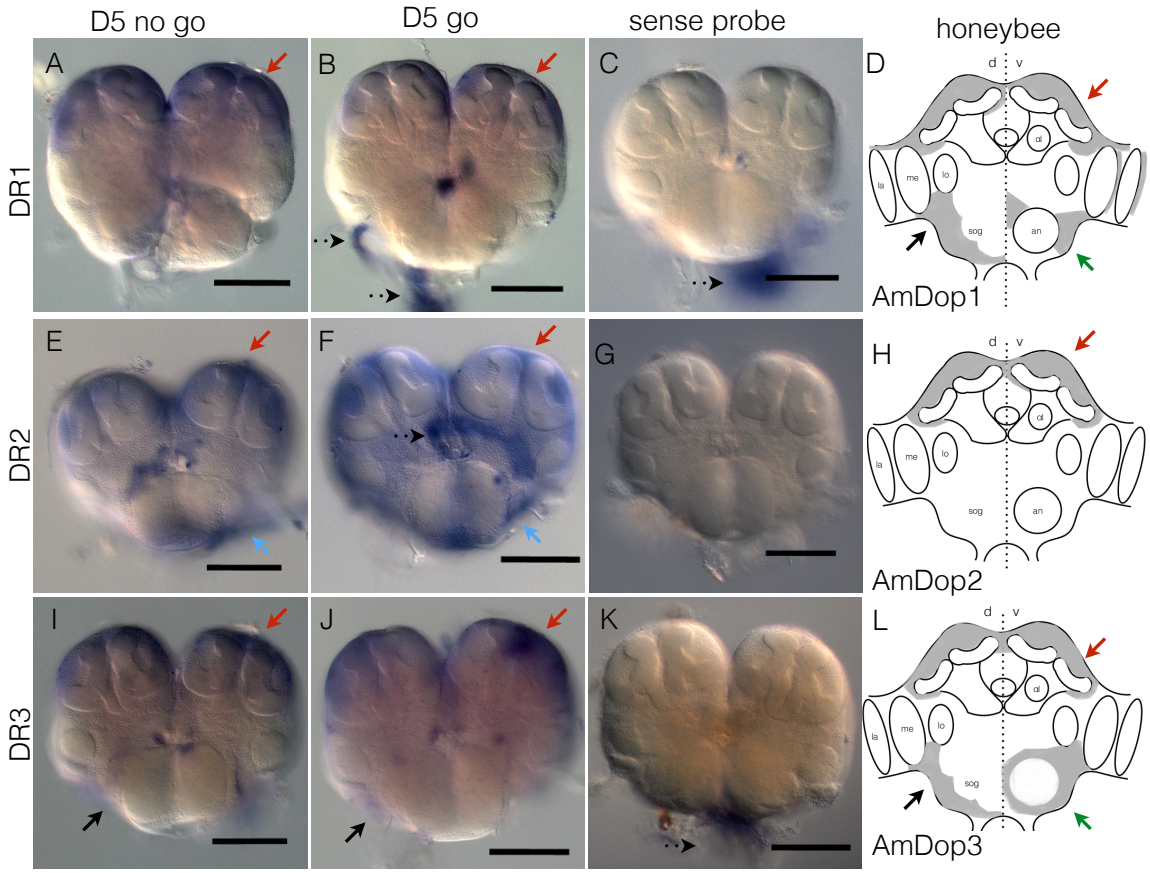

Figure 4.4: **Spatial expression of dopamine receptors DR1, DR2 and DR3**. Dopamine receptor 1: **A** *insitu* hybridization for DR1 in *no go* ants, **B** *insitu* hybridization for DR1 in *go* ants, **C** *insitu* hybridization for DR1 sense probe, and **D** Schematic representation of spatial distribution of honeybee DR1 (based on *insitu* hybridizations), Dopamine receptor 2: **E** *insitu* hybridization for DR2 in *no go* ants, **F** *insitu* hybridization for DR2 in *go* ants, **G** *insitu* hybridization for DR2 sense probe, and **H** Schematic representation of spatial distribution of honeybee DR2 (based on *insitu* hybridizations), Dopamine receptor 3: **I** *insitu* hybridization for DR3 in *no go* ants, **J** *insitu* hybridization for DR3 in *go* ants, **K** *insitu* hybridization for DR3 sense probe, and **L** Schematic representation of spatial distribution of honeybee DR3 (based on *insitu* hybridizations). Scale bar indicates 100 micrometers. Magnification used 20X. Orientation: anterior is up and posterior down. Comparative focal plane based on sharp boundaries of mushroom body cups. Solid arrows (red, black and green) indicate conserved areas relative to honeybee. Dotted black arrows indicate unspecific staining. Solid blue arrows indicate expression not found in honyebees.

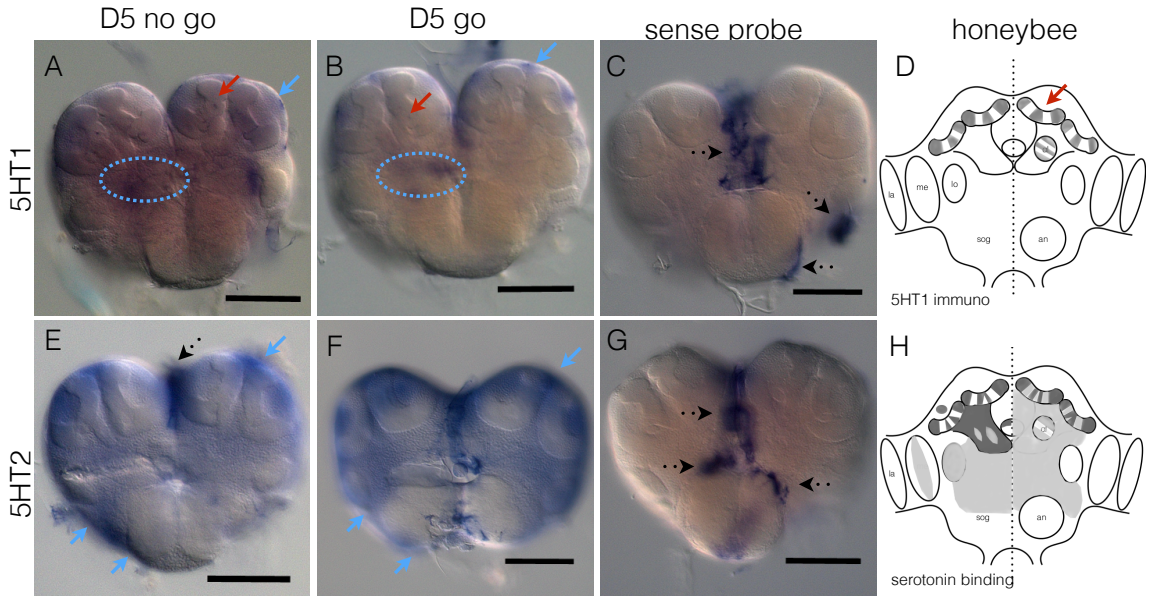

Figure 4.5: **Spatial expression of serotonin receptors 5HT1 and 5HT2**. Serotonin receptor 1: **A** *insitu* hybridization for 5HT1 in *no go* ants, **B** *insitu* hybridization for 5HT1 in *go* ants, **C** *insitu* hybridization for 5HT1 sense probe, and **D** Schematic representation of spatial distribution of honeybee serotonin receptor 5HT1, based on immunohistochemistry. Serotonin receptor 2: **E** *insitu* hybridization for 5HT2 in *no go* ants, **F** *insitu* hybridization for 5HT2 in *go* ants and **G** *insitu* hybridization for 5HT2 sense probe and **H** Schematic representation of serotoin binding (the expression in honeybee has not been described). Scale bar indicates 100 micrometers. Magnification used 20X. Orientation: anterior is up and posterior down. Comparative focal plane based on sharp boundaries of mushroom body cups. Solid red arrows indicate conserved areas relative to honeybee. Dotted black arrows indicate unspecific staining. Solid blue arrows and dotted blue circles indicate expression not previously described in honyebees.

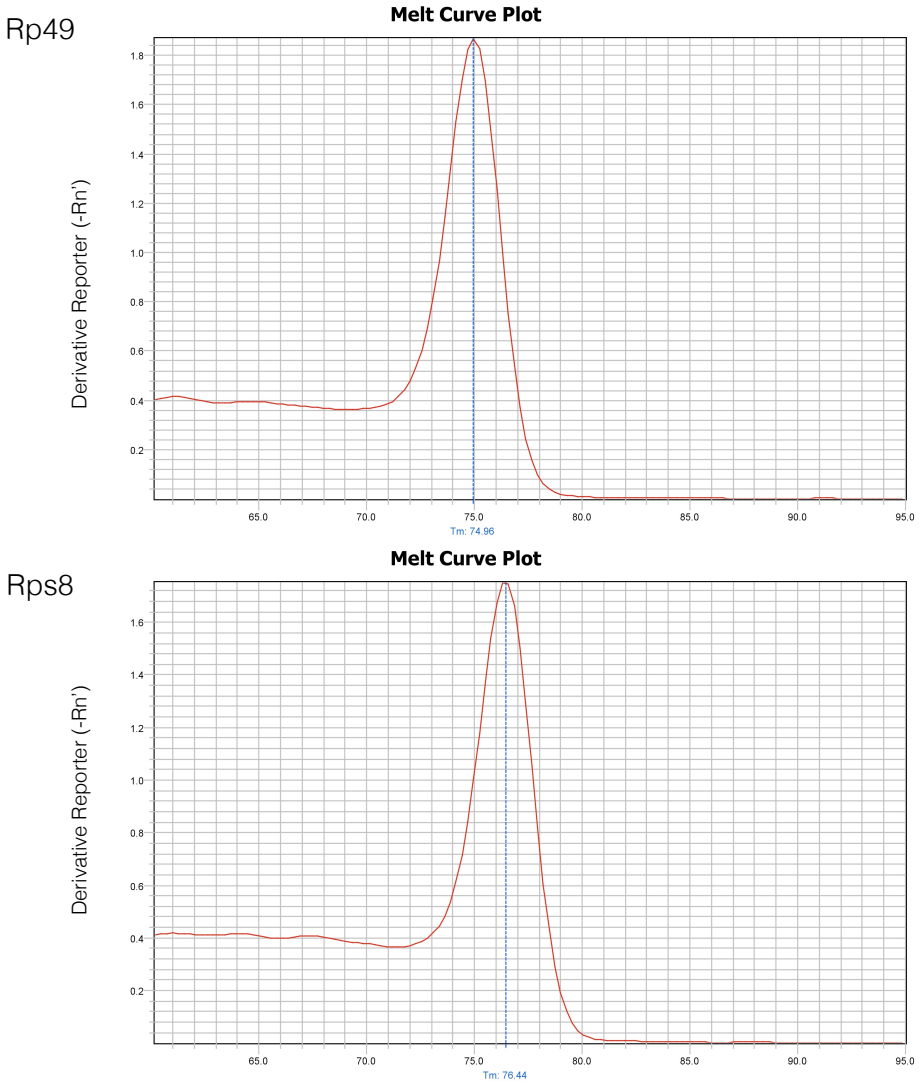

Figure 4.6: **Melting curves for reference genes: Rps8 and Rp49** X-axis temperature, y-axis derivative reporter.

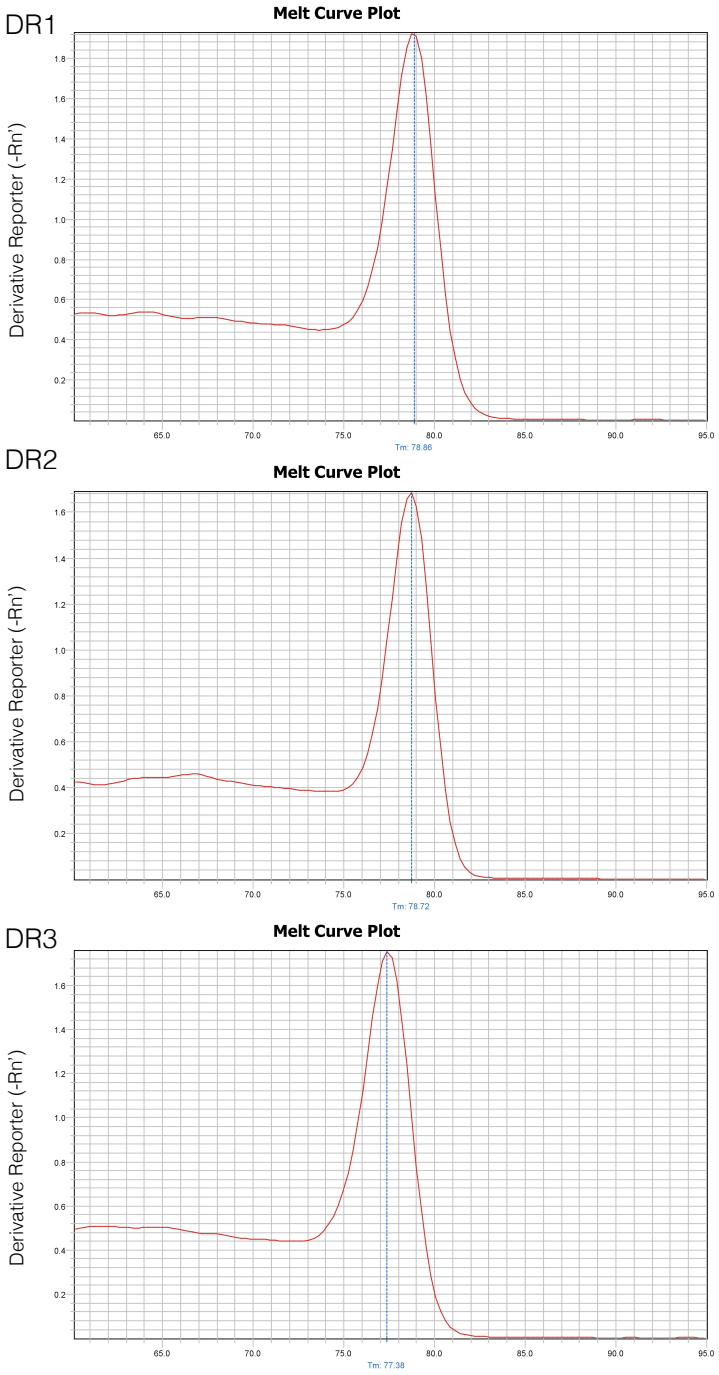

Figure 4.7: **Melting curves for dopamine receptor genes** X-axis temperature, y-axis derivative reporter.

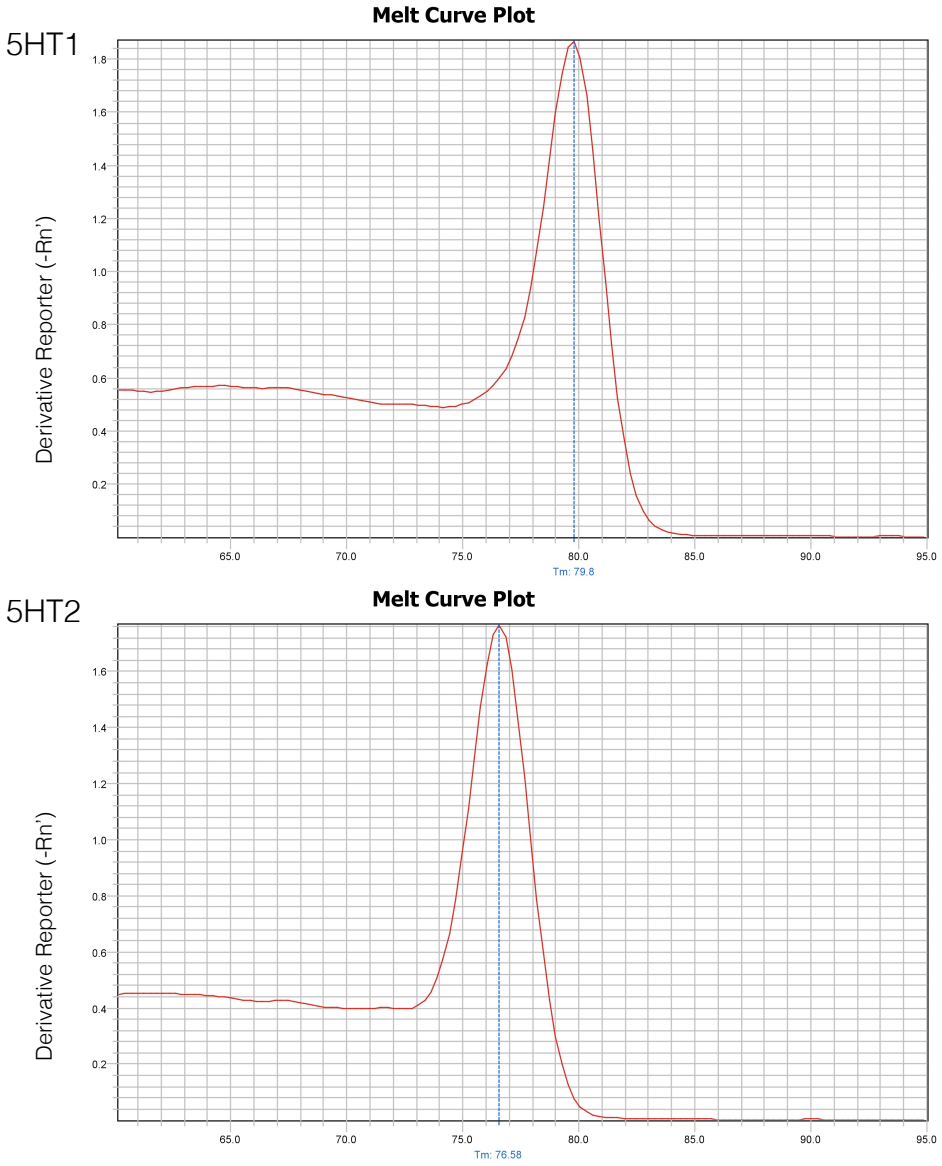

Figure 4.8: **Melting curves for serotonin receptor genes** X-axis temperature, y-axis derivative reporter.

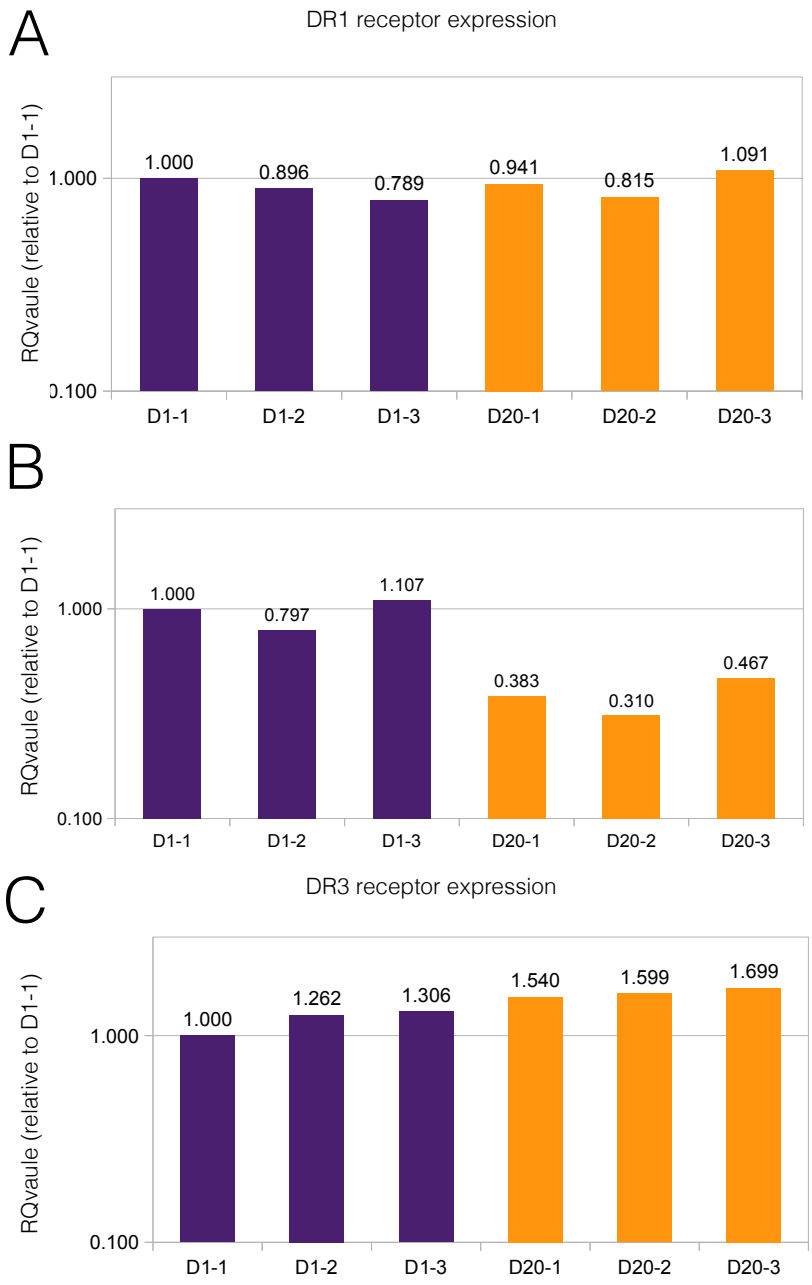

Figure 4.9: **Quantitative expression of dopamine receptors DR1, DR2 and DR3 between D1 and D20 samples**. X-axis samples: day 1 old ants (samples D1-1, D1-2, D1-3) and D20+ ants (samples D20-1, D20-2 and D20-3). Y-axis RQ value or fold change compared to calibrator sample; in this case D1-1 is the calibrator sample. Statistical significance indicated by 0.5 *RQvalues* 2.0. **A** DR1 comparison between one day old (D1) and ants older than 20 days (D20), **B** DR2 comparison between one day old (D1) and ants older than 20 days (D20). **C** DR3 comparison between one day old (D1) and ants older than 20 days (D20)

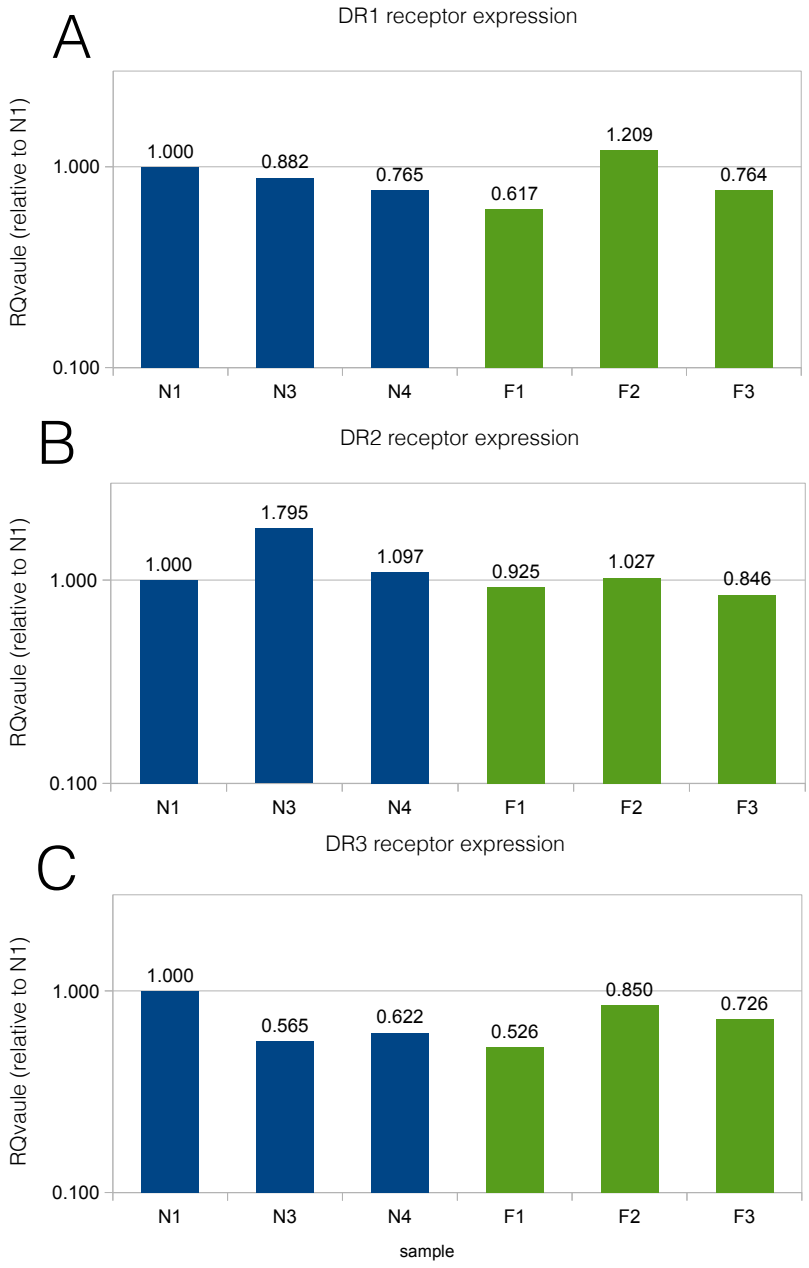

Figure 4.10: **Quantitative expression of dopamine receptors DR1, DR2 and DR3 in D5 samples**. X-axis samples: *no go* (samples N1, N2, N3) and *go* ants (samples F1, F2 and F3). Y-axis RQ value or fold change compared to calibrator sample; in this case N1 is the calibrator sample. Statistical significance indicated by 0.5 *RQvalues* 2.0. **A** DR1 comparison between 5 day old *no go* ants and *go* ants. **B** DR2 comparison between 5 day old *no go* ants and *go* ants. **C** DR3 comparison between *no go* ants and *go* ants.

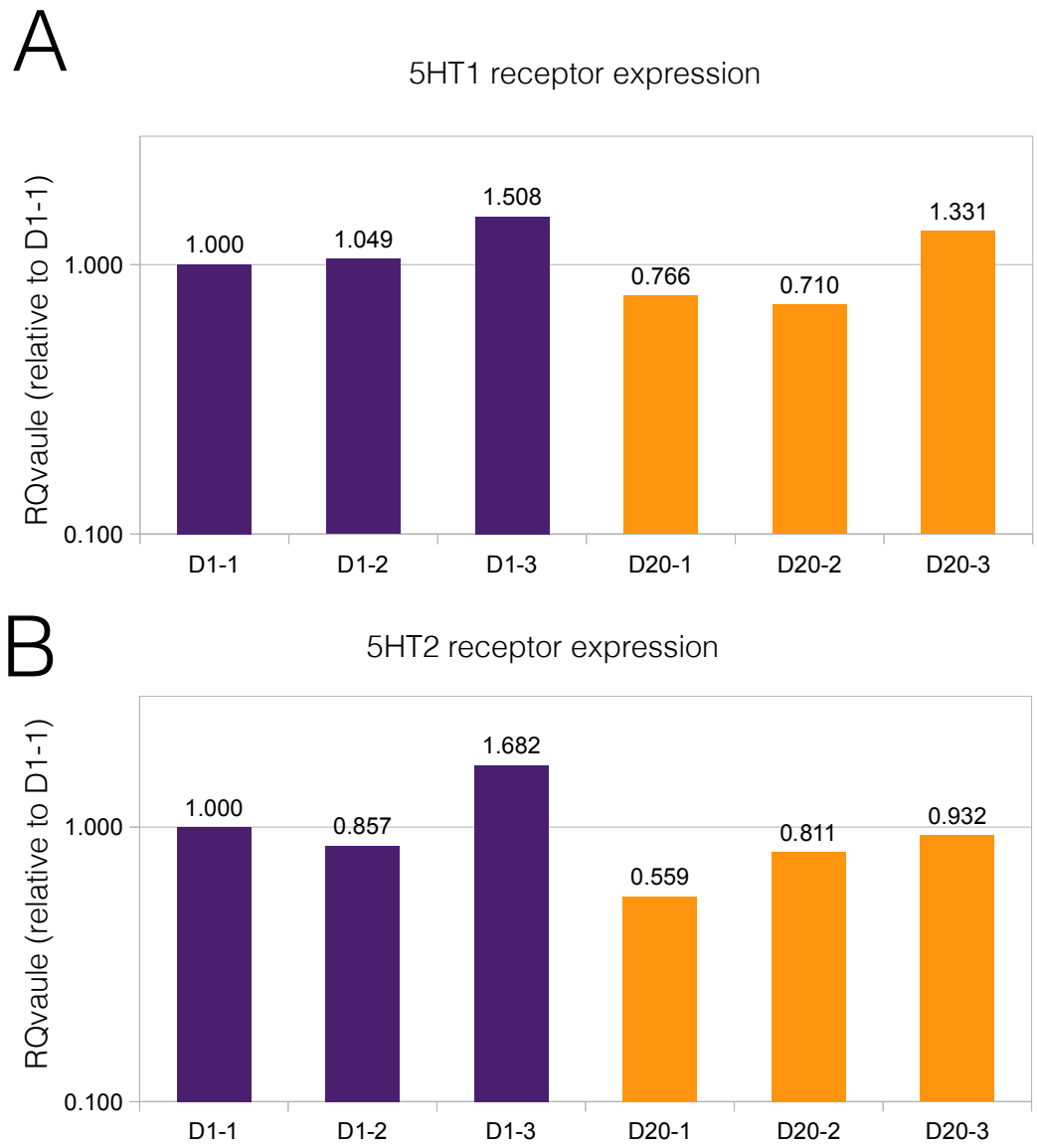

Figure 4.11: **Quantitative expression of serotonin receptors 5HT1 and 5HT2 in D1 and D20 samples**. X-axis samples: day 1 old ants (samples D1-1, D1-2, D1-3) and D20+ ants (samples D20-1, D20-2 and D20-3). Y-axis RQ value or fold change compared to calibrator sample; in this case D1-1 is the calibrator sample. Statistical significance indicated by 0.5 $RQvalues$ 2.0. **A** 5HT1 comparison between one day old (D1) and ants older than 20 days (D20). **B** 5HT2 comparison between one day old (D1) and ants older than 20 days(D20).

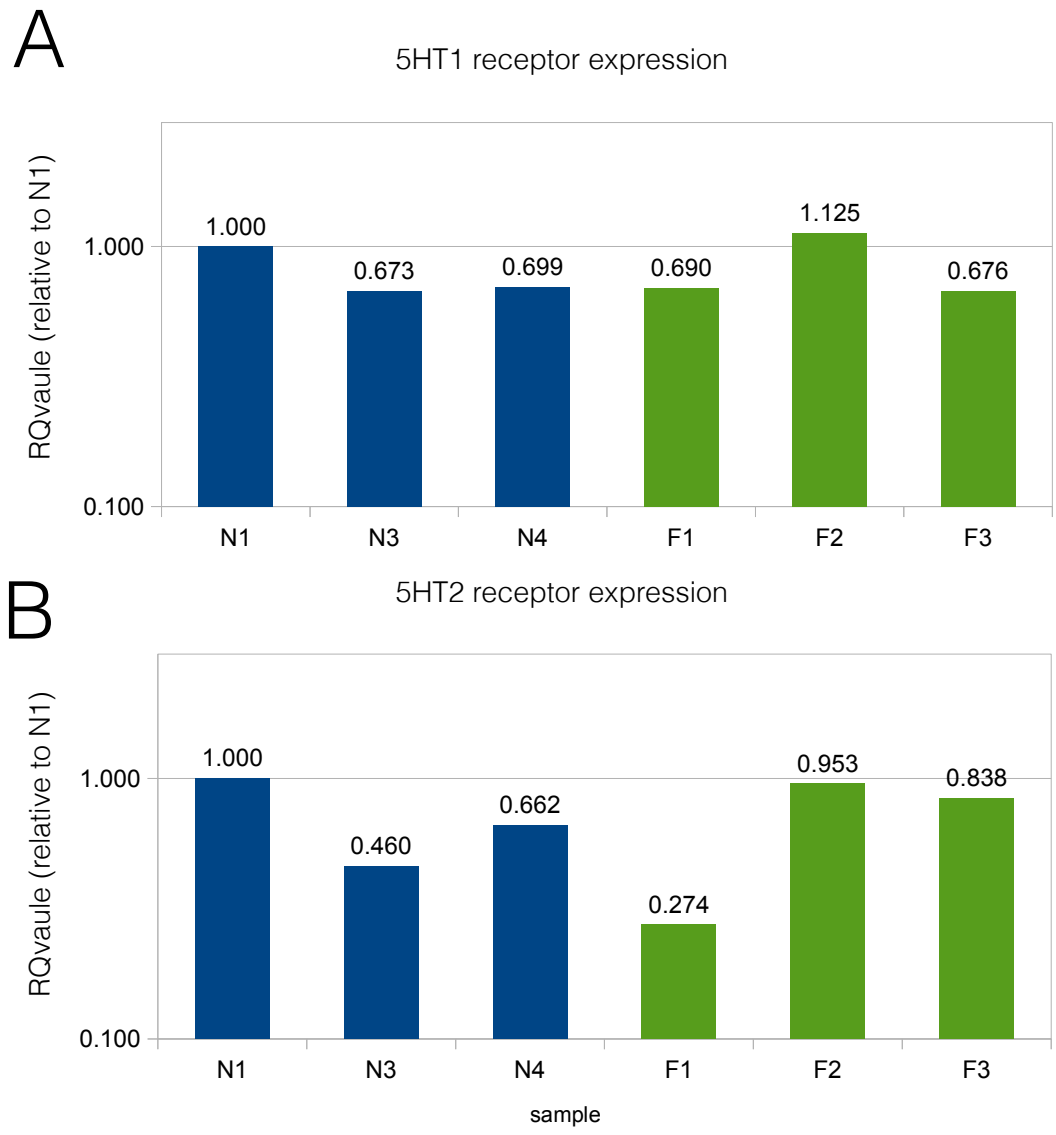

Figure 4.12: **Quantitative expression of serotonin receptors 5HT1 and 5HT2 in D5 samples**. X-axis samples: *no go* (samples N1, N2, N3) and *go* ants (samples F1, F2 and F3). Y-axis RQ value or fold change compared to calibrator sample; in this case N1 is the calibrator sample. Statistical significance indicated by 0.5 *RQvalues* 2.0. **A** 5HT1 comparison between *no go* ants ants (samples N1, N2, N3) and *go* ants (samples F1, F2 and F3). **B** 5HT2 comparison between *no go* ants and *go* ants.

Connecting statement

In the previous chapters, we found that inter-individual variability in foraging behaviour is not accompanied by differences in the biogenic amine systems dopamine or serotonin. We now present final conclusions and the significance of the work presented in this thesis for the field of social insect sociobiology.

CHAPTER 5
Concluding remarks

5.1 Conclusions

Major findings and implications

My research has lead us to reassess the organizing principles of division of labour and to suggest plasticity-mediated inter-individual variability must be added as one of them. In the past, plasticity has been considered a secondary process, limited to producing robustness and resilience, important features of colony organization, but not necessarily as having a primary role in the organization of division of labour. Inter-individual variability on the other hand has been given little attention and when acknowledged, is considered as noise in the system. Previously age, physiology, spatial location and reinforcement have been proposed as organizing principles of division of labour. However I now propose it is necessary to incorporate inter-individual variability into models in order for them to more accurately explain the organization of division of labour in social insect colonies.

In Chapter 2, I provide evidence of abundant inter-individual variability among same age individuals in a shared social environment. My results show a number of novel features about division of labour in *Pheidole dentata* minor workers. First, it partially recapitulates the pattern of temporal polyethism previously reported in this species by Seid and Traniello [1]. As their repertoire expansion model (REM) suggests individuals add on tasks to their repertoire without abandoning previously performed tasks. However, individuals do not necessarily do it in a concerted timing or concordant fashion. I find individuals show diverse array of repertoires and vary in the order in which tasks are added to their repertoire. Second, tasks have

different temporal dynamics not only in terms of when individuals add them to their repertoire, but also the proportion of individuals engaged in them over time. Finally, a pattern similar to the one of repertoire expansion can arise from inter-individual variability and independently of age.

Chapter 3 provides evidence that inter-individual differences in behaviour that inter-individual differences in behaviour of same age individuals are not due to differences in behavioural capacities. I showed, through a suite of behavioural paradigms testing exploration, foraging behaviour and brood care, that same age individuals that perform different tasks within the social context of the social group have similar behavioural capacities, however, I do find differences with age. Additionally I find no preference for either brood or food at early eclosion. Therefore, I conclude same age individuals performing different roles within the social context are not due to differences in behavioural capacities. Together my results suggest that differentiation into *no go* and *go* ants is not likely either a completely genetically predetermined or random process. Rather they support a plastic process or a stochastic process with reinforcement.

Finally in Chapter 4, I incorporate biogenic amines into the study of inter-individual variability. The role of biogenic amines as neuromodulators of behaviour has been widely studied. In social insects, the role of biogenic amines -mainly dopamine, serotonin, and octopamine- in social behaviours has lead us to consider a potential role for them in the organization of division of labour. I provide evidence

that supports an age-related changes in the titres of both dopamine and serotonin and in one of the dopamine receptors. However, I find no evidence of whole-brain level changes in biogenic amines related to task differences between same age individuals. My results lead me to propose that biogenic amines might have evolved different roles in the division of labour of ants and honeybees.

As a whole, my work provides evidence supporting there is an environmental influence on the generation of inter-individual variability in behaviour, which does not rule out the existence of a genetic component. Regardless of the process generating this inter-individual variability, the more important outcome of my work is that it indicates inter-individual variability is a fundamental part of the organization of division of labour, rather than being the result of division of labour. I propose inter-individual variability as an organizing principle of division of labour; an idea which could lead us to reassess the way we have traditionally thought about the organization of social insect societies.

Future Directions

As with all research projects, in the process of addressing a question we are left with answers that lead to more questions. I will expand on some of the questions arising from this work, and which I would like to address in the future.

What mechanism underlies the generation of inter-individual variability?

So far my research has provided evidence supporting a platicity-mediated process underlying inter-individual variability. However, the precise mechanisms remains elusive. One avenue I am specially interested in pursuing is the potential role of social interactions in the generation of inter-individual variability. Previous research lead by Gordon (reviewed in [2]) shows interaction rates inform the decision by foragers to actively engage in foraging trips. However the question remains whether social interactions can differentiate individuals into *no go* and *go* ants. The study of social interactions remains a challenge, so far most approaches are based on interactions defined by distance between individuals. Only recently have the first attempts been made to study interactions through specific behaviours and to date, this is limited to the study of trophallaxis. Aside from a number of mechanically distinguishable behaviours, ants are known to interact through a number of chemical cues that remain elusive to merely visual methods of assessing social interactions, which adds an additional level of complexity to the issue.

What else is hiding in the data?

So far we have only explored the tip of the iceberg of the data from the 20 days of behavioural progression. As mentioned my data has suggested a number of features that have not been previously addressed in terms of the temporal polyethism in *Pheidole dentata*. One of those features is the complex in temporal dynamics of tasks; I am interested in producing a mathematical model which would represent it

appropriately.

Are behavioural differences between *no go* and *go* ants due to transient and/or spatially-restricted changes in biogenic amine systems?

The sampling methods used for the biogenic amine work can quantify long-term systemic changes in biogenic amine titres or receptor expression. It is possible that differences in biogenic amine titres or its receptors could be transient and therefore would require a time-course approach in order to be detected. This approach would be challenging in terms of logistics because samples would need to be processed over a period of days and therefore variation in results could be confounded by day to day variation of experimental conditions. Alternatively a very large scale experiment could potentially allow the sample size to be large enough within a day, however it would remain challenging. Additionally, differences could be at a regional scale and therefore when comparing whole-brain titres and receptor expression the differences averaged out. Dissecting specific brain regions and measuring titres and receptor expression is technically extremely challenging at least with the methods we used. Due to the detection sensibility of the HPLC equipment we used measuring titres in subparts of a brain would bring us near the detection limit and therefore compromise the accuracy of the measurements. For the quantitative measurement of receptor expression, the amount of RNA we are able to obtain from a single whole brain is barely enough for qPCR, therefore having enough RNA for qPCR on brain compartments would be a challenge. However methods for extracting RNA from single cells are available and could be explored.

Relative abundance of biogenic amine receptor types and isoforms

I showed that the titres of biogenic amines are not different between same age individuals performing different tasks, I would be interested in further analyzing the relative abundance of receptor types and isoforms within each single brains. This would ideally be done through qPCR using multiple probes at the same time. Although technically possible, the challenge lies again in the small amount of RNA which can be extracted from a single ant brain.

Characterization of the biogenic amine receptors

I identified the dopamine and serotonin receptors of *Pheidole dentata* by sequence homology through alignment of receptors from *Drosophila melanogaster, Apis melifera, Nasonia vitripensis*, and *Leptinema humile*. For the sequence alignment, I designed degenerate primers used for cloning. The validation of the receptors thus far is based on sequence homology confirmed through BLAST. However in order to fully characterize the receptors approaches such as heterologous expression, pharmacological characterization and domain annotation would be required. All this is technically possible and could potentially be done in collaboration with a lab already doing this kind of work (potentially the at the laboratory of Prof. Alison Mercer at the University of Otago in New Zealand).

Is inter-individual variability generated through plasticity or stochasticity followed by reinforcement?

My results support the environment has a role in the generation of inter-individual variability. However, the environment could have a role through plasticity or as the

reinforcing element in the case of a stochastic process followed by reinforcement. Distinguishing between the two is not an easy task. Behavioural selection experiments could help us get a step closer to differentiating between a plastic process and a stochastic process followed by reinforcement. First, I would classify individuals based on their behavioural profile in a control social context. A second phase would involve individuals getting rearranged into a groups containing individuals with closely similar profiles. Finally, a second phase of behavioural observations would allow us to determine whether individuals change their behavioural profile to adjust to the new social context. As a first approach we could use individuals with different activity levels (Barnes [3], Kolmes [4] and Dornhaus et al. [5]), such as highly active versus *lazy* ants (based on their movement and whether they engage or not in identifiable tasks, Charbonneau and Dornhaus [6]).

Inter-individual variability: Why?

Thus far my research focused on addressing how inter-individual variability is generated. The question I would now ask is the importance of inter- individual variability for the organization of the colony. If inter-individual variability is a core organizing principle, are there differences between species which have different degrees of division of labour? Large scale experiments across species could allow us to determine this. Sampling widely across the ant phylogeny from basal to advanced ants, would also allow us to explore the evolution of inter-individual variability. Finally, is there a relationship between caste diversification and inter-individual variability? Is variability increased within castes in ant species where morphology has become

more *restrictive*?

Is plasticity-mediated inter-individual variability in the process of becoming a fixed trait?

In an environment with long-term stable conditions, one could argue it would be adaptive to fix inter-individual variability instead of having as a plasticity-mediated trait. However ant colonies, as superorganisms, have an extremely long lifespan compared with any other non-social insect. In this situation keeping the generation of inter-individual variation responsive to environmental conditions might be better. Additionally due to the long lifespan of ant colonies, even if the environment were stable, the internal environment of the colony is constantly changing as the colony as a whole develops.

Broader implications of my work: zooming out from ants

> **N**o man is an island entire of itself...
>
> - *John Donne*

The present work lead me to propose environmentally mediated inter-individual variability as an organizing principle of division in labour in the ant species *Pheidole dentata*. This idea, in turn, could lead us to rethink how we study division of labour not only in other ant species but within other social insect systems; and permeate the sphere of social evolution.

Inter-individual variability and social behaviour are both present throughout the animal kingdom. Wherever two individuals interact and respond to each other's presence, they influence each other's behaviour. Therefore the range of variability of individuals involved ultimately has an effect on the outcome. If this is true at the level of interacting pairs of individuals, it is even more so in groups. Collective behaviour is ubiquitous, from the emerging collective behaviours in a flock of birds and school of fish to collective decision making in human societies. Collective behaviour depends on the composition of groups, and individuals are, to a large extent, a product of their social context. Recognizing the fundamental importance of inter-individual variability in the organization of groups has far reaching implications beyond gaining understanding of social insect societies; ranging from wild life management and conservation to collective decision making by international organizations.

Ant colonies are highly complex systems but have a smaller scale than ecosystems, and therefore provide a opportunity to extract key principles shared with other complex biological systems. Finally, insight into division of labour in social insects can be used to improve optimization algorithms in computer science, and thus have the potential to solve problems not only in biology, but also in engineering (e.g. routing) and industry (e.g. scheduling), as has been documented.

References

[1] Seid M, Traniello J (2005) Age-related changes in biogenic amines in individual brains of the ant *Pheidole dentata*. *Naturwissenschaften* 92:198–201.

[2] Gordon D (2010) *Ant encounters: interaction networks and colony behavior*, Primers in complex systems (Princeton University Press).

[3] Barnes T (1941) Rhythms of activity in ant colonies. *Journal of General Psychology* 25:249–255.

[4] Kolmes SA (1985) An ergonomic study of *Apis mellifera* (hymenoptera: Apidae). *Journal of the Kansas Entomological Society* 58:pp. 413–421.

[5] Dornhaus A, Holley J, Franks N (2009) Larger colonies do not have more specialized workers in the ant *Temnothorax albipennis*. *Behavioural Ecology* 20:922–929.

[6] Charbonneau D, Hillis N, Dornhaus A (2014) lazy in nature: ant colony time budgets show high inactivity in the field as well as in the lab. *Insectes Sociaux* pp 1–5.