Variation in behavioural traits and their molecular basis in the globally invasive Argentine ant

by

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Abstract

A salient question in invasion biology is to elucidate what traits make some species invasive and not others. Recently, the introduction process has been hypothesised to function as a filter, selecting for traits that promote invasiveness in introduced populations. Behaviour has been increasingly recognised as a significant driver of animal invasions, but few studies focus on how the introduction process may affect behavioural variation across an invader's range. This thesis aims at investigating the consequences of the introduction process on behavioural variation along an introduction pathway in the globally widespread Argentine ant.

In Chapter I, I investigated transcriptome-wide variations associated with morphological caste as well as worker foraging propensity. I found a number of transcripts showed caste and behaviour-specific expression profiles, and that foraging workers exhibit higher expression of genes related to chemosensory receptors and neurological pathways known to be associated with foraging behaviour and aggression. In chapter II, I investigated octopamine-mediated modulation of foraging behaviour and aggression using pharmacological manipulations using synthetic octopamine (OA) and one of its antagonists. I found that OA was associated with colony-level foraging activity, but did not appear to similarly drive changes in individual behaviour. However, my results suggest that OA is associated with individual aggressiveness, and I also found an association between OA and foraging propensity. In chapter III, I tested the hypothesis that the introduction process selected for increased risk-taking behaviour along the Argentine ant introduction pathway. I used behavioural assays combined with OA diet supplementation in four regions along the introduction pathway, and showed that both behavioural variation and plasticity were consistent across the range, not in support of our hypothesis. Finally, in chapter IV I investigated transcriptome-wide expression profiles in workers along the introduction pathway and discovered distinct transcriptomic signatures associated with range. I found that specific immune and neural pathways were differentially regulated in the introduced range compared to the native range.

Overall, my results contribute to a better understanding of caste differentiation and neuromodulation of key behaviours in invasive ants. While I did not find behavioural differentiation between native and invasive Argentine ant populations, I show that they are

characterised by specific transcriptomic signatures perhaps underlying the ant's invasion success. This is one of the first global and integrative studies to focus on changes associated with the introduction of invasive species, and may have important implications for the understanding of biological invasions.

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Statement of authorship

I hereby declare that this thesis is my own work and that all sources quoted, paraphrased or otherwise referred to, have been properly acknowledged in the references at the end of each respective chapter. To the best of my knowledge, this thesis neither contains material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institutes of higher learning.

All data chapters have been written as individual research papers, so there is inevitable repetition between the introductions and discussions of these chapters. As all chapters contain contributions from collaborators, they are written in first person plural tense. In all cases, I wrote the manuscripts. Dr Monica Gruber and Prof Phil Lester provided advice throughout the experimental work and contributed to editing of the manuscripts, and are therefore co-authors on all manuscripts¹.

Chapter I. AF, MG and PL conceived the study. AF performed the laboratory work, data analysis, and wrote the manuscript with contributions from MG and PL.

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General introduction

Invasive species in a changing world

Biological invasions are tightly associated with globalisation, and there has been dramatic increase in the introduction of exotic species with recent economic growth (Hulme, 2009). The tonnage that is exchanged among continents has never been greater than it is today (Findlay and O'Rourke, 2009). The greater the degree of international trade, the higher number of invasive species (Westphal et al., 2008). Biological invasions are highlighted – together with habitat destruction, pollution and climate change – as a key factor contributing to the loss of biodiversity worldwide and human-caused environmental change (Vitousek et al., 1996). Invasion biologists are interested in what makes some species invasive, and not others (Davis, 2009; Elton, 1958). Elucidating how specific sets of traits can facilitate invasion success is a primary goal of invasion biology, and can have implications in both evolutionary biology and applied pest management strategies (Sakai et al., 2001). In this thesis, I investigate the changes associated with the introduction of the widespread invasive Argentine ant (*Linepithema humile*), with a focus on behavioural traits and their molecular basis.

What makes an introduced species invasive?

The introduction of exotic species in a new environment can be associated with population bottlenecks and loss of intraspecific genetic diversity because the number of founders is often low (Duglosch and Parker 2008). Limited genetic diversity can be harmful for a population, with problems associated with inbreeding depression and ultimately higher probability of extinction, which is why the success of introduced species has been frequently framed as a paradox (Allendorf and Lundquist, 2003; Sax and Brown, 2000). It is even more surprising that despite the consequences of introduction on diversity, invasive species are still frequently able to outcompete locally adapted native species that have evolved in this environment for many generations. A wide array of factors may however contribute to make a species invasive, and a key aim of invasion biology is to shed light on the mechanisms that can explain their success (Kolar and Lodge, 2001; Sakai et al., 2001). The literature on the subject is rich, and briefly summarised below by covering suggested roles for exaptation,

resource availability, enemy release and rapid evolution in the success of invasive species, with a focus on research regarding ant invasions.

Exaptation

Exapted traits are traits that can enhance fitness, but that were not shaped by natural selection for their current role (Gould and Vrba, 1982). Sometimes called ‘pre-adaptation’, exaptation has been suggested to explain the success of invasive species, especially in plant species (Schlaepfer et al., 2010). Often, exaptation in invasive species entails life-history traits such as high growth and fecundity, a generalist lifestyle, or ability to spread in disturbed environments (Baker, 1974; Lozon and MacIsaac, 1997; Schlaepfer et al., 2010). In invasive ants, exaptation of traits associated with social structure, body size and dispersal (Cremer et al., 2008), as well as traits associated with life in human-disturbed environments (Konorov et al., 2017) have been suggested to further drive their spread and success outside their native range.

Access to new resources

The access to new resources is a major driver for the success of invasive species. In New Zealand, the brushtail possum (*Trichosurus vulpecula*) was introduced by humans and quickly became out of control in an ecosystem where trees and birds evolved without such predatory pressure, and where they had little competition for resources (Allen and Lee, 2006). Many species of ants rely on mutualistic interactions with aphids and scale insects for their carbohydrate source, which provide them with honeydew in exchange of protection against predators. Invasive ant species can exhibit diet changes in their introduced range and rely even more heavily on these mutualisms, which has been linked to their ecological dominance and invasion success (Shik et al., 2014; Styrsky and Eubanks, 2007; Wilder et al., 2011).

Enemy release

Enemy release is another commonly invoked factor in the success of invasive species, and has been well observed for parasites in a wide range of introduced animal species (Torchin et al., 2003). Under the Evolution of Increased Competitive Ability hypothesis (Blossey and Notzold, 1995), introduced populations can re-allocate resources not needed for immunity into other functions that can contribute better to fitness. Although it has not been shown to be strongly associated with invasion success in ants, enemy-release has been

described in the Red Imported Fire Ants (Yang et al., 2010). Furthermore, microbial communities were shown to vary among regions in Argentine ant populations (Lester et al., 2017; Sébastien et al., 2015).

Rapid evolution

Species that arrive in new ranges often experience new sets of selective pressures compared to their native range, which are likely to promote rapid evolution (Suarez & Tsutsui 2008). In addition to new selective pressures, reduced genetic diversity of propagules could contribute to this phenomenon through genetic drift (Cox, 2004; Suarez and Tsutsui, 2008). Accelerated evolution rates after introduction has been observed for traits such as body morphology or associated to life history (Cox, 2004; Stockwell and Weeks, 1999; Yeh and Benkman, 2004). Rapid evolution can be directly associated with the spread of a species through a new range, leading to spatial sorting of phenotypes associated with activity and dispersal, enhanced by assortative mating at the invasion edge (Shine et al., 2011). A notorious example of spatial sorting of phenotypes has been observed in the cane toad in Australia, where populations at the invasion front were found to have longer legs and were faster at travelling than long-established populations (Phillips et al., 2010). In Red Imported Fire Ants (*Solenopsis invicta*) exploratory behaviour has been shown to vary along a spatial gradient consistent with the spatial sorting hypothesis, although data collection at a larger scale along an introduction pathway is needed (Bockoven et al., 2015). One aspect that has not been well studied in introduced species is the contribution of evolution of gene regulation or plasticity in gene expression to behavioural variation in introduced species. These mechanisms are thought to be important for the evolution of social behaviour (Robinson and Ben-Shahar, 2002).

The introduction process as a selective filter

The aims of this thesis are primarily towards investigating how behavioural traits and their molecular basis can be influenced by the introduction process to facilitate invasion success. I explore the hypothesis that the introduction process selects for behavioural trait variation in introduced populations, constraining which individuals or propagules become successful invaders and perhaps promoting spread and further introductions.

In the case of inadvertently introduced exotic species, the propagules have to go through successive stages (transportation, introduction, settlement, spread) to eventually settle and spread in a new range (Blackburn et al., 2011; Chapple et al., 2012; Chapple and Wong, 2016). If a given trait promotes survival and fitness throughout the process, individuals that do not possess the trait are more likely to be filtered out and fail to reach or persist in the new range. Under this scenario, the individuals that eventually manage to settle and spread in new areas represent a non-random subset of the native population. The propagules entrained in the invasion process would represent phenotypes that facilitate invasion success and promoted survival throughout the introduction process. Traits associated with invasiveness would therefore be inadvertently selected by human actions over the introduction process.

The Argentine ant

Introduced ant species are often particularly damaging invaders. The Argentine ant is especially striking in this regard given its large distribution and negative impacts in the introduced range (Holway et al., 2002). It is particularly harmful for ecosystems where it is introduced, primarily through outcompeting native ant species. Many ants are keystone organisms, and the Argentine ant-mediated disruption of native ant communities has been shown to have further ecological consequences across trophic hierarchies (Bond and Slingsby, 1984; Sanders et al., 2003; Suarez and Case, 2002). Furthermore, because the Argentine ant extensively tends aphids for honeydew, it can have significant impact on agriculture by indirectly lowering plant productivity (Vega and Rust, 2000). The Argentine ant is also an infamous household pest, and considerable amounts of money is spent each year on pest control, including in New Zealand (Ward et al., 2010).

The Argentine ant is native to the Paraná River basin in South America. It was introduced to Australia in the 1930s and New Zealand in the 1990s (Wetterer et al., 2009). In Europe, it was first detected outside its native range in the Atlantic island of Madeira in 1958 (Wetterer et al., 2009). Later in the early 1900s, it was reported all around the Southern Europe Mediterranean coast as well as in California (Wetterer et al., 2009). Genetic and behavioural analyses show that a single population – or global supercolony – has invaded Australia, California, Europe, Japan, and New Zealand (Van Wilgenburg et al., 2010; Vogel et al., 2010), pointing to serial introductions from one region to another. It appears that the

New Zealand population derived from Australia (Corin et al., 2007), itself likely derived from Europe (Suhr et al., 2011). It has been suggested that the Californian population was first introduced in Louisiana (Buczkowski et al., 2004). However, it is not clear whether they went to Louisiana from 1) Madeira – which was an important hub at the time, 2) Europe or 3) directly from South America through a distinct introduction pathway of the same native population that invaded Europe. Nevertheless, the Argentine ant's introduction pathway remains overall well documented, and comprises successive introductions. This characteristic makes it an ideal species to investigate the changes associated with the introduction process, as they may be increasingly detectable along the introduction pathway.

The importance of behavioural traits throughout the introduction process

Behaviour has been highlighted as a key feature of invasion success in animals (Holway and Suarez, 1999; Suarez and Cassey, 2016). For instance, increased activity, aggressiveness and boldness have been suggested to be important factors in promoting fitness in a wide range of species (Biro and Stamps, 2008; Smith and Blumstein, 2008), and specifically invasiveness (Chapple et al., 2011). Many invasive ants are characterised by an “invasive ant syndrome” that includes behaviours such as generalist feeding habits (e.g. omnivory, around the clock foraging activity, reliance on mutualisms), efficient colony spread (e.g. flexible nesting habits and frequent relocation, proximity with humans, polygyny), high interspecific aggression, and low intraspecific aggression (Cremer et al., 2008; Silverman and Buczkowski, 2016).

The introduction process can be divided into successive stages that are usually broadly classified under transport, introduction, establishment and spread. Different selective pressures associated with these stages are likely to differentially affect the invaders' survival depending on their behavioural phenotype (Blackburn et al., 2011; Chapple et al., 2012; Chapple and Wong, 2016). For instance, while hyperactivity may promote intake into a cargo ship, it is likely to become a deadly strategy when traveling long distances, in a hostile environment that may not offer resources (reviewed in Chapple and Wong, 2016). The evolutionary forces acting on propagules in terms of selecting specific traits or maintaining behavioural variation are therefore likely to be complex.

Foraging behaviour is risky because when a forager is out of its nest, it is more prone to predation, desiccation or disorientation (Nonacs, 1990; Wilson, 1971). Similarly, aggressiveness is likely to increase the chance of physical injury or death. However, increased foraging and interspecific aggression could also bring forth benefits in terms of resource discovery and competitiveness, crucial factors when invading novel environments (Blackburn et al., 2009; Martin and Fitzgerald, 2005; Pintor et al., 2008; Rowles and O'Dowd, 2006; Weis, 2010; reviewed in Chapple and Wong, 2016). During the introduction process, it is arguable that the more active individuals may be more likely to be transported in cargo, or successfully settle in a new environment by discovering and exploiting resources quicker than less active individuals (reviewed in Chapple et al., 2012; Chapple and Wong, 2016). Similarly, interspecific aggression is likely to favour ecological dominance and therefore enhance spread in a new range (Chapple et al., 2012; Chapple and Wong, 2016). In the Argentine ant, the benefits of interspecific aggression are likely to be enhanced by its unicolonial behaviour (i.e. the formation of extensive networks of interconnected nests – supercolonies – where workers co-operate and lack intra-specific aggression). However, at the early stages of the introduction process, a low-key behaviour may facilitate settlement by limiting risk-taking. It is likely that foraging activity and aggressiveness are key determinants of the fitness of ant colonies. Therefore, foraging behaviour and aggression are prime candidates to investigate behavioural variation along an introduction pathway.

Molecular basis of behaviour and division of labour in ants

In order to understand how the introduction process may select for specific behaviours, it is crucial to understand their molecular basis. One extraordinary feature of social insect societies is the extreme division of labour within a colony, primarily in terms of reproduction with often-distinct morphological castes, i.e. queens and workers. Workers are often sterile in ants and undertake most tasks within the colony, except egg-laying. In ants, the worker caste can be divided into sub-castes that fulfil different functions within the colony, which is sometimes associated with polymorphism (Holldobler and Wilson, 1990). Argentine ant workers are monomorphic, but are likely to exhibit age-related division of labour like most other ant species (Holldobler and Wilson, 1990). The underlying mechanisms of division of labour in ants are associated with variation in genotype (Libbrecht and Keller, 2013; Smith et al., 2008; Wiernasz and Cole, 2010), epigenetic regulation (Simola et al., 2016, 2013; Yan et al., 2014; but see Libbrecht et al., 2016), gene expression

(Mikheyev and Linksvayer, 2015; Walsh et al., 2017) and neurochemical pathways (Giraldo et al., 2016; Kamhi et al., 2015; Kamhi and Traniello, 2013).

The orchestration hypothesis postulates that neuromodulators can activate specific central neuronal circuits underlying specific behaviours (Hoyle, 1985; Libersat and Pflueger, 2004). Compounds such as biogenic amines are known to be key behavioural neuromodulators in ants and other invertebrates, and also function as key neurotransmitters and neurohormones (Kamhi and Traniello, 2013; Libersat and Pflueger, 2004). Octopamine is an important biogenic amine in invertebrates, and has been shown to be associated with various behaviours and neural processes in social hymenopterans, including foraging-related behaviours and aggression in ants and honey bees (Kamhi and Traniello, 2013; Schulz et al., 2002). The understanding of the molecular basis of behavioural variation is likely to help understanding behavioural variation among native and introduced populations in invasive species.

In this thesis, I investigate how successive introduction events have affected behavioural variation of the Argentine ant along an introduction pathway, primarily to test the hypothesis that the introduction process has selected for traits that promote invasiveness in introduced populations. I postulated that if behaviours likely to promote invasiveness, such as increased foraging activity and aggression, were selected for along the introduction pathway, introduced populations would express these behaviours to a greater extent than native populations. Furthermore, the underlying molecular basis of behavioural variation would similarly exhibit differences between native and introduced populations. After characterising the molecular basis of castes in the Argentine ant and the effect of octopamine on worker behaviour, I used behavioural assays to measure variation in foraging behaviour and aggression, as well as neurochemical manipulations and RNA-sequencing to investigate if key traits in the Argentine ant were affected by successive introductions.

Thesis outline

Chapter I investigates the molecular basis of risk-taking and division of labour in the Argentine ant. First, I discuss the transcriptomic signature of queens and workers of two different age cohorts to identify genes differentially expressed in distinct morphological castes. Second, I discuss the transcriptomic signatures of workers of known age after

categorising them depending on their foraging behaviour as scouts, foragers or within-nest workers.

Chapter II investigates the role of the octopaminergic system in the Argentine ant behaviour by using three different doses of octopamine as well a synthetic antagonist of octopamine – epinastine – to measure the effect on foraging behaviour and aggression, both at the collective and individual levels.

Chapter III tests the core hypothesis of this thesis, i.e. if key behaviours were selected for along the Argentine ant introduction pathway. I used experimental manipulations of the octopaminergic system (octopamine supplementation) and environmental conditions (sugar availability) to investigate behavioural variation and plasticity along the Argentine ant's introduction pathway.

Chapter IV consists of a global RNA-seq analysis of the populations studied in the previous chapter to investigate changes associated with the introduction events at the transcriptomic level.

Chapter I: Transcriptomic signatures associated with morphological caste, age and social behaviour in the Argentine ant

Abstract

The evolution of division of labour is considered a key factor in the success of social insects. Beyond the reproductive division of labour between workers and queens, the worker caste is also often characterised by behavioural variation, with different groups of workers engaging in different tasks. Foraging is a key factor influencing the fitness of a colony, especially in the context of biological invasions. Here, we investigate the transcriptomic basis of morphological caste differentiation (i.e. queens versus workers), age-dependent variations in workers and worker foraging behaviour.

We found that morphological caste, worker age and worker foraging propensity were associated with distinct transcriptomic signatures. Specific neural pathways such as dopaminergic, tyraminerbic and GABAergic systems were upregulated in workers compared to queens, as well as genes associated with chemoreception. We also found a positive association between foraging propensity and upregulation of genes associated with octopaminergic, dopaminergic, serotonergic, GABAergic and glutamatergic neural pathways, as well as olfactory reception.

Our results demonstrate that behavioural variation has a molecular basis in Argentine ants, and provide directions to further investigate the complexity of the social organisation of ants.

Key words

Argentine ant, Castes, Chemoreception, Foraging behaviour, Neural pathways, RNA-seq, Social behaviour

Introduction

Social insects are amongst the most ecologically successful organisms on the planet in terms of diversity, distribution and abundance. The evolution of division of labour – or polyethism – is considered a key factor in their success (Holldobler and Wilson, 1990). Understanding the underlying mechanisms of both morphological caste differentiation and social behaviour is a major goal in sociobiology and is greatly improving with recent advances in next-generation sequencing and computational biology (Nygaard and Wurm, 2015). The identification of caste-specific genes is crucial to unravel the complex organisation of social insects.

The most apparent form of division of labour in social insect colonies is the reproductive division of labour, which is in most species associated with marked morphological differences. The vast majority of individuals within a colony are female workers that often lack functional reproductive organs, while a subset of individuals – the queens – specialise in reproduction. Furthermore, polyethism also exists within the worker caste, which is sometimes also associated with polymorphism (Oster and Wilson, 1978). In many species, morphological variation among castes is a polyphenic trait where environmental factors such as nutrient intake or temperature during development can determine the fate of totipotent female larvae (Holldobler and Wilson, 1990). Polyethism within the worker caste is usually age dependent in social hymenopterans, with young workers performing tasks inside the nest (e.g. nursing) and older workers engaging in riskier tasks outside of the nest such as foraging (Holldobler and Wilson, 1990). Several mechanisms have been shown to underpin caste ontology and specificities in ants, spanning from genetic effects (Hughes et al., 2003; Libbrecht and Keller, 2013), epigenetic regulation (Simola et al., 2013), transcriptomic variation (Mikheyev and Linksvayer, 2015) to variations in neurophysiology (Amador-Vargas et al., 2015; Kamhi and Traniello, 2013). Such variations ultimately result in behavioural differences among castes as well as inter-individual variation, and shape the collective organisation of a colony (Bengston and Jandt, 2014; Jandt et al., 2014; Jeanson and Weidenmüller, 2014).

Several key behaviours are differentiated according to caste. For instance, foraging is likely to require higher performance in chemoreception, learning and memory capabilities than inside-nest workers (Page et al., 2006; Perez et al., 2013; Riveros and Gronenberg,

2010). Certain neural pathways have been shown to be associated with social behaviour in social insects (Kamhi and Traniello, 2013), including modulation of sensory functions (Farooqui, 2007), and learning and memory (Gauthier and Grünewald, 2012). Compounds that serve as neuromodulators or neurotransmitters such as octopamine, dopamine, serotonin, tyramine, GABA or glutamate are associated with behavioural variations, and differential expression of genes associated with these neural pathways have been shown to exhibit caste-biased variations (Liang et al., 2014, 2012).

Social insects are likely to be particularly sensitive to pathogens as colonies constitute dense groups of related individuals, which may facilitate pathogen transmission (Schmid-Hempel, 1998). Social immunity plays an important role in defending the colonies against infections, and includes mechanisms such as spatial nest compartmentalisation and behavioural structuring (Cremer et al., 2007). Differential exposure to pathogens among castes may therefore be associated with differences in the activation of immune pathways, as queens and in-nest or young workers may be challenged by infections to a lesser extent than foragers.

The Argentine ant is one of the most damaging invasive ants in subtropical to temperate climates around the world (Holway et al., 2002; Wetterer et al., 2009; Wild, 2004). It is a highly polygyne species, and forms extensive networks of interconnected nests – supercolonies – to an incredibly larger extent in its introduced range compared to its native range (Ingram, 2002a, 2002b; Van Wilgenburg et al., 2010; Vogel et al., 2010). The worker caste is monomorphic and sterile (Passera et al., 1988), which is a common characteristic in invasive ants (Holway et al., 2002). Hence, beyond the sole interest of understanding the fascinating social organisation of ants, the study of traits associated with castes in Argentine ants may give further insights on what contributes to the success of invasive ants. The Argentine ant's genome is sequenced (Smith et al., 2011), which makes it a good invasive ant species model to explore the molecular basis underlying their social behaviour.

Here, our goal was to characterise the transcriptomic signatures of morphological castes, worker age as well as worker foraging propensity in the Argentine ant. We used mRNA sequencing of queens and workers to investigate 1) the genes associated with morphological castes, 2) the genes associated with worker age and 3) the transcriptomic signatures associated with foraging propensity in workers of known age. We extracted RNA

from head tissue, including antennae, in order to target genes associated with brain, gland and sensory functions. We also undertook a candidate gene approach to test the hypothesis that genes associated with specific functions – neural pathways, chemoreception and immunity – are differentially regulated among castes, worker age and foraging propensity and underlie social behaviour within Argentine ant colonies.

Methods

Transcriptomic signatures of morphological caste and worker age

Queens, workers and brood were collected in Oakura, New Zealand (-39.119632, 173.939618) in February 2016 and maintained in a soil-free plastic container (30x30x20 cm) with darkened test tubes for nesting. Overall, we collected about 80 queens and 20,000 workers. The ants were kept for two months, fed *ad libitum* with 20 % w/v sugar water and frozen mealworms under a 14/10 light/dark cycle at 23°C. Mature workers were sampled outside of the nest in the foraging area, and queens and callow workers were sampled after having emptied the nest tube into a clear box lined with Fluon. Callow workers were selected based on cuticle coloration, which remains light for about a week after emergence (personal observations).

Transcriptomic signatures of behavioural caste

Pupae, queens and workers were collected in Paraparaumu, New Zealand (-40.882410, 174.994083) in late summer (March 2017) and maintained as described above. Newly emerged workers were sampled based on cuticle coloration. We were able to form three experimental colonies of approximately 300 workers of known ages by pooling together two queens and newly emerged workers every week for three weeks. Ants were maintained for 12 weeks under standard conditions until processed for RNA-seq library preparation. Colonies were housed in 20x15 cm plastic boxes lined with Fluon®, in a single 12 cm darkened glass test tube as nesting site and fed three times a week with ~ 1 gram of Bhaktar-Whitcomb jelly (Bhatkar and Whitcomb, 1970). In order to select workers based on their foraging behaviour propensity, we starved the colonies for 3 days before sampling, and then placed a 30 cm wooden tree (see Chapter III for further description of the setup) in their 20x15 cm foraging area. Ants that were exploring the tree were carefully sampled as “scouts”. The sampling of scouts stopped when ants reached the tree at a rate of less than one every five minutes for half an hour. Subsequently, the tree was removed and a drop of honey was placed in the foraging area. 10 minutes after the honey was discovered, all the ants that recruited to the food source were sampled as “foragers”. For an hour, we then progressively removed all the ants that were found outside of the foraging area until no ants were exiting the nest. The ants that remained inside the nest together with the queens and brood constituted roughly half of the experimental colonies and were sampled as “insiders”.

RNA-seq library preparation

Samples for the RNA-seq library preparation of worker ants were prepared by pooling the heads of 20 individuals. Queen libraries included the heads of 5 individuals. Ants were killed immediately after sampling by placing them in tubes on dry ice and then placed at -80°C until processed. Heads including antenna were separated from bodies under a microscope with fine forceps. Temperature was maintained under 0°C with dry ice throughout the dissection. RNA was extracted using an in-column Trizol-based purification kit using the manufacturer's recommended methods (DirectZol MicroPrep, Zymo Research, Irvine, USA). Extracted RNA was stored in RNASTable (Biomatrica Inc., San Diego, USA) and sent to BGI (BGI, Shenzhen, China) for Illumina Hi-Seq sequencing (Illumina Inc., San Diego, USA). Samples were sequenced as 150 paired-end barcoded mRNA TruSeq libraries, aiming at 4 Gb of data per sample. Overall, five libraries per experimental group were sequenced on two different lanes (i.e. for queens, callow and mature workers, and insiders, foragers and scouts, respectively). Post-processing at BGI included the removal of reads with more than 10% of missing values (i.e. bp), reads with more than 10% of bases with quality scores $Q < 20$ and removal of adapters.

Genome-guided transcriptome assembly

We undertook two distinct transcriptome assemblies for queens, callow and mature workers and insiders, foragers and scouts, respectively. Computationally demanding analyses were performed on Victoria University of Wellington Science Faculty's High Performance Computing Facility. Clean paired-end reads (128,829,637 in the queens, callow and mature workers assembly and 391,070,304 in the insiders, foragers and scouts assembly) were merged and mapped to the Argentine ant reference genome (Smith et al., 2011) with *BBMerge* from the *BBTools* suite 36.0 (Bushnell, in prep). The output was a single sorted BAM file that was then fed into the *Trinity* 2.2 pipeline for a genome-guided assembly (Haas et al., 2013). First, we used *BBMerge* to merge the paired-end reads. In order to account for non-overlapping paired-end reads but reducing the number of false-positives, we used a large maximum insert size of 1000 in *BBTools*. *Trinity* assembled 93,714 transcripts in the queens, callow and mature workers assembly, and 73,261 transcripts in the insiders, foragers and scouts assembly. Average contig length was 1,688 bases and 960 bases, with an overall alignment rate to the Argentine ant genome of 95.15 % and 96.23 %, respectively.

Transcript quantification, SIPCA and differential expression analysis

Assembled *Trinity* transcripts were quantified using the alignment-based quantification method *eXpress* 1.5.1 (Roberts and Pachter, 2012). Clean reads were aligned to the transcriptome assembly using *Bowtie2* 2.2.9 (Langmead and Salzberg, 2012) and abundance was then estimated using *eXpress*. The *eXpress* outputs were leveraged to produce a transcripts per million (TPM) matrix containing all expressed transcripts for all samples, which was then cross-normalised to obtain a weighted trimmed mean of the log expression ratios (i.e. TMM-normalised TPM values; Robinson and Oshlack, 2010).

First, in order to investigate transcriptome-wide differences among groups, we ran a Sparse Independent Principal Component Analysis from the *mixOmics* R package (Le Cao et al., 2017) using all expressed transcripts filtered based on expression value (i.e. >1 TPM). We selected the number of components based on their kurtosis value, which has been shown to be a post-hoc indicator of the relevance of the number of components to choose (Rohart et al., 2017). We presented a visual description of the analysis by plotting samples across the components landscape.

Second, we ran a differential expression analysis with the *voom* function in the *limma* R package (Ritchie et al., 2015), and the highest differentially expressed transcripts were selected using various values for the false discovery rate p-value (FDR) and the fold-change (FC). For the queens/workers comparison as well as the foraging propensity, we used a FC cut-off value of 4 as we expected gene expression associated with morphological castes to be highly variable. For the other workers/workers comparison (age or behaviour), we used a FC cut-off value of 2. In each case, the FDR was adjusted to retrieve the most consistently differentially expressed transcripts across conditions. The differential expression analysis was performed within the *Trinity* workflow.

BLAST searches

BLAST searches were performed with the *BLAST+* suite 2.4 (Camacho et al., 2009). In order to predict protein-coding transcripts from the Trinity-assembled transcripts, we used *TransDecoder* 2.0.1 (Haas et al., 2013). First, we annotated as many differentially expressed transcripts as possible using *BLASTp* of the *TransDecoder*-predicted coding transcripts on the Argentine ant and the honey bee official gene sets (Elsik et al., 2014; Smith et al., 2011)

downloaded from the *HymenopteraMine* database (Elsik et al., 2016). When a transcript retrieved no hit neither to the Argentine ant nor the honeybee gene sets, we performed a *BLASTx* search using the original *Trinity*-assembled nucleotide sequence against the non-redundant *nr* NCBI database (<ftp://ftp.ncbi.nlm.nih.gov/blast/db/>) downloaded 16 May 2017 (NCBI Resource Coordinators, 2017). In addition, we also ran *BLASTp* on the Swissprot database (The Uniprot Consortium, 2017) using the output produced by *TransDecoder*. When there were ambiguous matches with e-values lower than 1.10^{-5} , we selected the best hit based on the lowest e-values and highest bit scores, as well as sequence length and sequence percent identity.

Candidate gene approach

We created a list of candidate genes from the Argentine ant official gene set to investigate their expression pattern based on their likely association with extranidal activities and foraging behaviour using existing annotations of the Argentine ant genome (Smith et al., 2011). More specifically, we hypothesised that genes associated with neural pathways and chemoreception may underly variation in social behaviour, and that immune pathways may also exhibit differences associated with castes and behaviour. We investigated variation in genes associated with the octopaminergic (OA), dopaminergic (DA), serotonergic (5-HT), tyraminergetic (TYR), GABAergic (GABA) and glutamatergetic systems (MSG) for the neural pathways, olfactory (OR) and gustatory receptors (GR) for chemoreception, and genes associated with the TOLL, JAK/STAT and RNAi immune pathways. The lists of candidate genes are reported in Table I.S3 (queen, callow and mature workers) and S8 (insiders, foragers and scouts). We used the output of the *BLASTp* search on the Argentine ant official gene set to match specific genes with their *Trinity* transcript in both experiments. First, we retrieved TMM-normalised counts for each assembled transcripts isoforms corresponding to our target candidates, and then summed the counts of the different isoforms to obtain gene-level counts that were subsequently log-transformed and scaled across samples. We analysed differential gene expression using Generalised Linear Models (GLMs) assuming a Normal distribution with morphological caste, age group or foraging propensity as a dependent variable. We tested for post-hoc pairwise differences among groups using the *glht* function of the *multcomp R* package (Hothorn et al., 2016).

Results

Comparison of morphological castes: queens and workers

Our SIPCA analysis revealed that three independent principal components were needed to explain the distinction between queens, callow and mature workers, despite the clusters overlapping to some extent (Figure I. 1). We first compared gene expression profiles of queens and workers by pooling both callow and mature workers. We found 6,001 differentially expressed transcripts between queens and workers (i.e. all differentially expressed transcripts with $FDR < 0.05$), a large proportion of which were overexpressed in queens (Figure I.2). Increasing the FC threshold to 4 and decreasing the FDR threshold to 1.10^{-5} resulted in a subset of 109 most differentially expressed transcripts (Figure I.3). *BLAST* searches revealed that 95/109 of the most differentially expressed transcripts matched unambiguously to the Argentine ant genome (and in most cases the honey bee genome as well). Three additional transcripts matched to other ant species, as well as *Drosophila melanogaster* genes. Ten transcripts were not assigned to either Argentine ant or honey bee genes, and did not match significantly to known proteins. From the 109 most differentially expressed transcripts two clusters were defined based on expression values, characterised by upregulated genes in queens and workers, respectively (Figure I.S1, Table I.S1). The majority of significant differentially expressed transcripts were upregulated in queens (i.e. 102/109 differentially expressed transcripts).

Worker-specific transcripts were dominated (i.e. 5/7 transcripts significantly upregulated in workers) by transcripts coding for cytochrome P450 enzymes, which are involved in detoxification processes. Two other transcripts had more ambiguous annotations, with one matching to an uncharacterised locus of the Argentine ant genome and the second one matching to the *Drosophila brahma* gene, involved in transcription regulation.

Queen-specific transcripts included a number of genes associated with cellular components, metabolism, general neuronal functions, or uncharacterised Argentine ant loci. Specific functions associated with such transcripts are difficult to elucidate, if not completely unknown. However, a subset of transcripts upregulated in queens are interesting in terms of their known association with caste differentiation or behaviour. Three *vitellogenin* transcripts were upregulated in queens, as well as a transcript coding for an enzyme associated with the synthesis of juvenile hormone, i.e. farnesol dehydrogenase. Queen specific transcripts also

included a transcript putatively associated with circadian rhythm (Smith et al., 2011). Two transcripts coding for an UDP-glucuronosyltransferase, an enzyme associated with *Drosophila* male aggressive behaviour, were upregulated in queens (The Uniprot Consortium, 2017). We also found two copies of cytochrome P450 upregulated in queens as well as a putative immune gene, 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase transcript (The Uniprot Consortium, 2017).

Figure I.1. Scatter plot of individual samples showing clustering of queens, callow and mature workers after a Sparse Independent Principal Component Analysis. Samples are plotted as a function of the first three components (IPC1, IPC2, IPC3) chosen based on their kurtosis value. For representation purposes, ellipses are drawn assuming a multivariate t-distribution.

Figure I.2. Volcano plots showing differentially expressed transcripts for queens versus workers (both callow and mature worker) for various false discovery rates (FDR) and fold changes (FC). 6,001 transcripts were differentially expressed between queens and workers for a FDR < 0.05 (in red and orange). We restricted the differential expression analysis to 109 most differentially expressed transcripts by reducing the FDR to 1.10^{-5} and the increasing the FC threshold to 4 (in red only).

Figure I.3. Details of the transcripts differentially expressed between queens and workers (both callow and mature), showing log2-transformed TMMs with $p < 1.10^{-5}$ and fold change $FC > 4$. Annotations are given derived from initial *BLASTp* searches on the Argentine ant official gene set, and completed with *BLASTp* on the Swissprot database and *BLASTx* on the *nr* NCBI database. Colours in the top band represent castes (mature and callow workers in dark and light green, respectively, and queens in gold), and shades of grey in the lateral band represent gene clusters. Trees are based on expression similarity (clusters of samples and genes).

Comparison of callow and mature workers

Samples used in this analysis were restricted to mature and callow workers only. We found 871 transcripts differentially expressed between callow and mature workers (i.e. all differentially expressed transcripts with $FDR < 0.05$; Figure I.4). We decreased the FDR threshold to 1.10^{-3} and the FC cut-off to 2 in order to restrict our analysis to the most differentially expressed transcripts (Figure I.5, Table I.S2). *BLAST* searches revealed that 83/85 most differentially expressed transcripts matched unambiguously to the Argentine ant official gene set (Table I.S2). Two transcripts were neither assigned to Argentine ant nor the honeybee genomes, and did not significantly match any protein on the Swissprot database. We derived from these most differentially expressed transcripts two clusters, characterised by upregulated transcripts in mature and callow workers respectively (Figure I.S2, Table I.S2).

The majority of differentially expressed transcripts between callow and mature workers coded for proteins associated with a broad range of possible functions. Both callow and mature workers were characterised by multiple differentially expressed transcripts coding for cytochrome P450 enzymes among age groups. In the callow worker-specific expression cluster, we found a differentially expressed circadian clock transcript, as well as another vitellogenin transcript. The mature worker-specific expression cluster contained a Janus kinase transcript, associated with the JAK/STAT immune pathway (The Uniprot Consortium, 2017).

Figure I.4. Volcano plots showing differentially expressed transcripts between callow and mature workers for various false discovery rates (FDR) and fold changes (FC). 871 transcripts were differentially expressed between queens and workers for a FDR < 0.05 (in red and orange). We restricted the differential expression analysis to the 85 most differentially expressed transcripts by reducing the FDR threshold to 0.001 and increasing the FC threshold to 2 (in red only).

Figure I.5. Details of the transcripts differentially expressed between callow and mature workers, showing log2-transformed TMMs with $p < 0.001$ and fold change $FC > 2$. Annotations are derived from initial BLASTp searches on the Argentine ant official gene set, completed with BLASTp on the Swissprot database and BLASTx on the nr NCBI database. Colours in the top band represent castes (mature and callow workers in dark and light green, respectively), and shades of grey in the lateral band represent gene clusters. Trees are based on expression similarity (clusters of samples and genes).

Candidate gene approach: gene expression associated with age and caste

We found no differences in gene expression associated with the octopaminergic, serotonergic and glutamatergic neural pathways among morphological castes and worker age groups (OA: $p > 0.258$; 5-HT: $p > 0.106$; MSG: $p > 0.123$; Figure I.6, details in Table I.S4). However, genes associated with dopaminergic, tyraminergetic and GABAergic pathways were downregulated in queens compared to workers (DA in queens versus callow workers: -0.599 , $z = -4.355$, $p < 0.001$; DA in queens vs mature workers: -0.528 , $z = -3.839$, $p < 0.001$; TYR in queens versus callow workers: -0.650 , $z = -4.479$, $p < 0.001$; TYR in queens versus mature workers: -0.583 , $z = -4.017$, $p < 0.001$; GABA in queens versus callow workers: -0.882 , $z = -5.053$, $p < 0.001$; GABA in queens versus mature workers: -0.418 , $z = -2.399$, $p < 0.05$; Figure I.6, Table I.S4). Furthermore, genes associated with GABAergic pathways were downregulated in mature workers compared to callow workers (-0.463 , $z = -2.655$, $p < 0.05$; Figure I.6, Table I.S4).

Genes coding for olfactory receptors were downregulated queens compared to workers (queens versus callow workers: -0.281 , $z = -5.107$, $p < 0.001$; queens versus mature workers: -0.430 , $z = -7.804$, $p < 0.001$) and were upregulated in mature compared to callow workers (0.149 , $z = 2.697$, $p < 0.05$; Figure I.7, Table I.S5). Gustatory receptors were also downregulated in queens compared to workers (queens versus callow workers: -0.360 , $z = -3.605$, $p < 0.001$; queens versus mature workers: -0.361 , $z = -3.608$, $p < 0.001$; Figure I.7, Table I.S5).

We found no overall difference in expression of immune genes among queens and workers ($p > 0.175$; Figure I.8, details in Table I.S6). However, genes associated with the RNAi pathway were significantly upregulated in queens and mature workers compared to callow workers (mature workers versus callow workers: 0.587 , $z = 5.438$, $p < 0.001$; queens versus callow workers: 0.701 , $z = 6.502$, $p < 0.001$; Figure I.8, Table I.S6).

Figure I.6. Log-centred TMM-normalised TPMs for genes associated with a) octopaminergic, b) dopaminergic, c) serotonergic, d) tyraminergetic and, f) glutamatergic neural pathways in queens, callow and mature workers. Results of the post-hoc pairwise comparisons after GLMs are given in Table I.S4, and the associated p-values are reported on the plot. Details of the genes included in the datasets are found in Table I.S3.

Figure I.7. Log-centred TMM-normalised TPMs for genes associated with a) olfactory receptors and b) gustatory receptors in queens, callow and mature workers. Results of the post-hoc pairwise comparisons after GLMs are given in Table I.S5, and the associated p-values are reported on the plot. Details of the genes included in the datasets are found in Table I.S3.

Figure I.8. Log-centred TMM-normalised TPMs for a) pooled immune genes, and genes specifically associated with the b) TOLL pathway, c) JAK/STAT pathway and d) RNAi pathway in queens, callow and mature workers. Results of the post-hoc pairwise comparisons after GLMs are given in Table I.S6, and the associated p-values are reported on the plot. Details of the genes included in the datasets are found in Table I.S3.

Comparison of behavioural worker castes

Our SIPCA analysis revealed that three independent principal components enabled distinction between insiders, foragers and scouts based on all transcripts expressed at more than 1 TPM (Figure I.9). Overall, we found 79 differentially expressed transcripts among foragers and insiders, 237 differentially expressed transcripts between scouts and insiders but no significant difference between foragers and scouts for a $FDR < 0.05$ (Figure I.10). After setting the FC threshold to 2, 114 transcripts remained significantly differentially expressed between scouts and insiders, including 35 transcripts also differentially expressed in foragers versus insiders (for $FC > 2$ and $FDR < 0.05$; Figure I.10). These most differentially expressed transcripts separated into two clusters, characterised by upregulated transcripts in insiders versus foragers and scouts, respectively (Figure I.11, Table I.S7). The expression profiles of foragers and scouts were very similar (Figure I.12). *BLAST* searches revealed that 104/114 most differentially expressed transcripts matched unambiguously to the Argentine ant official gene set, meaning that only 10 transcripts were assigned to neither Argentine ant nor honeybee genes (Table I.S7).

We found five transcripts associated with epigenetic regulation (The Uniprot Consortium, 2017) to be differentially expressed. Three transcript isoforms coding for the isoform B of *msta* that were upregulated in insiders compared to foragers and scouts, and another transcript coding for a similar protein was upregulated in insiders compared to scouts only. Conversely, a transcript coding for a methyltransferase was found to be upregulated in scouts compared to insiders. We found six cytochrome P450-associated loci upregulated in scouts compared to insiders, including four also upregulated in foragers. On the other hand, two cytochrome P450 loci were upregulated in insiders compared to scouts (but not foragers), including the cytochrome P450-6k1 enzyme for which other transcript isoforms were found to be upregulated in scouts. A hexamerin-like transcript was found to be upregulated in insiders, as well as a gonadotropin-releasing hormone. We found that a Gp-9 transcript, associated with social structure in the Red Imported Fire Ant (Lucas et al., 2015), was upregulated in foraging workers and scouts. An uncharacterised Argentine ant locus upregulated in foragers and scouts versus insiders closely matched *Drosophila takeout* gene, associated with circadian clock and feeding behaviour (The Uniprot Consortium, 2017). Conversely, another circadian-clock associated transcript was upregulated in insiders.

Figure I.9. Scatter plot of individual samples showing clustering of insiders, foragers and scouts after a Sparse Independent Principal Component Analysis. Samples are plotted as a function of the first three components (IPC1, IPC2, IPC3) chosen based on their kurtosis value. For representation purposes, ellipses are drawn assuming a multivariate t-distribution.

Figure I.10. Volcano plots showing pairwise differentially expressed transcripts among insiders, foragers and scouts below false discovery rate (FDR) of 0.05 and fold change (FC) of 2. After post-hoc corrections for pairwise comparisons, no transcript was significantly differentially expressed between foragers and scouts. We restricted the differential expression analysis to overall 114 most differentially expressed transcripts by increasing the FC threshold to 2 (in red only).

Figure I.11. Details of the transcripts differentially expressed between insiders, foragers and scouts, showing log₂-transformed TMMs with $p < 0.001$ and fold change $FC > 2$. Annotations are given based on initial *BLASTp* searches on the Argentine ant official gene set, completed with *BLASTp* on the Swissprot database and *BLASTx* on the *nr* NCBI database. All transcripts were differentially expressed between insiders and scouts, and transcript names followed by octothorps indicate a significant difference between insiders and foragers as well. Colours in the top band represent castes (dark, mid-range and light green for scouts, foragers and insiders, respectively), and shades of grey in the lateral band represent gene clusters. Trees are based on expression similarity (clusters of samples and transcripts).

Figure I.12. Detail of expression pattern of transcripts assigned to the a) forager/scout-specific and b) insider-specific clusters. Individuals are indicated on the X-axis. Log-centred TMM-normalised TPMs are shown on the Y-axis. The blue line indicates the mean expression level for the cluster and the grey lines indicates individual values. Note the similarity between foragers and scouts and the contrast between these and the insiders.

Candidate gene approach: gene expression associated with foraging propensity

We selected genes related to key neural pathways, chemoreception and immunity to further investigate gene expression profiles associated with foraging propensity. We found that genes associated with OA, DA, 5-HT, GABA and MSG were overall upregulated in scouts compared to insiders (OA: 0.281, $z = 2.583$, $p < 0.05$; DA: 0.506, $z = 3.205$, $p < 0.01$; 5-HT: 0.402, $z = 5.549$, $p < 0.001$; GABA: 0.301, $z = 5.495$, $p < 0.001$; MSG: 0.221, $z = 3.90$, $p < 0.001$; Figure I.13, Table I.S9). Furthermore, genes associated with 5-HT were also upregulated in scouts compared to foragers (0.443, $z = 6.109$, $p < 0.001$; Figure I.13, Table I.S9), and genes associated with OA exhibited a marginally significant upregulation in scouts compared to foragers as well (0.236, $z = 2.166$, $p = 0.077$). In addition, genes associated with GABA were significantly downregulated in insiders compared to foragers (0.271, 4.957, $p < 0.001$; Figure I.13, Table I.S9).

We found that gene expression of olfactory receptors was associated with foraging propensity. Genes related to ORs were upregulated in scouts compared to foragers (0.090, $z = 3.053$, $p < 0.01$) and insiders (0.537, $z = 18.228$, $p < 0.001$). Furthermore, ORs were also upregulated in foragers compared to insiders (0.447, $z = 15.175$, $p < 0.001$; Figure I.14, Table I.S10). We found no difference in overall expression of genes associated with gustatory receptors ($p > 0.358$; Figure I.14, Table I.S10).

We found no overall difference in immune genes associated with foraging propensity ($p > 0.368$; Figure I.15, details in Table I.S11). Furthermore, the absence of association between foraging propensity and immune gene expression held true for specific immune pathways ($p > 0.174$; Figure I.15, details in Table I.S11).

Figure I.13. Log-centred TMM-normalised TPMs for genes associated with a) octopaminergic, b) dopaminergic, c) serotonergic, d) tyraminergetic, e) GABAergic, and f) glutamatergic neural pathways in insiders, foragers and scouts. Results of the post-hoc pairwise comparisons after GLMs are given in Table I.S9, and the associated p-values are reported on the plot. Details of the genes included in the datasets are found in Table I.S8.

Figure I.14. Log-centred TMM-normalised TPMs for genes associated with a) olfactory receptors and b) gustatory receptors in insiders, foragers and scouts. Results of the post-hoc pairwise comparisons after GLMs are given in Table I.S10, and the associated p-values are reported on the plot. Details of the genes included in the datasets are found in Table I.S8.

Figure I15. Log-centred TMM-normalised TPMs for a) pooled immune genes, and genes specifically associated with the b) TOLL pathway, c) JAK/STAT pathway and d) RNAi pathway in insiders, foragers and scouts. Results of the post-hoc pairwise comparisons after GLMs are given in Table S11, and the associated p-values are reported on the plot. Details of the genes included in the datasets are found in Table I.S8.

Discussion

Here, we first discuss our findings relative to the most differentially expressed genes between queens and workers, as well as associated with worker age and foraging propensity. Then, we revisit the transcriptomic signatures of castes or behaviour by exploring patterns of gene expression using count data for sets of annotated genes associated with neural pathways, chemoreception and immune pathways, selected based on their likely association with social behaviour. While this candidate gene approach has to be taken with caution, it can provide useful exploratory insights to investigate variation based on prior biological knowledge.

We found significant differences in transcript expression profiles of queens compared to workers, as well as transcriptomic signatures of worker age and foraging propensity. The principal component analyses that we ran based on all expressed transcripts clearly distinguished between callow workers, mature workers and queens in one analysis and worker behavioural caste based on foraging propensity in the second analysis. The top hundred genes differentially expressed between queens and workers as well as within worker groups (age or foraging propensity) also show clearly distinct expression profiles, as did candidate gene groups associated with neural pathways and chemoreception.

Genes associated with morphological castes

Differential transcript expression analysis between queens and workers revealed a set of genes mostly upregulated in queens, with fewer genes significantly upregulated in workers at the defined thresholds. Queen-specific differentially expressed features included vitellogenin transcripts. Vitellogenin has long been known to be associated with fecundity in ants (Engels, 1974), and was shown to be differentially regulated in honeybee queens and paper wasps (Corona et al., 2007; Manfredini et al., 2018). Furthermore, vitellogenin expression in the head of a sub-social beetle was associated with parental care (Roy-Zokan et al., 2015). Recent advances in bioinformatics allowed for the discovery of vitellogenin duplication events in ants and subsequent neo- or sub-functionalisation, with different copies showing different caste-specific expression profiles (Corona et al., 2013; Morandin et al., 2014; Wurm et al., 2011). Interestingly, these studies altogether point to species-specific expression profiles of the various copies of vitellogenin across castes, suggesting that different genes may have adapted to different functions depending on the species through sub-functionalisation or neo-functionalisation (Morandin et al., 2014).

We also found a farnesol dehydrogenase transcript was upregulated in queens. Farnesol dehydrogenase is an enzyme involved in the synthesis of juvenile hormone in the insect brain (Mayoral et al., 2009). Juvenile hormone is a key hormone that has a number of different regulatory roles in insects. In ant queens, it has been suggested that juvenile hormone influences reproductive functions and immunity (Pamminger et al., 2016). Our results further support juvenile hormone having a key role in ant queen physiology and caste differentiation.

A transcript putatively associated with circadian rhythm was upregulated in queens compared to workers. There are a number of processes within a social insect colony that show cyclicity in both queens and workers, which has been extensively studied in the honeybee (Bloch, 2010). In ants, the study of locomotor activity in queens has shown plastic activity patterns, suggesting the existence of cyclicity in their daily activity as well (Sharma et al., 2004). The transcript in question may therefore be associated with queen biological cycles.

A transcript coding for an UDP-glucuronosyltransferase, an enzyme associated with the inactivation and excretion of endogenous and xenobiotic compounds (Bock, 2016), was comparatively upregulated in queens. Interestingly, the enzyme is also associated with *Drosophila* male aggressive behaviour (Edwards et al., 2009). While its function remains elusive in ants, many neural pathways are conserved among insects (Reaume and Sokolowski, 2011) and UDP-glucuronosyltransferase may also have a role in modulation of behaviour in the Argentine ant.

Five out of seven genes upregulated in workers were cytochrome P450 transcripts. However, other cytochrome P450 transcripts were also upregulated in queens. Numerous cytochrome P450 enzymes have been identified in animals and are known to be associated with detoxification of a broad range of compounds. Because their expression is in part induced by exposure to toxic compounds (Giraud et al., 2010), and such exposure may vary greatly among morphological or behavioural groups within a colony and even among individuals within groups, it is not surprising that many transcripts were differentially expressed between groups, perhaps reflecting differences among individuals within samples. The Argentine ant genome encodes for a large number of cytochrome P450s compared to other hymenopterans (Smith et al., 2011). We found cytochrome P450 transcripts in every

differential expression analysis, which may indicate that they are indeed major components of the Argentine ant metabolism as an adaptation to a generalist diet and life in human-disturbed habitats (Smith et al., 2011).

Genes associated with worker age

Interestingly, another vitellogenin-like transcript was upregulated in callow compared to mature workers, which may suggest a role in late ant worker development as well. In honeybees for instance, vitellogenin was shown to be associated with the behavioural transition with age from nursing to foraging (Guidugli et al., 2005).

Mature workers exhibited upregulation of a transcript coding for a Janus kinase, which is associated with the JAK-STAT signalling pathway. The JAK-STAT pathway is known to be associated with growth factors and immune response in honeybees, especially antiviral defence mechanisms (Brutscher et al., 2015). Given that mature workers are more likely to perform extranidal tasks and therefore more likely exposed to pathogens, their immune system may be more proficient than that of newly emerged workers. This would be consistent with what we know of honeybees, whose foragers exhibit higher immune and detoxification gene expression than nurses (Vannette et al., 2015). Alternatively, differential regulation of immune genes could be a consequence of increased exposure to pathogens, as has also been suggested in honey bees (Johnson et al., 2009).

Genes associated with foraging propensity

Transcripts associated with epigenetic regulation were differentially expressed among worker behavioural groups. Several transcripts coding for *Drosophila* msta proteins were upregulated in insiders, which may indicate differential epigenetic regulation (The Uniprot Consortium, 2017). A methyltransferase transcript also potentially associated with histone methylation (The Uniprot Consortium, 2017) was found to be upregulated in scouts compared to insiders. In ants, epigenetic mechanisms are known to underpin morphological caste differentiation both between queens and workers as well as determining worker polymorphism and behaviour (Alvarado et al., 2015; Bonasio et al., 2010; Simola et al., 2013). While techniques such as chromatin immunoprecipitation are necessary to infer which genomic regions are regulated by epigenetic mechanisms, our results hint that polyethism in Argentine ants may be under such regulation.

A transcript coding for a hexamerin-like protein was upregulated in insiders. In the honeybee, hexamerin expression in the fat bodies appear to be under the control of juvenile hormone and was found to be upregulated in workers compared to drones and queens, suggesting a role in foraging activity (Martins et al., 2010). There is further support for hexamerin as a storage protein in the carpenter ant *Camponotus festitanus*, where it was associated with colony foundation in queens (Martinez and Wheeler, 1994). In *Nasonia vitripennis*, hexamerin was mostly expressed in the thorax and abdomen but it was also expressed in the head (Cristino et al., 2010). While the quality of the Argentine ant annotations allows only speculation on the exact function of the transcript discussed here, hexamerin may similarly serve as a storage protein in the Argentine ant. So-called “lazy ants” can function as nutrient reserve for the colony (Charbonneau et al., 2017), although it is not known if the Argentine ant exhibits such social behaviour. Furthermore, we found a transcript coding for a gonadotropin-releasing hormone was upregulated in insiders compared to scouts. Gonadotropin is part of a family of hormones that is associated with juvenile hormone (Robinson and Vargo, 1997). The upregulation of a gonadotropin-releasing hormone transcript in insiders may further suggest a role for juvenile hormone-associated control of worker behaviour and function.

A transcript upregulated in foragers and scouts compared to insiders had a significant match with the *Drosophila takeout* gene, that has been associated with male courtship behaviour (Dauwalder et al., 2002) and feeding behaviour associated with circadian rhythm (Sarov-Blat et al., 2000). Furthermore, another transcript under circadian-clock control was found to be downregulated in insiders. These differentially expressed features may reflect cyclicity in foraging activity. Interestingly, it was shown that honeybee and bumblebee nurses typically lack circadian rhythm (Bloch, 2010; Yerushalmi et al., 2006). Here, differential expression of transcripts associated with circadian rhythm may as well indicate differential rhythmicity associated with foraging propensity.

We found that a Gp-9-like transcript was significantly upregulated in foragers and scout compared to insiders. Gp-9 is an odorant binding protein that is part of a larger non-recombining genomic region associated with social structure in the Red Imported Fire Ant *Solenopsis invicta* (Wang et al., 2013). However, contrary to our findings, Gp-9 was upregulated in inside-nest fire ant workers (Lucas et al., 2015). Unlike fire ants, established Argentine ant colonies are always polygyne and the role of Gp-9 in the social organisation of

our study species remains unclear. However, as an odorant-binding protein it may reflect increased olfactory sensitivity, as discussed below.

Neural pathways

Foraging behaviours, and more broadly division of labour and social behaviour, are known to be associated with key neural pathways in social insects (Barron and Robinson, 2005; Kamhi and Traniello, 2013; Schulz and Robinson, 2001). More specifically, genes related to the octopaminergic, dopaminergic, tyraminerbic, serotoninerbic, GABAergic and glutamaterbic systems have been shown to be associated with scouting behaviours in honeybees (Liang et al., 2014, 2012). In ants, OA, 5-HT and DA in particular are known to be associated with worker caste division of labour, social behaviour and aggression (Kamhi et al., 2015; Smith et al., 2013; Vander Meer et al., 2008). Both callow and mature workers exhibited overall upregulation of genes associated with DA, TYR, and GABA compared to queens, which may suggest that they are important neural pathways in worker behaviour. OA, DA, 5-HT, GABA and MSG were upregulated in scouts compared to insiders, as well as upregulated in foragers compared to scouts in the case of DA, GABA and MSG. While these results provide useful insights on which neural pathways may be associated with social behaviour in Argentine ants, future research should focus on individual genes to improve resolution of our analysis.

Chemoreception

Olfactory receptors were overall upregulated in workers compared to queens. Furthermore, foraging workers and scouts also exhibit upregulated olfactory receptors transcripts compared to insiders. Interestingly, olfactory receptors were also upregulated in scouts compared to foragers, but to a lesser extent than compared to insiders. Far fewer transcripts associated with gustatory receptors were found, and they were only significantly upregulated in workers compared to queens. Most of the widely-accepted models for division of labour in social insects are based on inter-individual variability in response thresholds (Beshers and Fewell, 2001; Jeanson and Weidenmüller, 2014), in which individual response threshold to task-associated stimuli determine its propensity to engage in a specific task. While most, if not all, tasks performed by ants require a sense of smell, foraging is likely to be particularly demanding in terms of odour and taste sensitivity. Evidence for the importance of smell and taste in foraging through inter-caste and inter-individual comparisons have been reported in honeybees (Page et al., 2006; Riveros and Gronenberg,

2010) and ants (Perez et al., 2013). Here, our results seem to indicate that the foraging caste (i.e. workers) and foraging propensity within the worker caste are both associated with increased olfactory reception.

Immune pathways

We found no significant difference in transcript abundance associated with foraging propensity when looking at the overall expression of known immune genes, as well as when focusing on specific pathways. However, genes associated with the RNAi immune pathway were downregulated in callow workers. In honeybees, there is theoretical and empirical support for immune strength being overall higher in older bees (Bull et al., 2012; Durrer and Schmid-Hempel, 1994; Wilson-Rich et al., 2008). However, it remains unclear why the RNAi immune pathway may be specifically inhibited in callow ants, and more research is needed to better understand individual and social immunity in the Argentine ant.

Overall, we found distinct transcriptomic signatures of head tissues associated with morphological castes (queens and workers), worker age (callow and mature) and foraging propensity (within-nest and scouting/foraging workers). Differential gene expression analysis revealed a number of transcripts in the Argentine ant that are homologous to genes that have already been identified in other hymenopterans as associated in division of labour. Furthermore, our exploratory analysis revealed that specific sets of genes including olfactory receptors and transcripts associated with specific neural pathways were upregulated in workers compared to queens, and in foraging workers to an even larger extent. Here, we provide material for future research to further investigate the complexity of the social organisation of ants.

Chapter II: Octopamine modulates foraging behaviour and aggression in Argentine ants

Abstract

Risk-taking behaviours such as boldness, increased activity or aggression have been suggested to promote invasiveness across a wide range of exotic species. The Argentine ant is a successful global invader, whose behaviour has been suggested to be a key factor contributing to its spread. The orchestration hypothesis postulates that neuromodulators such as biogenic amines can underlie specific behaviours. Octopamine (OA) is a biogenic amine associated with social behaviour in social hymenopterans, including foraging and aggression. Here, we studied the effect of OA on behaviour at multiple levels in the Argentine ant.

We used oral administration of OA and epinastine (EPN), an OA antagonist, to manipulate the ant octopaminergic pathways and measured behavioural variation in colony-level foraging activity, as well as individual boldness and interspecific aggression. Our results suggest that the octopaminergic system is associated with colony-level foraging activity (division of labour), as well as individual aggressiveness.

Our study contributes to the understanding of the molecular basis of key behaviours in order to establish a comprehensive framework of how variation at the physiological level can influence invasion success through behavioural variation.

Key words

Aggression, Argentine ant, Division of labour, Epinastine, Foraging behaviour, Octopamine

Introduction

Behaviour is increasingly recognised as a key driver of biological invasions (Holway and Suarez, 1999; Silverman and Buczkowski, 2016; Suarez and Cassey, 2016). Risk-taking behaviours such as boldness, increased activity or aggression have been suggested to promote fitness across a wide range of species (Biro and Stamps, 2008; Smith and Blumstein, 2008). In invasive species, such behaviours have been repeatedly directly associated with invasion success (Ashenden et al., 2017; Chapple et al., 2011; Cote et al., 2010; Hudina et al., 2014; Mutascio et al., 2017; Pintor et al., 2009, 2008). However, little is known about the underlying mechanisms that drive variation in these behaviours across the species range.

The orchestration hypothesis postulates that the neuromodulators can activate specific central neuronal circuits underlying specific behaviours (Hoyle, 1985; Libersat and Pflueger, 2004). Biogenic amines are known to be key behavioural neuromodulators in ants and other invertebrates, and also function as key neurotransmitters and neurohormones (Kamhi and Traniello, 2013; Libersat and Pflueger, 2004). Octopamine (OA), a major biogenic amine in invertebrates, is known to be associated with a wide range of processes in insect behaviour and physiology, including high-energy behaviours such as flying (Farooqui, 2007; Kamhi and Traniello, 2013). In honeybees, OA has been shown to be associated with foraging and the division of labour through an extensive array of methods, from correlative studies between brain OA titres and behaviour, as well as experimental neurochemical manipulations (Barron et al., 2002; Barron and Robinson, 2005; Liang et al., 2012; Reim and Scheiner, 2014; Schulz et al., 2002; Schulz and Robinson, 2001; Wagener-Hulme et al., 1999). In ants, OA brain titres have been shown to be higher in the foraging caste of leaf-cutting ants *Acromyrmex echinator* (Smith et al., 2013), and have been correlated with age-related division of labour in big-headed ants *Pheidole dentata* (Seid and Traniello, 2005). Experimental neurochemical manipulations also suggest that OA is associated with neuromodulation of aggression in weaver ants *Oecophylla smaragdina* (Kamhi et al., 2015). Altogether, these reports indicate that octopaminergic neural pathways are likely to have conserved functions in social hymenoptera as a key determinant of foraging and aggressive behaviour.

The Argentine ant (*Linepithema humile*) is a widespread invasive species in Mediterranean-type and subtropical temperate climates (Wetterer et al., 2009). Behaviour is recognised as a key factor to contribute to the success of invasive ants (Holway and Suarez,

1999; Silverman and Buczkowski, 2016). During the early stages of the introduction process, increased activity, boldness (i.e. defined as the tendency of an individual to take risks and be exploratory in novel contexts; Wilson et al., 1994) and interspecific aggression have been suggested to facilitate the successful transportation of propagules, as well as their establishment and spread (Chapple et al., 2012; Chapple and Wong, 2016). The understanding of the molecular basis of key behaviours in invasive species is therefore crucial to elucidate how behaviour and the introduction process may interact.

Here, we used pharmacological diet manipulations of Argentine ant experimental colonies to investigate the effect of OA on behaviour at multiple levels. Epinastine (EPN) is a highly specific OA antagonist, although it can also bind to other receptors, such as dopaminergic receptors (Beggs et al., 2011; Roeder et al., 1998). In previous neurochemical studies in insects, EPN was shown to successfully inhibit OA receptors (Beggs et al., 2011), and manipulate insect behaviour (Kamhi et al., 2015; Pophof, 2000). We test the hypothesis that octopaminergic pathways are associated with foraging, boldness and aggression in Argentine ants. We treated the ants with three different doses of OA, as well as EPN. We measured the effects of our experimental manipulations on foraging activity at the colony-level, as well as boldness and aggressiveness in the individual level. We also performed HPLC analysis of biogenic amine titres associated with worker foraging propensity. We hypothesised that foraging workers exhibit higher OA titres than in-nest workers, and that OA-treated colonies display increased foraging activity at the colony level, as well as increased individual boldness and aggression. We postulated that if OA modulates foraging behaviour by increasing motor activity, mean walking speed may also be affected by our experimental treatments. Conversely, we hypothesise that EPN-treated ants will exhibit opposite patterns to that of OA-treated ants.

Methods

Ant collection and experimental colonies

Argentine ant queens, workers and brood were collected in Paraparaumu, New Zealand (-40.882410°S, 174.994083°E) in late summer (March 2017). Ants and brood were isolated from soil material following the flooding technique by Scholes & Suarez (2009) and kept as a single stock-colony. In the lab, ants were maintained at 23°C on a 14/10 light/dark cycle, and unless under experimental treatment, received a diet of Bhaktar-Whitcomb jelly *ad libitum* (Bhatkar and Whitcomb, 1970). We set up 40 experimental colonies overall.

We sampled ants in two different ways for neurochemical manipulation experiments (experiments 1-3 below) and biogenic amine quantification (experiment 4 below), respectively. For the neurochemical manipulations (experiments 1-3), we set up 40 experimental colonies of two queens, 400 workers, and a small amount of brood. As for the biogenic amine quantification experiment (experiment 4), newly emerged workers were sampled based on cuticle coloration and pooled together with two queens and early stage brood every week for three weeks. Cuticle coloration is a good indication of worker age, as newly emerged callow ants lack cuticle pigmentation and are distinguishable from older workers for a few days (personal observation). This way, we were able to form three single-cohort colonies of approximately 300 workers of known age. In both cases, colonies were housed in 20 × 15 cm boxes lined with Fluon[®] and containing a single test-tube as nesting site.

Four colonies of endemic New Zealand bush ants (*Prolasius advenus*) were collected in Kaitoke Regional Park (-41.055562, 175.191590), and maintained in similar conditions to that of Argentine ant colonies. These colonies were used for interspecific aggression assays (i.e. experiment 3, below).

Experimental manipulations with OA/EPN treatments

Experimental colonies were assigned to one of five groups: control, OA 2 mg.mL⁻¹, OA 4 mg.mL⁻¹, OA 8 mg.mL⁻¹ or EPN (i.e. 8 experimental colonies per treatment). Both OA and EPN were acquired in hydrochloride solid form (Sigma-Aldrich, USA) and delivered in 10% sugar water. Colonies received 200 µL of feeding solution every day throughout the

experiment, directly applied on a plastic film renewed daily in the foraging area. In order to evaluate the potential toxicity of OA and EPN administration, we monitored the effect of experimental treatments on survival at the end of the experiment by re-counting the workers that remained alive.

Experiment 1: Foraging activity at the colony-level

We measured foraging activity at the colony-level with a similar protocol as in chapter II and III. We used a structure novel to the ants – an artificial wooden tree made of a 12 × 12 cm base mounted with a 60 cm trunk (10 mm diameter wooden dowel) branched with 11 horizontal sticks every 5 cm (12 cm wooden skewer, 2 mm diameter, pictured in Figure S1 in chapter III). Experimental colonies were given access to the tree by placing it in their foraging area. We placed 0.1 mL of 10 % sugar solution on a $\approx 10 \text{ mm}^3$ piece of cotton wool at the top of the tree trunk to measure foraging activity in the context of the exploitation of a novel food source. We monitored foraging activity by counting the ants present on the entire tree every 5 minutes for 20 minutes. The colony-level experiment was divided in two phases. First, the number of ants foraging on the tree was monitored as described above during 5 days before the experimental treatments onset to measure baseline foraging activity. Then, 18 days after treatment onset, we measured colony-level foraging activity for 5 days in order to measure treatment effect. At the end of experiment 1, workers were sampled from the colonies for experiments 2-3.

We assessed the results of the colony-level foraging assays in two ways. First, we analysed the absolute foraging activity by counting the number of ants foraging on the tree. Second, we used the total number of live ants per colony at the end of the experiment as a proxy for colony size in the last 5 experimental days to calculate foraging activity relative to colony size, as the different experimental treatments were associated with different effects on survival, leading to different colony sizes at the end of the experiment (Figure II.S1). In both cases, the effect of treatments on colony-level foraging activity compared to control colonies were analysed using a GLM (R Core Team, 2015) with treatment and experimental phase interacting as independent variables.

Experiment 2: Boldness at the individual level

Individual workers were sampled from the foraging area of their colony and placed in the centre of a 85 mm diameter Petri dish arena, within a cylinder coated with Fluon[®] to prevent ants from escaping. A folded black paper shelter was placed within the cylinder prior to the ant so it could habituate to it. After 1 to 2 minutes of acclimation to the set-up, the cylinder was removed and the ant was left to explore the arena. The assays were recorded using a Raspberry PiCamera video camera (Raspberry Pi Foundation, Cambridge, UK), and snapshots extracted from the movies every second (Figure II.S3a). At each snapshot, the position of the ant was manually recorded using the *CellCounter* plug-in in *ImageJ* (Schneider et al., 2012). *ImageJ* outputs in pixels were converted to millimetres using the diameter of the Petri dish arena (85 mm) as a reference. The coordinates of the shelter were also manually recorded on one of the snapshots (Figure II.S3b). Using these coordinates, we computed the time to emerge from the shelter after the start of the assay, the time spent outside of the shelter, the average distance from the shelter and the total distance walked. Distances from the shelter less than one body length were filtered out. Similarly, speeds less than one body length per second were not included in the analysis, as well as data points when the ant stayed motionless. Variation in time to emerge from the shelter was analysed with a regression survival analysis with the *survival R* package, using treatment as independent variable (Christensen, 2015). We performed post-hoc pairwise comparisons after survival analysis using the *glht* function in the *multcomp R* package (Hothorn et al., 2016). Variation in time spent outside of the shelter, mean distance from the shelter and mean speed was analysed using a series of GLMs assuming normal distribution using treatment as independent variable. Overall, we performed 16 boldness assays per experimental colony, i.e. 128 assays per treatment.

Experiment 3: Interspecific aggression at the individual level

We performed dyadic interspecific aggression assays between workers from different treatments to measure the effect of OA and EPN on Argentine ant aggressiveness. Immediately after the boldness assay (experiment 2), a set of eight encounters per experimental colony were performed in small Petri dishes (60 mm diameter) and video-recorded from above as in experiment 2. In each Petri dish, we placed one foraging Argentine ant worker, isolated within plastic cylinders as in the assay for experiment 2. A *Prolasius advenus* worker was carefully placed in the arena as far as possible from the Argentine ant (Figure II.S3). The interactions between Argentine ant and *Prolasius* workers were then

recorded for three minutes, after which we also recorded both ants' survival and extent of damage. We scored behaviours from the Argentine ant's perspective using a scale ranging from 0 to 4 as following:

- 0 Avoidance. Quick change of direction after a contact with the opponent
- 1 Neutral. No discernible response after a contact with the opponent
- 2 Reactive attack. Bite or use of chemicals after an attack from the opponent
- 3 Proactive attack. Proactive bite or use of chemicals on the opponent.
- 4 Prolonged fight. Continuous aggression for more than 5 seconds

Aggression data was analysed using a cumulative link model with flexible thresholds with the *clm* function in the *ordinal R* package (Christensen, 2015), with treatment as independent variable. Damage and individual survival was also analysed using a cumulative link model with four ordered categories: “intact” (i.e. no visible damage), “slight injuries” (i.e. one or less limb or antenna missing, no visible effect on locomotion), “heavy injuries” (i.e. more than one limb or antenna missing, impaired locomotion) and “death”. Overall, we performed 16 aggression assays per experimental colony, i.e. 128 assays per treatment.

Experiment 4: Foraging propensity and OA and serotonin (5-HT) head titres

We aimed at measuring variation in OA and 5-HT worker head titres in relation to foraging propensity. Single-cohort colonies were maintained for 12 weeks under standard conditions until worker ants were sampled depending on their foraging propensity: “scouts”, “insiders” or “foragers”. We starved the colonies for 3 days before sampling, and then placed a 30 cm artificial wooden tree in their 20 × 15 cm foraging area. Ants that were exploring the tree were carefully sampled as “scouts”. The sampling of scouts stopped when ants reached the tree at a rate of less than one every five minutes for half an hour. Subsequently, the tree was removed and a drop of honey was placed in the foraging area. Ten minutes after the honey was discovered, all the ants that recruited to the food source were classified and sampled as “foragers”. For an hour, we then progressively removed all the ants that were found outside of the foraging area until no ants were exiting the nest. The ants that remained inside the nest together with the queens and brood constituted roughly half of the experimental colonies and were sampled as “insiders”, i.e. workers that tend to stay within

the nest and perform tasks such as nursing (Holldobler and Wilson, 1990). OA and 5-HT head titres were measured as outlined below.

HPLC analysis (Experiments 1-4)

We ran HPLC analysis in order to measure 1) the effect of experimental treatments relative to experiments 1-3 and 2) biogenic amine titres in relation to foraging propensity (experiment 4). Worker ants were collected from experimental colonies used in experiments 1 and 4, respectively. Samples were prepared under a stereomicroscope on a glass plate chilled with dry ice. Five heads were detached from the bodies with two fine forceps (Drummond #5, Switzerland) and the antennae removed and discarded. Extracts were prepared on the same day as the HPLC runs. For each sample, the five ant heads were homogenised in a 2 mL Eppendorf tube in 60 μL of ice-cold 0.2 M perchloric acid containing 10 $\text{pg}\cdot\mu\text{L}^{-1}$ of 2,3-Dihydroxybenzoic acid (DHBA) as an internal standard. The tubes were centrifuged for 15 minutes at 15,000 rpm at 0°C, after which 50 μL of the supernatant was transferred into a vial glass insert of an amber-tinted vial chilled on ice. We used a C18 column (HypersilTM BDS, 100 x 4.6 mm, 5 μm particle size) with an ESA Coulochem[®] III detector (guard cell 5020 and analytical cell 5011). The mobile phase was composed of 15% v/v methanol, 15% v/v acetonitrile, 75 mM monobasic sodium phosphate, 5 mM sodium citrate and 1.5 mM sodium dodecyl sulfate. The final pH was adjusted to 5.6 with phosphoric acid. We set up the flow rate to 1.2 $\text{mL}\cdot\text{min}^{-1}$, the column temperature at 28°C, guard cell voltage at 920 mV and analytical cell at -200 mV and 850 mV for channel 1 and 2, respectively. A series of external standards with known concentrations of OA were run before and after each set of samples, and calibration was performed in R using raw peak areas from the Agilent software automatic peak integration. The sample injection volume was 40 μL . Differences among experimental treatments were tested with two GLMs on compound amount per worker head, using treatment as independent variable and assuming normal distribution. OA titres were up to two orders of magnitude higher in OA-treated ants, so we used log-transformed amounts. In the case of foraging propensity, we performed post-hoc pairwise comparisons using the *multcomp* R package (Hothorn et al., 2016). Overall, we ran 24 samples per experimental treatment in the samples from experiments 1-3, and 40 samples per behavioural group from experiment 4.

Results

Effect of experimental treatments on OA, 5-HT levels and ant survival

Epinastine (EPN) is a highly specific OA antagonist. Accordingly, EPN-treated ants exhibited significantly lower OA head titres than untreated ants (-0.568 , $t = -2.282$, $p < 0.05$; Figure II.S2a). Conversely, OA-treated ants had OA head titres two orders of magnitude higher than untreated ants ($p < 0.001$ in all cases; Table II.S2a). Although there was a trend in mean OA head titres to increase with OA dose, data were extremely variable in OA-treated groups. Serotonergic pathways are not known to be a target of EPN, and as expected we found no effect of any experimental treatment on 5-HT levels (Figure II.S2b; Table II.S2b).

Over the course of the colony-level experiment, about a quarter of the ants in the control colonies died. Furthermore, we found that the two highest OA doses (i.e. 4 and 8 mg.mL⁻¹) had a significant effect on ant survival compared to control, with approximately two thirds and half of the colony dying when treated with 4 and 8 mg.mL⁻¹ of OA, respectively (EPN: -9.875 , $t = -0.588$, $p = 0.560$; OA2: -22.625 , $t = -1.347$, $p = 0.187$; OA4: -80.375 , $t = -4.786$, $p < 0.001$; OA8: -122.750 , $t = -7.309$, $p < 0.001$; Figure II.S1, Table II.S1).

Experiment 1: Foraging activity at the colony-level

The aim of this assay was to measure the effect of experimental treatments on colony-level foraging activity. Because experimental OA treatments affected ant survival, we analysed both absolute foraging activity, as well as relative foraging activity (i.e. proportion of the colony foraging). Surprisingly, colonies assigned to the EPN treatment were significantly less active in foraging before treatment onset (-7.425 , $t = -2.794$, $p < 0.01$; Figure II.1a). However, the difference between baseline activity and activity after treatment was significantly larger in EPN-treated colonies compared to control colonies (-9.600 , $t = -2.554$, $p < 0.05$; Figure II.1b). There was no significant effect of any OA treatment on the absolute number of ants foraging (Figure II.1b; Table II.S3a).

When considering foraging activity in terms of proportion of the colony foraging, there was no significant difference among groups prior to treatment onset (Figure II.1c; Table II.S3b). After treatment, EPN significantly decreased relative foraging activity by more than 50% (-0.033 , $t = -2.479$, $p < 0.05$; Figure II.1d), and the two highest OA doses were

associated with increased relative foraging activity in a dose-dependent fashion (OA2: 0.015, $t = 1.125$, $p = 0.261$; OA4: 0.036, $t = 2.670$, $p < 0.01$; OA8: 0.058, $t = 4.332$, $p < 0.001$; Figure II.1d, Table II.S3b).

Figure II.1. Colony-level foraging activity is influenced by octopamine (OA) and an antagonist (EPN). Data are shown as a) absolute baseline foraging activity (i.e. before treatment onset) and b) absolute foraging activity after treatment. As mortality among experimental groups was significantly different, we also present the data as c) relative baseline foraging activity and d) relative foraging activity after treatment. The mean value in each group is represented by red dots. Only the p-values from the GLM outputs are shown, all groups compared to control, with full outputs in Table II.S3.

Experiment 2: Boldness at the individual level

The aim of this assay was to measure the effect of experimental treatments on individual boldness and walking dynamics (i.e. speed). We found significant differences among experimental groups on the time to emerge from the shelter ($X^2 = 19.79$, $df = 4$, $p < 0.001$; Figure II.2a, Table II.S2). Post-hoc pairwise comparisons revealed that ants treated with 8 mg.mL⁻¹ of OA took significantly more time to emerge from the shelter than control groups and groups treated with the lowest dose of OA (Table II.S4b). There was no significant effect of EPN, or the two lowest OA doses on the time spent under the shelter (Figure II.2b, Table II.S5). However, the highest OA doses increased the time that ants spent under the shelter (-0.079 , $t = -2.088$, $p < 0.05$). OA treatments did not influence the mean distance of the ant from the shelter (Figure II.2c, Table II.S6) but EPN-treated ants stayed significantly closer to the shelter (-2.531 , $t = -2.538$, $p < 0.05$). EPN-treated ants as well as OA-treated ants under the 4 and 8 mg.mL⁻¹ doses walked on average 80% slower than control ants (EPN: -5.220 , $t = -5.189$, $p < 0.001$; OA2: 0.460 , $t = 0.460$, $p = 0.646$; OA4: -2.108 , $t = -2.091$, $p < 0.05$; OA8: -2.109 , $t = -2.083$, $p < 0.05$; Figure II.2d, Table II.S7).

Experiment 3: Interspecific aggression at the individual level

We found that EPN-treated ants were significantly less aggressive than control ants in one-on-one interspecific interactions (-0.414 , $z = -3.644$, $p < 0.001$; Figure II.3a, Table II.S8). Conversely, there was a marginally significant effect of the lowest OA dose administration on aggressiveness but no difference in the higher doses (OA2: 0.192 , $z = 1.742$, $p = 0.081$; OA4: -0.075 , $z = -0.692$, $p = 0.489$; OA8: 0.026 , $z = 0.234$, $p = 0.815$). Argentine ants suffer less damage when treated with EPN and high doses of OA (EPN: -0.889 , $z = -3.456$, $p < 0.001$; OA4: -0.477 , $z = -1.822$, $p = 0.068$; OA8: -0.551 , $z = -2.087$, $p < 0.05$; Figure II.3b, Table II.S9a) but there was no significant effect of OA 2 mg.mL⁻¹ (-0.252 , $z = -0.934$, $p = 0.350$). Bush ants survival was significantly higher when confronted with Argentine ants treated with EPN, OA 4 mg.mL⁻¹ and 8 mg.mL⁻¹ (EPN: -0.561 , $z = -2.402$, $p < 0.05$; OA2: 0.026 , $z = 0.110$, $p = 0.912$; OA4: -0.580 , $z = -2.577$, $p < 0.01$; OA8: -0.636 , $z = -2.702$, $p < 0.01$; Figure II.3c, Table II.S9b).

Figure II.2. Octopamine (OA) and an antagonist (EPN) do not have major effects on individual foraging dynamics. Data are expressed as a) time to emerge from the shelter (in seconds), b) proportion of time spent outside the shelter, c) mean distance from the shelter (in millimetres) and d) mean walking speed (in millimetres per second). In boxplots, the mean value in each group is represented by red dots. Only the p-values from the GLM outputs are shown, all groups compared to control, with full outputs in Tables S4 to S7.

Figure II.3. Epinastine (EPN), an octopamine (OA) antagonist decreased aggressiveness in the Argentine ant and influences encounter outcome. Data are shown as a) proportion of categories of interactions recorded during the assay from the Argentine ant's perspective, b) Argentine ant and c) bush ant damage. Only the p-values from the CLM outputs are shown, all groups compared to control, with full outputs in Tables S8 and S9.

Experiment 4: Foraging propensity and OA and serotonin (5-HT) head titres

We found significantly higher OA head titres in foragers compared to insiders, but surprisingly also in foragers compared to scouts (insiders versus foragers: -0.404 , $z = -7.013$, $p < 0.001$; scouts versus foragers: -0.392 , $z = -6.245$, $p < 0.001$; scouts versus insiders: 0.011 , $z = 0.201$, $p = 0.978$; Figure II.4a, Table II.S10a). Conversely, we found no difference in 5-HT levels across worker behavioural groups (insiders versus foragers: 0.345 , $z = 1.823$, $p = 0.162$; scouts versus foragers: 0.282 , $z = 1.353$, $p = 0.365$; scouts versus insiders: -0.063 , $z = -0.337$, $p = 0.939$; Figure II.4b, Table II.S10b).

Figure II.4. Foraging workers are characterised by higher octopamine (OA) head titres compared to insiders and scouts. Conversely, serotonin (5-HT) titres are no significantly different among behavioural groups. The mean value in each group is represented by red dots. Only the p-values from the GLM outputs are shown, all groups compared to control, with full outputs in Table II.S10.

Discussion

Here, we reported experimental manipulation of Argentine ant behaviour at group and individual levels using OA and EPN administration. Although EPN is one of the OA-antagonists with the highest affinity to OA receptors (Roeder et al., 1998) it has also been shown to bind with other receptors in the honey bee brain (Beggs et al., 2011). However, our HPLC analysis of OA head titres showed a reduction in OA in EPN-treated ants, which appears to indicate that EPN did indeed target octopaminergic systems in our experiments. We showed that OA and EPN administration have opposite effects on colony-level foraging activity, as well as individual aggressiveness. Our results indicate that the octopaminergic system is likely to be associated with these key behaviours in the Argentine ant.

We measured the effect of neurochemical manipulations with OA and EPN on foraging activity of experimental colonies. Our experiments showed that OA increased foraging activity at the colony level (when corrected for colony size), while EPN decreased this foraging activity. The contrasting effects of OA and EPN that we observed are also in accordance with previous studies on honey bees and other social insects, which showed that OA brain titres were associated with foraging and scouting behaviour (Liang et al., 2012; Schulz et al., 2003; Schulz and Robinson, 2001; Wagener-Hulme et al., 1999). Furthermore, when OA was orally administered to worker bees, it was shown to increase foraging activity at the colony-level in a dose-dependent manner (Schulz and Robinson, 2001). In ants, studies show a positive association of OA with foraging (Smith et al., 2013) or age-related polyethism (Seid and Traniello, 2005). However, other ant species have shown an opposite trend, with nurses having higher OA brain titres than foragers (Wnuk et al., 2011). Here, we show that foragers, but not scouts, had higher OA head titres than in-nest workers, in accordance with the above studies. The difference between foragers and scouts remains puzzling as both behavioural groups engage in activities outside the nest, but may reflect a dose-dependent effect of OA on modulation of division of labour. Substantial variation has been shown in the molecular basis of behaviour in scouts versus non-scouts for foraging honeybees (Liang et al., 2012) as well as among types of scouts, i.e. food scouts versus nest scouts (Liang et al., 2014). Interestingly, these studies indicate differentially expressed genes associated with neuromodulation and neurotransmission, including octopaminergic pathways (Liang et al., 2014). In the previous chapter, we only found significant differences in genes associated with OA between scouts and insiders, but not between foragers and insiders or

scouts. It is worth noting here that mRNA levels are known to be far from perfectly correlated with protein levels (Maier et al., 2009). Nevertheless, our results on the effect of OA and EPN administration on colony-level foraging behaviour together with changes in OA titres associated with foraging propensity suggests that OA is likely to be associated with division of labour within Argentine ant colonies.

We observed a significant effect of octopamine (OA) treatments on Argentine ant mortality. These results indicate that ants may have been poisoned with OA, as suggested by non-physiological OA head titres in OA-treated colonies. While our results are to be taken with caution as a consequence, the experiments still provide valuable insights on the behavioural effects of OA in Argentine ants, especially when focusing on the lowest dose of OA, which did not show significant effect on ant survival.

We also measured the effect of neurochemical manipulations with OA and EPN on individual boldness and walking dynamics. We postulated that, in an open environment such as the arenas used, shy ants would prefer a distinctive feature (i.e. the shelters; Chapman et al., 2011). Exploratory ants would therefore spend less time under the shelter, emerge sooner from it and be more mobile – using speed and distance from the shelter as proxies. In the individual boldness assays, EPN-treated ants stayed significantly closer to the shelter, although they did not spend more time under it and took a similar amount of time to emerge from it at the start of the assay compared to control ants. EPN-treated ants also walked significantly slower than the control ants. Altogether, these results may suggest an inhibitory effect of EPN on boldness by disrupting the octopaminergic neural pathways. However, ants from colonies treated with low OA doses did not exhibit any difference in boldness compared with control ants. High OA doses significantly increased the time to emerge from the shelter, decreased the time spent outside the shelter, and decreased their walking speed. The results exhibit an opposite trend as what we expected, and may reflect poisoning of the ants at the dose of 8 mg.mL^{-1} of OA. Nevertheless, the comparison of ants treated with the lowest dose of OA with control groups does not indicate an association of OA with individual boldness. Furthermore, observations in EPN-treated ants indicate only a weak negative association with boldness. Beyond its association with division of labour in social insects, OA is known to be involved in a wide range of behavioural and physiological processes in other insects, including high-energy behaviours such as locomotion and flight (Farooqui, 2012). Lower

walking speed, and perhaps distance from the shelter may indicate that OA serves similar functions in modulating such behaviours in Argentine ants.

We then measured the effect of neurochemical manipulations with OA and EPN on individual interspecific aggression. We did not find a significant effect of OA on aggression. However, EPN significantly decreased the frequency of aggressive interactions, which supports the presumed association of OA neural pathways and aggressive behaviour. Instances of OA-modulated aggressive behaviour have been reported in various species of ants (Cuvillier-Hot and Lenoir, 2006; Kamhi et al., 2015; Vander Meer et al., 2008). In contrast, a few studies did not find an effect of OA on aggression in ants (Szczuka et al., 2013). Interestingly, we observed fewer aggressive interactions in the EPN-treated ants, which was associated with less fighting-related damage to both species. These findings suggest that the benefits of aggressive behaviour in terms of invasion success may be overstated, as increased aggression can be associated with increase damage. However, the Argentine ant's competitive abilities stem from high numbers and recruitment performance (Carpintero et al., 2007; Holway, 1999; Holway and Suarez, 2003; Human and Gordon, 1996), and high aggressiveness is likely to have a better outcome for Argentine ants in numerically unbalanced confrontations as they occur in the field. Interestingly, Argentine ant propagules have been shown to adjust their aggressive behaviour depending on propagule size, suggesting that behavioural plasticity may be particularly important in Argentine ant invasion success (Sagata and Lester, 2009).

The division of labour in social insects is thought to emerge from inter-individual differences in response thresholds (Beshers and Fewell, 2001; Jeanson and Weidenmüller, 2014). Interestingly, OA has been associated with neuromodulation of sensation (Farooqui, 2007). OA has been specifically shown to be positively associated with sensitivity to sucrose in stingless bees (Cabe et al., 2016), nest-mate recognition in ants and bees (Robinson et al., 1999; Vander Meer et al., 2008), and chemoreception of pheromones in several species of moths (Linn and Roelofs, 1986; Pophof, 2000). If the decision-making process to start foraging involves a threshold response to external stimuli, whether environmental, or associated with pheromonal communication, an association between OA and division of labour is therefore expected. The individual behavioural assays included in this study lacked both environmental cues relative to foraging (e.g. sucrose) and collective context, which may explain the discrepancy observed between the effects of OA administration in the colony-

level assays. Further investigations on how OA may affect chemoreception in Argentine ants are required to explore this hypothesis.

Here, we used experimental administration of OA and EPN to manipulate the octopaminergic neural pathways in the Argentine ant. Our results suggest that OA is likely to be associated with modulation of colony-level division of labour and individual aggressiveness. Foraging and aggressive behaviours are known to be drivers of invasion success in introduced species. The understanding of their molecular basis is therefore critical to establish a comprehensive framework of how variation at the physiological level can influence invasion success through behavioural variation.

Recommendations for further research

If I were to do this experiment again, there are several aspects that I would do differently. First, I would use lower doses of compounds in the experimental treatments. In a preliminary experiment as well as the experiment described in chapter III (that was performed prior to the present study), we used an OA dose of 4 mg.mL^{-1} . In both instances, in contrast with this experiment there was no observed effect on mortality. In the present experiment, the experimental colonies were three times smaller than in the chapter III experiment. Although the amount of feeding solution delivered was four-fold lower, smaller colony size may be associated with faster rates of food exchange throughout the colony and increase individual exposure to the drug. Second, I would also not get appendicitis during the course of the experiment, which considerably increased the treatment time from 5 to 18 days. Perhaps the combined effect of long treatment time and small colony size increased the exposure of individual workers to OA, proving it toxic for the ants.

Chapter III: Behavioural variation and plasticity along an invasive ant introduction pathway

Abstract

Once established in new areas, introduced species may exhibit changes in their biology due to phenotypic plasticity, novel selection pressures and genetic drift. Moreover, the introduction process itself has been hypothesised to act as a selective filter for traits that promote invasiveness. We tested the hypothesis that risk-taking behaviours thought to promote invasiveness are selected for during invasion by comparing traits among native and introduced populations of the widespread Argentine ant (*Linepithema humile*).

We studied Argentine ant populations in the native range in Argentina, and in three invaded regions along an introduction pathway: California, Australia and New Zealand. In each study region, we set up 32 experimental colonies to measure foraging activity and interspecific aggression in a subset of the study regions. These colonies were subject to experimental manipulation of carbohydrate availability and a biogenic amine supplementation (octopamine) to measure behavioural plasticity.

We found variation in foraging activity among populations, but this variation was not consistent with selection on risk-taking behaviour in relation to the invasion process. We found that colonies with limited access to carbohydrates exhibited unchanged exploratory behaviour, but higher exploitation activity and lower aggression. Colonies given octopamine increased foraging behaviour (both exploration and exploitation) and aggression. We did not find difference in behavioural plasticity in relation to country along the introduction pathway.

We did not find support for selection of traits associated with invasiveness along the Argentine ant's introduction pathway, indicating that mechanisms promote behavioural variation in a similar fashion both in the native and invaded ranges. Our results also challenge the assumption that invasive species perform better in their introduced range.

Key words

Behavioural plasticity, Behavioural variation, Biological invasions, Introduction Pathway, Invasive species

Introduction

Species transported by humans often undergo population bottlenecks because the number of initial founders is typically low, resulting in the introduced populations harbouring a subset of the genetic diversity of native populations and rendering them more susceptible to genetic drift (Dlugosch and Parker, 2008; Sakai et al., 2001; Tsutsui et al., 2000). Despite the potential problems associated with reduced genetic variation and a lack of local adaptation, introduced populations often outperform native populations in key traits associated with growth and reproduction (Parker et al., 2013), creating a paradox for their success (Allendorf and Lundquist, 2003; Sax and Brown, 2000). While many mechanisms have been proposed to explain the success of invasive species (Kolar and Lodge, 2001; Sakai et al., 2001), a possible solution to this paradox is that introduced species must pass through a succession of critical stages (i.e. association with humans, transportation, establishment and spread) with each stage having specific barriers that may filter out maladaptive phenotypes (Blackburn et al., 2011; Chapple et al., 2012; Sakai et al., 2001; Suarez and Tsutsui, 2008). Under this scenario, the introduction process would lead to newly established populations being non-random subsets of the native populations by promoting traits associated with invasion success. In this study, we investigated behavioural variation and plasticity in native and introduced Argentine ant populations to test the hypothesis that the introduction process acts as a filter, leading to the inadvertent selection of traits that promote invasiveness.

Behaviour is a key predictor of invasion success in animals and behavioural traits that are likely to influence the competitive abilities of an invasive species are diverse (reviewed in Chapple et al., 2012; Holway and Suarez, 1999; Phillips and Suarez, 2012). For example, activity, aggressiveness and boldness (i.e. risk-taking behaviours) have been suggested to be important factors in promoting fitness in a wide range of species (Biro and Stamps, 2008; Smith and Blumstein, 2008), and specifically invasiveness in the delicate skink compared to the closely-related but non-invasive garden skink (Chapple et al., 2011). Different behavioural traits may influence invasion success at different stages of the introduction process (i.e. transportation, establishment, spread), and a single trait can have either positive and/or negative effect on survival depending on the stage considered (Chapple et al., 2012; Chapple and Wong, 2016; Clobert et al., 2009). In new environments, increased risk-taking behaviour could translate into increased foraging success through faster resource discovery and exploitation, or increased aggressiveness towards competitors. On the other hand,

increased behavioural plasticity could be favoured in invaders, especially in early stages of an invasion by accelerating evolution of optimal phenotypes in the new range (Chevin and Lande, 2010). We suggest that behaviours associated with risk-taking, such as foraging and aggression, are therefore good candidates to test our hypothesis by measuring variation and plasticity in these traits along an introduction pathway.

The Argentine ant (*Linepithema humile*) is a global invader that can have damaging effects on agriculture, ecosystems and human activities (Holway et al., 2002). Outside its native range in the Paraná River basin in South America, the ant was first recorded on the Atlantic island of Madeira in the 1850s (Wetterer et al., 2009) and its likely invasion pathway has been partially reconstructed from historical records as well as behavioural and genetic data (Tsutsui et al., 2001). Half a century later, the ant was detected in warm temperate and Mediterranean regions, including North America and Southern Europe (Vogel et al., 2010), and from the latter likely invaded Australia in the 1930s (Suhr et al., 2011). From Australia, it then colonised New Zealand in the 1990s (Corin et al., 2007). Population genetics and behavioural data altogether show evidence for successive introductions from a single origin in Argentina (Van Wilgenburg et al., 2010; Vogel et al., 2010). The well-documented invasion pathway of the Argentine ant from its native range to New Zealand makes it an ideal species to test the hypothesis of trait selection during the introduction process.

In this study, we examined behavioural variation of Argentine ants in four regions along an invasion pathway: its native range in Argentina and three invaded locations (California, Australia and New Zealand). In each region, we measured foraging behaviour (exploration activity and recruitment to a food source) of experimental colonies given a novel foraging arena. We also quantified aggressive behaviour towards local ants in a subset of the study regions. Artificially manipulating modulators of behaviour may provide insights on the extent of behavioural plasticity in different Argentine ant populations. Colonies were subject to an experimental manipulation of carbohydrate availability (high and low sugar) and octopamine (OA), both of which can influence foraging behaviour and aggression in ants. OA is a major biogenic amine known to influence insect behaviour and physiology, including bees and ants (Kamhi and Traniello, 2013). If OA is associated with risk-taking behaviours, we predicted that OA-treated ants will be more exploratory and aggressive. Similarly, carbohydrate availability has been shown to influence ant activity and aggression (e.g. Grangier and Lester, 2014; Grover et al., 2007) and we predict colonies with high sugar

availability will also be more exploratory and aggressive. If selection for risk-taking behaviours has occurred as Argentine ants were introduced from one region to another, we predict increased foraging activity and aggression in introduced populations relative to native populations. Physiological processes such as neuronal signalling are always limited in their response. Therefore, if an increase in foraging activity and aggression in introduced populations stems from increased signalling in the ant's octopaminergic system, we predict a reduced response to OA experimental manipulations along the introduction pathway.

Methods

Sampling and experimental colonies maintenance

We studied Argentine ant populations in their native range in Argentina and in three introduced regions – California, Australia, and New Zealand (Figure III.1a). In each region, ants were collected within a week of initiating the experiment at four different sites separated by a distance ranging from 5 to 300 km, in summer (Figure III.1b, c, d, e; Table III.S1). A minimum of 20,000 adult workers were collected at each site, as well as queens and brood. In the laboratory, colonies were then isolated from soil material by connecting the collection box to a clean one using a metal wire, and slowly flooding the former to encourage migration (Scholes and Suarez, 2009). We set up eight experimental colonies per site, each comprising 1,200 workers, four queens and a small amount of brood for a total of 32 colonies per study region. To equalize proportions of foragers versus within-nest workers, for each collection site, we constituted experimental colonies with ants collected from intermediary boxes that contained well-mixed groups of workers and offered no shelter for behavioural subgroups (e.g. nurses or guards). Two experimental colonies per site were assigned to each treatment (referred as “site replication” in Fig. 2). Colonies were housed in nest tubes placed in 30x30x12 cm plastic boxes (Figure III.S1).

Feeding solutions used for experimentally treating the ants were designed to investigate the effect of OA and sugar availability on the Argentine ant behaviour in a 2x2 factorial design (Table III.1). Ants were fed 16 hours before the experiments of the next day. In order to measure the exposure of the ants to OA in our experimental treatments, we sampled workers in New Zealand at the end of the experiment in order to perform a HPLC quantification of OA on pooled heads (Method S1, Figure III.S2).

Table III.1. Composition of the experimental treatments, designed to study the behavioural effects of octopamine (OA) and sugar availability. Experimental colonies were randomly assigned to a treatment and had daily access to 1 mL of corresponding feeding solution plus a mealworm (*Tenebrio molitor*) (≈ 150 mg) cut into three pieces.

	Acclimation (days 1 to 5)	Treatment (days 6 to 20)
High sugar (control)	20 % sugar	20 % sugar
Low sugar	20 % sugar	1 % sugar
High sugar + octopamine	20 % sugar	20 % sugar with 4 mg.mL ⁻¹ OA
Low sugar + octopamine	20 % sugar	1 % sugar with 4 mg.mL ⁻¹ OA

a. Locations of study regions

b. Argentina

c. California

d. Australia

e. New Zealand

Figure III.1. (a) Regional populations included in the study. Earliest record of the Argentine ant in the three invaded regions are given in brackets (Wetterer et al., 2009). Regional locations of the collection sites in (b) Argentina, (c) California, (d) Australia and (e) New Zealand. Site names and descriptions are given in Table III.S1.

Behavioural assays

Foraging assays. Foraging assays measured both the exploration of a novel structure as well as the exploitation of a food source. The novel structure was an artificial wooden tree made of a 12x12 cm base mounted with a 60 cm trunk (10 mm diameter wooden dowel) branched with 11 horizontal sticks every 5 cm (12 cm wooden skewer, 2 mm diameter). Experimental colonies were given access to the tree daily during the course of the 20-day experiment by simply placing it in their foraging area (Figure III.S1). We monitored foraging activity by counting the ants present on the tree every 10 minutes for an hour, as well as recording branches that foragers were visiting.

The 20-day experiment was divided in three phases. First, foraging activity was monitored as described above during a 5-day acclimation phase before the OA and sugar treatments onset to allow experimental colonies to get used to the laboratory environment. Then, after the treatment onset on day 5, we continued monitoring foraging activity without changing the protocol for 10 more days (i.e. exploration phase). During these first 15 days of the experiment, the trees offered no resource and the number of workers counted were considered as a proxy for exploration activity. For the remaining 5 days of the experiment, we placed 0.1 mL of 20 % sugar solution on a $\approx 10 \text{ mm}^3$ piece of cotton wool at the top of the tree trunk to measure foraging activity in the context of the exploitation of a novel food source (i.e. exploitation phase). At this stage, we recorded the number of workers feeding at the food source separately from the workers counted on the tree but not feeding. Foraging activity (i.e. exploration or exploitation activity) was expressed as the total number of ants counted on the tree for the first 20 minutes of the assay in order to capture foraging behaviour in the context of a novel environment. We also recorded the time when an ant was first observed on the top branch, which was only analysed for the exploration phase as the presence of the sugar reward during the exploitation phase can be considered as a confounding factor. In the majority of trials during the exploitation phase, ants discovered the new food source before the first count 10 minutes after the start of the assay, so we could not use resource discovery time as a relevant measure. Instead, we used the number of ants feeding after 10 minutes as a proxy for foraging performance associated with resource discovery. After the treatment onset on day 5, we excluded the next two days to allow experimental treatments to come into effect.

All analyses were performed in R (R Core Team, 2015). Unless specified otherwise, a significance threshold of 0.05 was used throughout the study. We analysed foraging activity using generalised mixed-effects model (Bates et al., 2017) on the number of workers counted on the tree during the first 20 minutes as well as on foraging performance associated with resource discovery. We ran models restricted to experimental phases using region and treatment as fixed effects (interacting if significant), and colony nested within sites as well as observation day as random effects. To further compare exploration activity, we also performed a series of Cox proportional hazard regression models (Therneau and Lumley, 2017) on the time for each experimental colony to reach the top branch of the tree during the exploration phase, using treatment and region interacting as fixed effects. A consequence of the population bottlenecks associated with the introduction process could be a decrease in behavioural variation along the introduction pathway. To measure this, variance in our main variables – i.e. exploration and exploitation activity – was compared in control groups among regions with a single Fligner-Killeen test followed by a series of six post-hoc pairwise comparisons with Bonferroni corrections.

Aggression assays. We performed dyadic interspecific aggression assays between workers to measure the regional differences in Argentine ant aggressiveness as well as the effect of OA and sugar deprivation on aggression. In Argentina and Australia, we selected two species of local *Pheidole*, a widespread genus that co-occurs with the Argentine ant both in its native and invaded ranges. It was not possible to use the same *Pheidole* species in the two regions so we chose species with minor workers that are similar in size to *L. humile* workers (*Pheidole triconstricta* in Argentina and *Pheidole vigilans* in Australia). At the end of both the exploration and the exploitation phases (i.e. on days 15 and 20), a set of 8 encounters per experimental colony (i.e. 64 assays per treatment) were performed in small Petri dishes (60 mm diameter) and filmed from above with a digital camera (Lumix DMC-TZ8, Panasonic). In each Petri dish, we placed one foraging Argentine ant and one *Pheidole* minor worker, both isolated within plastic cylinders. The inner sides of both the plastic cylinders and the Petri dish were coated with Fluon[®] to prevent escapes. The cylinders were kept in place for at least one minute to allow the ants to habituate to the environment and once removed, the interactions between Argentine ant and *Pheidole* workers were recorded for three minutes. We scored behaviours using a scale ranging from 0 to 4 as following:

- 0 Avoidance. Quick change of direction after a contact with the opponent
- 1 Neutral. No discernable response after a contact with the opponent
- 2 Reactive attack. Bite or use of chemicals after an attack from the opponent
- 3 Proactive attack. Proactive bite or use of chemicals on the opponent.
- 4 Prolonged fight. Continuous aggression for more than 5 seconds

Variation in the Argentine ant aggressiveness among treatments and regions was tested with a cumulative link model with flexible thresholds (Christensen, 2015) using treatment and region as independent variables. The interaction between treatment and region was included in a first version model but eventually removed, as there was no significant interaction between treatment and region.

Results

Behavioural variation along the introduction pathway

In control colonies (high sugar solution, no OA added), we found no consistent differences in behaviour at the regional level in relation to the introduction pathway. There was no significant difference in foraging activity in populations in California (exploration: -0.110 , $z = 0.399$, $p = 0.670$; exploitation: 0.048 , $z = 0.184$, $p = 0.854$) and New Zealand (exploration: 0.094 , $z = 0.344$, $p = 0.731$; exploitation: 0.133 , $z = 0.514$, $p = 0.607$) compared to Argentina but experimental colonies in Australia overall exhibited lower exploration (-0.507 , $z = -2.157$, $p = 0.031$; Figure III.2a, Table III.S2) and exploitation activity (-0.987 , $z = -3.784$, $p = 0.001$; Figure III.2b, Table III.S3) and also took more time to reach the top branch of the artificial tree when exploring compared to ants from Argentina (-0.410 , $z = -2.135$, $p = 0.033$; Table III.S4). However, we did not find significantly different foraging performance associated with resource discovery in Australia (-0.318 , $z = -1.501$, $p = 0.133$), but it was significantly higher in New Zealand compared to Argentina (0.477 , $z = 2.265$, $p = 0.024$; Figure III.S3, Table III.S5). We found high variability among collection sites within regions and even among experimental colonies from a single collection site (Figure III.2c), but we found no clear directional change in variance along the pathway. Variance in exploration activity was only significantly lower in Australia compared to California after pairwise comparisons with Bonferroni corrections ($\chi^2 = 12.750$, $df = 1$, $p < 0.001$), similar to exploitation activity ($\chi^2 = 7.5175$, $df = 1$, $p = 0.006$; Figure III.2, Table III.S6). We found higher aggression in Australia compared to Argentina (0.246 , $z = 5.204$, $p < 0.001$; Figure III.5, Table III.S7), but these results have to be taken with caution as we used different species of *Pheidole* in the two regions.

Figure III.2. Colony-level foraging activity was very variable but there was no directional variation along the introduction pathway. (a) Colony-level foraging activity when exploring a new environment that offered no resource. (b) Colony-level foraging activity in the context of the exploitation of a newly discovered food source in a new environment. (c) Detailed plotting of (b) with data broken down per site to illustrate variability among site within region and within site. Data are shown for control groups only ($n = 8$ colonies per region, with 8 observations for each colony during the exploration phase, and 5 during the exploitation phase) to illustrate innate variation in foraging activity along the introduction pathway. We observed no directional variation along the introduction pathway, with only significantly lower exploitation activity in Australia. If presented, the same letters indicate no significant differences within phases, notches represent 95% confidence interval on the median and outliers are represented with black dots (i.e. beyond 1.5 interquartile lower and upper ranges), or circled in black in plot (c). Site name abbreviations are described in Table III.S1.

Determinants of foraging activity and aggression

HPLC analysis on worker heads revealed that ants treated with the low sugar combined with OA were potentially exposed to higher amounts of OA compared to high sugar/OA-treated colonies (Figure III.S3). We found that sugar-deprivation did not affect exploration activity ($0.286, z = 1.554, p = 0.120$), except in California where sugar-deprivation decreased exploration activity ($-0.589, z = -2.253, p = 0.024$; Figure III.3a, Table III.S2). However, more ants were foraging during the exploitation phase in these colonies in all regions ($0.695, z = 3.851, p < 0.001$; Figure III.3b, Table III.S3). Sugar-deprivation reduced the time for the first ant to be counted on the top branch in Argentina, Australia and New Zealand ($0.406, z = 2.218, p = 0.027$). In California, the pattern was different and sugar-deprived groups reached the top branch later than control groups ($-0.986, z = -3.677, p < 0.001$; Figure III.4b, Table III.S4). Sugar deprivation increased resource discovery in all regions ($0.853, z = 7.111, p < 0.001$; Figure III.S3, Table III.S5). Likewise, both OA treatments increased exploration activity in a similar fashion in all regions (high sugar + OA: $0.800, z = 4.377, p < 0.001$; low sugar + OA: $1.282, z = 7.039, p < 0.001$; Figure III.3a, Table III.S2). Activity outside the nest was always higher in OA-treated colonies during the exploitation phase, and the differences among treatments followed an overall similar trend to that of the exploration phase (high sugar + OA: $0.800, z = 4.377, p < 0.001$; low sugar + OA: $1.282, z = 7.039, p < 0.001$) except in Australia where there was a significant positive interaction between region and both OA treatments (high sugar + OA in Australia: $0.614, z = 2.413, p = 0.016$; low sugar + OA in Australia: $0.736, z = 2.897, p = 0.004$; Figure III.3b, Table III.S3). The time for the first ant to be counted on the top branch was also shorter in OA-treated colonies compared to control groups (high sugar + OA: $0.449, z = 2.462, p = 0.014$; low sugar + OA: $0.877, z = 4.798, p < 0.001$; Figure III.4, Table III.S4). Both OA treatments increased foraging performance associated with resource discovery in all regions tested (high sugar + OA: $1.262, z = 10.603, p < 0.001$; low sugar + OA: $1.825, z = 15.434, p < 0.001$; Figure III.S3, Table III.S5). Sugar-deprived ants displayed less aggressive behaviour than controls ($-0.171, z = -2.532, p = 0.011$). There was only a marginally significant effect of OA supplementation alone on aggression ($0.126, z = 1.879, p = 0.060$), but sugar-deprived OA-treated ants were more aggressive than controls ($0.176, z = 2.584, p = 0.010$; Figure III.5, Table III.S7). There was no consistent significant interaction between region and experimental treatment on risk-taking behaviour (i.e. both foraging and aggression), indicating that OA administration modulated behaviour in a similar fashion in native and introduced regions.

Figure III.3. Colony-level foraging activity, including exploration activity and exploitation of a food source, was generally increased by our octopamine (OA) treatments. Sugar shortage only increased foraging activity in the context of the exploitation of a food source. Data are shown as foraging activity (n = 8 colonies per region, with 8 observations for each colony during the exploration phase, and 5 during the exploitation phase) for all treatments during (a) the exploration phase and (b) the exploitation experimental phases in the native range in Argentina, and three invaded regions in California, Australia and New Zealand. Symbolic results of the GLMMs are shown (i.e. * < 0.001 < ** < 0.01 < * < 0.05 < NS). Different letters (i.e. a, b) indicate significant interaction between treatment and region, and the details are given in italics below the corresponding letter. Notches represent 95% confidence interval on the median. Outliers (i.e. beyond 1.5 interquartile lower and upper ranges) are indicated with black dots. Note the difference in the y-axis between the two phases. Note that data for control groups are also shown in Figure III.2.**

Figure III.4. The time for the first ant to reach the top branch of the artificial tree during the exploration phase decreased in all the octopamine-treated groups (OA), but sugar deprivation differed by region. Data are shown for (a) the native range in Argentina, and three invaded regions in (b) California, (c) Australia and (d) New Zealand. Sample size was $n = 8$ colonies treatment in each region, with 8 observations for each colony. Shaded areas indicate 95% confidence intervals for the hazard ratio. Symbolic results of the Cox-proportional hazard models are shown in each plot, and refer to the comparison with controls within region (i.e. $*** < 0.001 < ** < 0.01 < * < 0.05 < \text{NS}$).

Figure III.5. Individual Argentine ant aggressiveness was lower in Argentina (a) compared to Australia (b). Octopamine (OA) supplementation increased aggression when combined with low sugar and sugar-deprivation reduced aggression in a similar fashion in the two regions. Non-aggressive and aggressive interactions are depicted in shades of blue and red, respectively. We performed 128 assays for each treatment in both Argentina and Australia.

Discussion

We hypothesised that if the introduction process acted as a selective filter for traits that promote invasiveness, Argentine ants would increasingly express risk-taking behaviours along an introduction pathway. Although we found considerable variation in behaviour, we did not find a directional response in support of selection for risk-taking associated with successive introductions, suggesting that other mechanisms are associated with behavioural variation. We also investigated the potential environmental and physiological basis of risk-taking by manipulating carbohydrate availability and OA supplementation, with the prediction that OA-treated ants would be more exploratory and aggressive, and that sugar availability would also affect such behaviours. Our predictions were consistently supported for the effect of OA on foraging activity across both the native and invaded range. The similarity in behavioural plasticity in terms of the ants' response to our experimental manipulations in the native and introduced regions suggest that the mechanisms associated with the modulation of risk-taking are conserved in the different populations despite successive bottlenecks.

Behavioural variation along the introduction pathway

We found no directional variation in colony-level foraging behaviour that would be consistent with selection for risk-taking, a set of behavioural traits likely to promote invasiveness along the Argentine ant introduction pathway. Our results need to be considered in the context of the various selective forces that may act against each other at different stages of the introduction process. The benefits associated with specific behavioural traits are likely to be context-dependent and vary depending on the stage of the introduction process (Clobert et al., 2009). Some traits may be regarded as an advantage during some stages of the introduction process, but deleterious in others and may or may not get carried over the subsequent stages of the introduction process. For instance, while bold and exploratory behaviours might promote human-mediated dispersal by increasing the chances of uptake on cargo, these traits could also be selected against at other steps along the introduction process (Chapple et al., 2012). Indeed, even though exploratory and aggressive phenotypes may be an advantage in terms of competition in established populations, they may also be risky approaches at early stages of invasion when colony size is likely to be smaller. Interestingly, the Argentine ant does show a behavioural response to colony size, with small colonies displaying less aggressive behaviour than bigger ones (Sagata and Lester, 2009). Behavioural

variation within a propagule would therefore be critical for it to successfully invade a new range (Chapple and Wong, 2016). Selection for risk-taking during the introduction process may thus be complex as selective pressures on a given trait may vary along the process.

We found that foraging activity was very variable among sites within region, as well as among experimental colonies within sites to some extent. A recent study on another major unicolonial invasive ant, the polygyne form of the red imported fire ant *Solenopsis invicta*, similarly showed high variability among experimental colonies, even when collected at a single site (Bockoven et al., 2015). The causes of such high variability remain unknown and could arise from a variety of factors including colony genetics, environmental factors and colony composition (reviewed in Jandt et al., 2014). Regardless of the underlying proximate mechanisms, our data suggest that selection could operate to maintain intermediate levels of risk-taking in a similar way throughout the native and invasive ranges. Behavioural plasticity within a population can persist because different strategies are associated with life-history trade-offs (Biro and Stamps, 2008), or because of context-dependent consequences of behavioural types on fitness (Sih et al., 2004; Smith and Blumstein, 2008). It is remarkable that, despite serial introductions likely associated with strong population bottlenecks, the behavioural variation appears unchanged in introduced populations compared to native ones.

The absence of directional variation in our observations on foraging behaviour along the introduction pathway could also be associated with the foraging strategy of the Argentine ant. It has been suggested in multiple studies across the introduced range that the Argentine ant competitive abilities arise from high performance in terms of both exploitation and interference competition. In both field and laboratory studies, the Argentine ant was shown to perform better than native species by discovering and recruiting to food sources more quickly, but also by monopolising these resources (Buczkowski and Bennett, 2007; Holway, 1999; Human and Gordon, 1996; Walters and Mackay, 2005). Numerical advantage appears to be a key component of the foraging success of the Argentine ant, which may thus primarily rely on nest size and connectivity. The nature of the Argentine ant foraging strategy may consequently relax selective pressure on foraging activity as we measured it, i.e. in relatively small and isolated experimental colonies. Interestingly, in its introduced range the Argentine ant exhibits polydomous behaviour (i.e. the formation of networks of interconnected nests) at a much larger scale than in the native range, to the point of unicoloniality (Suarez et al., 1999). The benefits associated with unicoloniality in terms of foraging may therefore

promote the Argentine ant ecological dominance in its introduced range, even without an increased individual foraging activity.

We observed more frequent aggressive interactions in Australia compared to Argentina. Our findings are consistent with other reports of the Argentine ant behaving aggressively towards local species in many introduced regions (Buczkowski and Bennett, 2007; Holway, 1999; Rowles and O'Dowd, 2006). However, we used two different species of *Pheidole* in our aggression assays, making comparison among regions potentially confounding. The only study to date that has directly compared risk-taking in native and introduced populations of Argentine ants showed that ants from the main European supercolony were more aggressive and exploratory than ants from native colonies of Argentine ant (Blight et al., 2016). Interestingly, in the same study Blight et al. (2016) did not find increased risk-taking in ants from the independently introduced Catalanian supercolony. Similarly, different introduced populations in California showed variable levels of aggression, although this was tested in an intraspecific context (Tsutsui et al., 2003). These two studies do not support a consistent association between successful introduction and increased aggression in the Argentine ant. However, our results add to the body of evidence indicating that invasive populations often appear to behave more aggressively than native ones.

Determinants of behaviour and behavioural plasticity

We found that sugar-deprived colonies were generally quicker than control colonies to reach the top branch of the tree during exploration, and performed better than control colonies in terms of resource discovery during the exploitation phase, although we did not find any difference in the number of workers exploring. We found higher foraging activity in sugar-deprived colonies during the exploitation phase, which is likely largely due to the nutritional state of the ants by enhancing recruitment to the food source when discovered. We also found an association between carbohydrate scarcity and aggression, with sugar-deprived ants displaying less aggressive behaviour than control ants. Previous studies report evidence for effects of carbohydrate availability on foraging and aggressiveness in ants (Grangier and Lester, 2014; Grover et al., 2007). Our results provide further evidence for carbohydrate availability as a key determinant of ant risk-taking behaviour in the Argentine ant. Carbohydrate availability has been pinpointed as a key feature of invasion success in ants (Shik and Silverman, 2013; Styrsky and Eubanks, 2007). Interestingly, the behavioural

response to carbohydrate scarcity was the same in the native and invaded range, suggesting that it is a key trait in Argentine ant throughout its native and introduced range.

Our dietary treatments showed that OA increased foraging activity at the colony level, at least when combined with low sugar. Increased foraging activity was consistently observed in all regions across both the native and the invaded range, suggesting a conserved association between OA and foraging in native and introduced populations. These results are also in accordance with previous studies on other social insects, including honeybees and ants, which showed that OA brain titres were often associated with forager behavioural traits (Liang et al., 2012; Schulz and Robinson, 2001; Smith et al., 2013; Wagener-Hulme et al., 1999). OA was shown to increase foraging activity in bees in a dose-dependent manner (Schulz and Robinson, 2001). In our study, the higher exploration activity in colonies treated with OA and deprived of sugar may likewise reflect a dose-dependent effect of OA. Sugar deprivation in the low sugar + OA colonies was likely to increase their solution consumption, thereby increasing OA intake, as suggested by the results of the OA titration we performed. We found only a marginally significant positive effect of OA alone on interspecific aggression. However, while sugar deprivation alone significantly decreased aggressiveness in Argentine ants, OA combined with low amounts of sugar increased the number of aggressive interactions. This difference in the effect of OA on interspecific aggression in the high versus low sugar groups likely reflects a dose-dependent effect of OA on aggression. OA-modulated aggressive behaviour has been reported in various species of ants (Kamhi et al., 2015; Vander Meer et al., 2008) and appears to perform similar functions in the Argentine ant. Our findings therefore point to conserved molecular basis of foraging behaviour and aggression among social hymenopterans.

We found a consistent effect of our experimental manipulations of sugar availability and OA-supplementation on behaviour in both native and introduced populations, suggesting that there was neither loss nor gain of behavioural plasticity along the introduction pathway. Behavioural plasticity has been theorised as a factor in successful adaptation to new environments, especially in early stages of colonisation and before the traits are canalised to a new optimum (Chevin and Lande, 2010; Lande, 2015). Our dataset does not include recent introductions, making any assumption on the evolution of plasticity over time difficult. However, our study does suggest that mechanisms maintained behavioural plasticity in the

introduced range, despite numerous serial introduction events likely associated with population bottlenecks.

Overall, we observed consistent risk-taking behaviour along the Argentine ant introduction pathway, as well as intact behavioural plasticity. It appears that successive introductions did not affect behavioural variation and plasticity in a globally successful invader, suggesting that evolutionary trade-offs maintain risk-taking at similar levels throughout the native and invaded ranges. Our results challenge the assumption that invasive species perform better than native species in key traits associated with invasion success, and does not support the hypothesis of selection of these traits during the introduction process. Further investigations of genomic and transcriptomic variation between native and introduced range of invasive species are needed to understand better how the introduction of species and their evolution interact.

Chapter IV: Invasive Argentine ant populations are characterised by distinct transcriptomic signatures associated with functional adaptations

Abstract

Behavioural traits are increasingly being regarded as a major driver of biological invasions, especially in the context of ant invasions. It is crucial to understand what factors can contribute to the success of invasive species, and next-generation sequencing has increased our capacity to investigate the genomic basis of the functional changes associated with biological invasions. Here, we investigate if native and introduced Argentine ant populations are characterised by distinct transcriptomic signatures associated with range, focusing on the molecular basis of behaviour. We used RNA-sequencing across the native range in Argentina, and the introduced range in California, Europe, Australia and New Zealand, respectively.

Our results show that native and introduced populations have distinct transcriptomic signatures with a large number of genes differentially expressed between the native and invaded ranges. Several genes associated with olfactory reception were differentially expressed across the range, and genes associated with neural pathways were overall downregulated in the introduced range. Native and introduced populations also exhibited differences in the overall expression of genes associated with the TOLL and RNAi immune pathways.

Altogether, our data suggest that functional adaptations are associated with the introduction of the Argentine ant, which may contribute to the success of invasive populations.

Key words

Argentine ant, Behaviour, Biological invasions, Introduction pathway, RNA-sequencing, Functional adaptations

Introduction

Exotic species are constantly transported around the world as inadvertent stowaways in our cargo. When introduced outside their native range, these species can become invasive and pose great threats to biodiversity and human activities. Introduced species experience new evolutionary forces both during the introduction process and once established in their new range, which can lead to rapid evolution and enhanced ecological success (Sakai et al., 2001; Suarez and Tsutsui, 2008). Next-generation sequencing has increased our capacity to investigate the genomic basis of the functional changes associated with biological invasions (Rius et al., 2015). Here, we undertake a first step towards the understanding of functional adaptations underlying the success of a globally invasive pest, using RNA-sequencing across the native and introduced ranges of the Argentine ant.

Behavioural traits are increasingly being regarded as a major driver of biological invasions, and have been specifically suggested in the context of ant invasions (Holway and Suarez, 1999). For instance, generalist feeding habits (e.g. omnivory, around the clock foraging activity, reliance on mutualisms), efficient colony spread (e.g. flexible nesting habits and frequent relocation, proximity with humans), high interspecific aggression and unicoloniality are all behaviours that are likely to enhance invasiveness in ants (Suarez and Cassey, 2016). In chapter III, we investigated if the introduction process was associated with changes in foraging behaviour along the Argentine ant's introduction pathway. We did not find evidence for such an association in that analysis. Another study that focused on the comparison of two European populations with two native colonies did find increased foraging activity and intraspecific aggression in one of the introduced populations (Blight et al., 2016). Loss of intraspecific aggression is thought to be a key factor in the success of the Argentine ant (Holway et al., 1998). Native Argentine ant colonies are highly polygyne and polydomous, as they are in the introduced range, but in Argentina they remain smaller than in the introduced range (Tsutsui et al., 2001). Conversely, introduced Argentine ant populations are characterised by loss of intraspecific aggression despite populations being dispersed over large distances, and retain this trait even when located on different continents (Giraud et al., 2002; Tsutsui et al., 2000; Van Wilgenburg et al., 2010; Vogel et al., 2010). Though the persistence of such large supercolonies inherently contributes to the short-term success of the Argentine ant, the underlying mechanisms and further consequences of the phenomenon remain unclear (Helanterä et al., 2009). Here, we further explore variation along the

Argentine ant's introduction pathway through the prism of the underlying molecular basis of behaviour.

Behaviour – at the individual level but also at the collective level in social insects – is regulated by variation in biogenic amine pathways (Kamhi and Traniello, 2013). In previous chapters, we studied the effect of octopamine (OA) in neuromodulation of foraging behaviour and aggression. We found that OA-treated ants tend to forage more and be more aggressive, a pattern that has also been found in bees and other species of ants (Barron and Robinson, 2005; Kamhi et al., 2015; Liang et al., 2012; Reim and Scheiner, 2014; Schulz and Robinson, 2001; Wagener-Hulme et al., 1999). Furthermore, behaviours associated with foraging and aggression have been shown to be modulated by other biogenic amines and neuromodulators in social insects including serotonin (5-HT), dopamine (DA), GABA and glutamate (MSG) (Dierick and Greenspan, 2007; Hunt et al., 2007; Liang et al., 2014; Smith et al., 2013; Szczuka et al., 2013). These neuromodulators also have major roles in learning and memory processes in the honeybees, critical in foraging-related activities (Gauthier and Grünewald, 2012). Variation in expression of genes related to specific neural pathways could be associated with behavioural differences across the ranges that contribute the Argentine ant's invasion success.

Complex behaviour such as division of labour is thought to stem from inter-individual variations within a colony, including sensitivity to certain stimuli (Jeanson and Weidenmüller, 2014). OA is known to modulate chemoreception in ants, together with other neuromodulators (Farooqui, 2007). In the context of foraging behaviour, OA has been shown to modulate both response to brood pheromone – associated with regulation of foraging in honeybees (Barron et al., 2002) – and odour memory (Farooqui et al., 2003). Furthermore, nestmate recognition in ants – a key mechanism in the social organisation – is associated with perception of olfactory cues, mainly cuticular hydrocarbons (Blomquist and Bagnères, 2010; Torres et al., 2007). Variation in genes associated with olfactory receptors across the Argentine ant's ranges may therefore also contribute to our understanding of loss of intraspecific aggression within intercontinental supercolonies.

The enemy release hypothesis postulates that the absence of natural pathogens and parasites can promote abundance and success of exotic species (Torchin et al., 2003). Invasion pathway bottlenecks have been shown to be associated with changes in microbial

communities of invasive ants, with both loss and gains of pathogens and endosymbionts (Lester et al., 2017; Sébastien et al., 2015; Yang et al., 2010). Interestingly, microbial community diversity has been shown to be associated with behaviour in the Argentine ant, including aggressiveness (Lester et al., 2017). An enemy-release, loss of beneficial symbionts or pathogen accumulation event, could be associated with differential regulation of immune pathways across the ranges. Such insights improve our understanding of how changing microbial communities of invasive species can contribute to their success.

Here, we investigate if native and introduced Argentine ant populations are characterised by distinct transcriptomic signature associated with range, with a focus on the molecular basis of behaviour. We used RNA-sequencing of worker heads and bodies across the native range and invasive range in Argentina, and California, Europe, Australia and New Zealand, respectively. More specifically, we test the hypotheses that native and introduced populations are associated with variation in expression of neural pathways, chemoreception, and immune pathways that may be key factors in explaining the invasion success of the Argentine ant.

Methods

Sampling and RNA library preparation

We used worker ants collected in Argentina, California, Australia and New Zealand from control colonies included in chapter III. Colonies were maintained in standardised conditions for 20 days as described in chapter III for the duration of the behavioural experiment, and foragers were sampled and stored in ice-cold RNALater. Tubes of ants in RNALater were kept at 6°C for 24 hours, and at -20°C in the country of origin until shipped to New Zealand where they were stored at -80°C for up to six months until RNA was extracted. Additional samples were collected from Southern Europe but not included in the behavioural experiment (Table IV.S1).

Heads and antennae were separated from ant bodies under a stereomicroscope in RNALater, and total RNA was extracted from either pooled heads and antennae (referred as head libraries) or the remaining thoraxes/legs/abdomens (referred as body libraries) of 20 workers from the same colony (Table IV.S1). RNA was extracted using an in-column Trizol-based purification kit using the manufacturer's recommended methods (DirectZol MicroPrep, Zymo Research, Irvine, USA). Samples were then washed with ice-cold PBS to remove RNALater that can affect the extraction quality of the RNA extracts. Extracted RNA was stored in RNASTable (Biomatrix Inc., San Diego, USA) and sent to BGI (BGI, Shenzhen, China) for Illumina Hi-Seq sequencing (Illumina Inc., San Diego, USA). Overall, 5 and 21 head libraries were sequenced in the native and introduced ranges, respectively, as well as 3 and 4 body libraries (details in Table IV.S1). Samples were sequenced as 150 paired-end barcoded mRNA TruSeq libraries, aiming at 4 Gb of data per sample. Post-processing at BGI included the removal of reads with more than 10% of missing values (i.e. bp), reads with more than 10% of bases with quality scores $Q < 20$ and removal of adapters.

Genome-guided transcriptome assembly and transcript quantification

Computationally demanding analyses were performed on Victoria University of Wellington Science Faculty's High Performance Computing Facility. Clean paired-end reads (761,733,075 in total) were merged and mapped to the Argentine ant reference genome (Smith et al., 2011) with *BBMerge* from the *BBTools* suite 36.0 (Bushnell, in prep) to produce a single sorted BAM file that was then fed into the *Trinity* 2.2.0 pipeline for a genome-guided assembly (Haas et al., 2013). First, we used *BBMerge* to merge the paired-

end reads. In order to account for non-overlapping paired-end reads while reducing the number of false-positives, we used a large maximum insert size of 1,000 in *BBTools*. *Trinity* assembled 62,623 transcripts in total, representing 46330 genes. Average contig length was 1,263 bases, with an overall alignment rate to the Argentine ant genome of 90.51 %.

Assembled *Trinity* transcripts were quantified using the alignment-based quantification method *eXpress* 1.5.1 (Roberts and Pachter, 2012). Clean reads were aligned to the transcriptome assembly using *Bowtie2* 2.2.9 (Langmead and Salzberg, 2012) and transcript abundance was then estimated using *eXpress*. The *eXpress* outputs were used as input to produce a transcripts per million (TPM) matrix containing all expressed transcripts for all samples, which was then cross-normalised to obtain a weighted trimmed mean of the log expression ratios (i.e. TMM-normalised TPM values; Robinson and Oshlack, 2010). In this chapter, all the analyses were performed at the gene level. The gene TMM-normalised TPM matrix was used in three analysis strategies: Weighted Gene Co-expression Network Analysis (WGCNA; Langfelder and Horvath, 2008), differential expression analysis and candidate genes approach.

Weighted Co-Expression Network Analysis of head libraries

In order to examine transcriptome-wide expression patterns associated with invasion status, we initially analysed the TMM-normalised TPM matrix using WGCNA. WGCNA allows the detection of modules of co-expressed genes that can be correlated to factors such as phenotypic traits. Here, we followed WGCNA guidelines to detect and remove genes with too many missing data and sample outliers (Figure IV.S1, Figure IV.S2). Then, we used a scale-free topology criterion to select a soft thresholding power to build the network (Figure IV.S3). We used range as a phenotypic trait (0 for native populations, and 1 for invasive populations) to investigate the correlation between the eigengene of each module and range.

Differential expression analysis

In order to detect the most differentially expressed genes among regions, we used the *voom* function from the *limma* R package (Ritchie et al., 2015). We ran two differential expression analyses including 1) the heads/antennas samples with region of origin as a discriminant condition, and 2) the heads as well as body samples with range (native and invaded) and library type (heads or bodies) as discriminant conditions. For the first analysis including the head libraries only, we chose a fold-change cut-off value of 2 and a false

discovery (FDR) of 0.001. For the second analysis including both head and body libraries, we chose $FC > 4$ and $FDR < 1.10^{-9}$. In the latter case, FC and FDR thresholds were set at higher and lower values, respectively, as heads and bodies were expectedly characterised by a much larger number of differentially expressed genes.

BLAST searches

In order to identify the most differentially expressed genes in the differential expression analysis, *BLAST* searches were performed with the *BLAST+* suite 2.4 (Camacho et al., 2009). In order to predict protein-coding transcripts from the *Trinity*-assembled transcripts, we used *TransDecoder* 2.0.1 (Haas et al., 2013). First, we identified as many differentially expressed transcripts as possible using *BLASTp* of the *TransDecoder*-predicted coding transcripts on the Argentine ant and the honey bee official gene sets (ref honeybee, hymenoptera-genomes.org). When a transcript retrieved no match to either to the Argentine ant or the honeybee gene sets, we performed a *BLASTx* search using the original *Trinity*-assembled nucleotide sequence against the *nr/nt* NCBI database (NCBI Resource Coordinators, 2017). In addition, we also ran *BLASTp* on the Swissprot database (The Uniprot Consortium, 2017) using the output produced by *TransDecoder*. In all cases, we used a maximum e-value threshold of 1.10^{-5} . When there were ambiguous matches, we selected the best hit based on the lowest e-values and highest bit scores, as well as sequence length and sequence per cent identity.

Candidate gene approach

We created a list of genes of interest based on their association with neural pathways, chemoreception and immunity using existing annotations of the Argentine ant genome (Smith et al., 2011). The lists of candidate genes are reported in Table IV.S4. We used the output of the *BLASTp* search on the Argentine ant official gene set to match specific genes with the corresponding *Trinity* transcripts. First, we retrieved TMM-normalised counts for each assembled gene, and subsequently log-transformed and scaled values across samples. We analysed differential gene expression using Generalised Linear Models (GLMs) assuming normal distributions, with region as an independent variable (R Core Team, 2015). We tested for post-hoc pairwise differences among groups using the *glht* function of the *multcomp R* package (Hothorn et al., 2016).

Results

Weighted Co-Expression Network Analysis of head libraries

WGCNA took as input 45,417 *Trinity*-assembled genes and produced 53 modules of co-expressed genes. We found that 13 of these modules were significantly ($p < 0.05$) and strongly correlated ($r > 0.5$) with range (Figure IV.1, Figure IV.S4). Furthermore, WGCNA-computed eigengenes for four of these modules were positively correlated with invasion status, while nine exhibited a negative correlation (invasion status: 0 for native populations, 1 for introduced populations).

Figure IV.1. Heatmap representing module-trait relationship, showing correlation coefficient of module eigengene expression with range and statistical significance. 13/53 modules were strongly ($r > 0.50$) and significantly ($p < 0.05$) correlated with range (in bold). Shades of red and blue indicate the strength of the positive and negative correlations, respectively (range is numerically coded as 0 for native, 1 for invasive). Modules are identified by colour names.

Differential expression analysis of genes expressed in heads

We found overall 5,337 differentially expressed genes across regions with $FDR < 0.05$ (Figure IV.2). The differentially expressed genes were mostly found between Argentina and New Zealand. Fewer genes were differentially expressed among populations in Europe, Australia and New Zealand, which all belong to the same introduction origin. We decreased the FDR to 0.001 in order to reduce this list to the 190 most differentially expressed genes (Figure IV.3, coloured in red in Figure IV.2, Table IV.S2). We then defined gene clusters based on expression pattern across the range for further scrutiny (see lateral bar colours in Figure IV.3).

We found 21 genes upregulated in Argentina compared to the introduced range (blue cluster; Figure IV.3). Among these, we found two genes coding for sensory neuron membrane proteins, as well as one gene associated with DNA methylation (i.e. methylated-DNA-protein-cysteine methyltransferase; The Uniprot Consortium, 2017). In California, 8 genes were strongly upregulated compared to other regions (orange, dark green, brown and light green clusters; Figure IV.3). However, these genes had either no available annotation, or their functions in the Argentine ant were unknown.

We found 114 genes that were upregulated in most introduced populations compared to Argentina (purple cluster; Figure IV.3). This cluster included 2 genes coding for pheromone-binding protein Gp-9 as well as two genes coding for two different types of odorant receptors (i.e. 46a and 63a). The cluster also contained a number of photoreceptor-related proteins (i.e. phosrestin-2, three opsins (blue and two UV), retinol dehydrogenase, chaoptin (photoreceptor cell morphogenesis), transient-receptor-potential-like (unknown sensory function, taste or photoreception), carotenoid isomeroxygenase and rhodopsin; The Uniprot Consortium, 2017). We also found a gene coding for an immune TOLL-like protein to be upregulated in the introduced range. We found 30 upregulated genes in Australia and New Zealand compared to Argentina and California (pink cluster; Figure IV.3). The cluster included many proteins associated with metabolism (i.e. carbonic anhydrase 2-like, proteases, lipases, glucose dehydrogenase, phospholipases, alpha-amylases, aldose epimerase; The Uniprot Consortium, 2017). Finally, 17 genes were strongly upregulated in Europe, as well as Australia and New Zealand (cyan cluster; Figure IV.3). Muscle-associated proteins were largely represented in this cluster (i.e. sarcoplasmic calcium-binding protein, muscle LIM protein, as well as light and heavy myosin chains). A msta protein related gene, associated

with epigenetic regulation (The Uniprot Consortium, 2017), was also found to be upregulated in Europe, Australia and New Zealand.

Figure IV.2. Volcano plots showing pairwise comparisons of genes differentially expressed among regions along the Argentine ant introduction pathway. Genes coloured in orange and red are differentially expressed for a $FDR < 0.05$. We restricted the functional analysis to the 190 most differentially expressed transcripts across the ranges by reducing the FDR to 1.10^{-3} and the increasing the FC to 2 (in red only). Country codes refer to the following regions: Argentina (AR), California (CA), Europe (EU), Australia (AU) and New Zealand (NZ).

gene set, completed with *BLASTx* on the *nr* NCBI database. Colours in the top band represent regions (native range in blue and invaded range in shades of red, i.e. Argentina (AR), California (CA), Europe (EU), Australia (AU) and New Zealand (NZ)), and colours in the lateral band represent gene clusters (from top to bottom as referred in the text: blue cluster upregulated in Argentina; orange cluster upregulated in Argentina and California; dark green, brown clusters upregulated in California, and light green, purple, pink, cyan clusters overall upregulated in the introduced range). Trees are based on expression similarity (clusters of samples and genes).

Range-specific differences in expression pattern associated with body part

The differential expression analysis performed with both head and body libraries was mostly characterised by body part-specific expression pattern across the range. However, a subset of genes was range-specific, upregulated in the native compared to the introduced range in both heads and bodies (i.e. green cluster, Figure IV.4, Figure IV.S5). We found 38 genes that followed this pattern for $FDR < 1.10^{-9}$ and $FC > 4$, of which 18 did not match to either the Argentine ant official gene set or the *nr/nt* NCBI database. We found 16 genes that matched to Argentine ant sequences, and 4 extra genes that matched to other ant species (Table IV.S3). Among the annotated genes upregulated in both heads and bodies in the native range, we found a Janus-kinase interacting protein 3 (associated with the JAK/STAT immune signalling pathway), two copies of sensory neuron membrane protein 1-like (olfactory neurons), and a likely Argentine ant homologue to histone deacetylase 4 associated with histone modifications.

Figure IV.4. Details of the body part-specific genes differentially expressed across the Argentine ant introduction pathway, showing log2-transformed TMMs with $FDR < 1.10^{-9}$. Colours on the y-axis represent gene expression clusters (light grey, dark grey and green). Colours in the top band represent regions (native range in blue, and invaded range in red, darker shade indicate body libraries). Genes in the green cluster followed an unusual pattern of expression, dominated by differences between ranges rather than body parts. Trees are based on expression similarity (clusters of samples and genes).

Candidate gene approach: neural pathways

Genes associated with octopaminergic neural pathway were downregulated in Europe and New Zealand compared to Argentina, but we found no difference between California and Australia (EU: -0.743, $t = -3.665$, $p < 0.01$; NZ: -0.458, $t = -2.260$, $p < 0.05$; CA: -0.339, $t = -1.672$, $p = 0.110$; AU: -0.348, $t = -1.716$, $p = 0.102$; Figure IV.5, Table IV.S5). Genes associated with dopamine were downregulated in Europe and New Zealand, and there was a marginally significant downregulation in Australia but not in California (EU: -0.507, $t = -2.682$, $p < 0.05$; NZ: -0.486, $t = -2.570$, $p < 0.05$; CA: -0.318, $t = -1.682$, $p = 0.108$; AU: -0.380, $t = -2.008$, $p = 0.058$; Figure IV.5, Table IV.S5). Genes associated with serotonin were consistently downregulated in the introduced range (CA: -0.263, $t = -2.438$, $p < 0.05$; EU: -0.463, $t = -4.292$, $p < 0.01$; AU: -0.386, $t = -3.582$, $p < 0.01$; NZ: -0.498, $t = -4.620$, $p < 0.001$; Figure IV.5, Table IV.S5), as well as genes associated with glutamate (CA: -0.334, $t = -5.459$, $p < 0.001$; EU: -0.192, $t = -3.140$, $p < 0.01$; AU: -0.281, $t = -4.585$, $p < 0.001$; NZ: -0.273, $t = -4.465$, $p < 0.001$; Figure IV.5, Table IV.S5). Genes associated with GABAergic pathways were significantly downregulated in New Zealand compared to Argentina (-0.237, $t = -2.505$, $p < 0.05$; Figure IV.5, Table IV.S5), and there was a marginally significant downregulation in California and Australia but not in Europe (CA: -0.199, $t = -2.106$, $p = 0.0615$; AU: -0.186, $t = -1.968$, $p = 0.077$; EU: -0.084, $t = -0.891$, $p = 0.394$; Figure IV.5, Table IV.S5). We found no difference in expression of genes associated with tyraminergetic pathways across the ranges ($p > 0.160$; Figure IV.5, details in Table IV.S5).

Candidate gene approach: chemoreception

Compared to Argentina, we found that olfactory receptor genes were downregulated in California and upregulated in New Zealand but there was no differential regulation in Europe and Australia (CA: -0.180, $t = -5.305$, $p < 0.001$; NZ: 0.162, $t = 4.775$, $p < 0.001$; EU: -0.012, $t = -0.355$, $p = 0.723$; AU: -0.062, $t = -1.821$, $p = 0.070$; Figure IV.6, Table IV.6). Gustatory receptors were also downregulated in California relative to Argentina (CA: -0.283, $t = -2.721$, $p < 0.05$; EU: -0.152, $t = -1.465$, $p = 0.156$; AU: -0.137, $t = -1.315$, $p = 0.200$; NZ: -0.180, $t = -1.731$, $p = 0.096$; Figure IV.6, Table IV.6).

Candidate gene approach: immune pathways

We found no overall difference in immune gene expression between the native and invaded range, except in California where immune genes were upregulated (CA: 0.131, $t = 2.037$, $p < 0.05$; EU/AU/NZ: $p > 0.478$; Figure IV.7, details in Table IV.S7). However, an

immune pathway-specific analysis revealed that genes associated with the RNAi pathway were downregulated in the introduced range compared to Argentina, though the difference was only marginally significant in California (CA: -0.269, $t = -1.905$, $p = 0.076$; EU: -0.501, $t = -3.554$, $p < 0.01$; AU: -0.318, $t = -2.252$, $p < 0.05$; NZ: -0.376, $t = -2.663$, $p < 0.05$; Figure IV.7, Table IV.S7). Conversely, genes associated with the TOLL pathway were upregulated in the introduced range (CA: 0.298, $t = 3.289$, $p < 0.01$; EU: 0.182, $t = 2.003$, $p < 0.05$; AU: 0.188, $t = 2.074$, $p < 0.05$; NZ: 0.220, $t = 2.428$, $p < 0.05$; Figure IV.7, Table IV.7). We found no significant variation in the JAK/STAT pathway expression across the ranges ($p > 0.567$; Figure IV.7, details in Table IV.7).

Figure IV.5. Log-centred TMM-normalised TPMs for genes associated with (a) octopaminergic, (b) dopaminergic, (c) serotonergic, (d) tyraminergetic, (e) GABAergic and (f) glutamatergic neural pathways. Results of GLMs are given above each boxplot in the introduced range, compared to Argentina. Details of the genes included in the datasets are found in Table IV.S3.

Figure IV.6. Log-centred TMM-normalised TPMs for genes associated with (a) olfactory receptors and (b) gustatory receptors. Results of GLMs are given above each boxplot in the introduced range, compared to Argentina. Details of the genes included in the datasets are found in Table IV.S3.

Figure IV.7. Log-centred TMM-normalised TPMs for (a) pooled immune genes, and genes specifically associated with the (b) TOLL pathway, (c) JAK/STAT pathway and (d) RNAi pathway. Results of GLMs are given above each boxplot in the introduced range, compared to Argentina. Details of the genes included in the datasets are found in Table IV.S3.

Discussion

Here, we investigated transcriptome-wide expression profiles associated with range in the Argentine ant, identified the most differentially expressed genes among regions across the introduction pathway, and investigated gene expression profiles associated with neural pathways, chemoreception and immune pathways. Our results show distinct transcriptomic signatures between native and invaded ranges. Indeed, WGCNA showed a large number of genes clustered in expression modules that were either positively or negatively correlated with range. Similarly, differential expression analysis of libraries also showed clear differences among regions, with samples showing perfect range and region-wise clustering. Interestingly, the largest differences in gene expression among regions were found between Argentina and New Zealand, both at the extremes of the introduction pathway included in this study, which is consistent with gradual changes associated with each step along the pathway. Differential expression analysis of head and body libraries across the ranges was dominated by body part-specific expression patterns. However, a number of genes appeared to be more influenced by range than body part, and were consistently expressed or upregulated in Argentina compared to the introduced range, which may indicate an association of these genes with variations between native and invasive populations. Functional analysis of differentially expressed genes in both analyses indicated a number of genes associated with a wide range of biological processes, including sensory systems. Further scrutiny of specific gene groups pointed to range differences in key neural and immune pathways.

Chemosensory functions are central in regulating the social behaviour of ants and other social insects, especially in terms of foraging behaviour and nestmate recognition (Leonhardt et al., 2016). Among the 190 genes most significantly differentially expressed in worker heads between the native and invaded ranges, we found 6/190 genes associated with chemoreception. Furthermore, one sensory neuron-associated gene was also upregulated in Argentina regardless of the body part considered. Interestingly, a pheromone-binding Gp-9 gene – known to be associated with social structure (i.e. queen number) in *Solenopsis invicta* (Keller and Ross, 1998) – was upregulated in the introduced range, although the function of Gp-9 is unknown in the Argentine ant. At least two different hypotheses have been suggested to explain loss of intraspecific aggression and variation in colony structure between native and invaded ranges, but both suggest alteration of nestmate recognition process in introduced

populations (Giraud et al., 2002; Tsutsui et al., 2003). Altogether, these differentially expressed genes may indicate a role for chemoreception in the differences between ranges, perhaps in terms of reducing intraspecific aggression. However, we found no consistent differences in genes associated with olfactory and gustatory receptors between the native and invaded range. Both olfactory and gustatory receptors were downregulated in California, and olfactory receptors were significantly upregulated in New Zealand. Nevertheless, different types of receptors may have different targets and therefore different roles in regulating the Argentine ant's social behaviour. Therefore, an analysis at the gene level following thorough functional annotations of individual chemoreceptors (and which classes of compounds bind to them) may be more appropriate to investigate the role of chemoreception in differences between native and invaded ranges.

Compounds that can function as neuromodulators and neurotransmitters regulate social behaviour in ants (Kamhi and Traniello, 2013) and may be of particular interest in terms of behavioural variations associated with invasiveness. Genes associated with the octopaminergic and dopaminergic systems did not follow a native/invasive range dichotomy, and were significantly downregulated only in Europe and New Zealand compared to Argentina. Genes associated with the serotonergic and glutamatergic systems were consistently downregulated in the introduced range. Interestingly, while we did not find behavioural differences in foraging activity between native and invaded ranges (Chapter III), many of the neural pathways associated with foraging behaviour in honeybee (Liang et al., 2014, 2012) exhibited significant differences between native and introduced Argentine ant populations in the present study. In insects, serotonergic pathways are often associated with aggressive behaviour, together with octopaminergic and dopaminergic pathways (Dierick and Greenspan, 2007; Hunt et al., 2007; Szczuka et al., 2013). The introduced Argentine ant populations are characterised by loss of intraspecific aggression over large distances (Tsutsui et al., 2000). The causes of such behavioural variation remains controversial, but it is possible that down-regulation of neural pathways such as the serotonergic pathway are associated with mediating intraspecific aggressive behaviour in the introduced range.

The introduction of species may be associated with changes in their microbial communities, which can affect their ecological success. While immune genes were overall only differentially regulated in California, a closer look at specific pathways revealed that the TOLL pathway was upregulated in the introduced range, while the RNAi pathway was

overall downregulated in the introduced range. We found no difference in the JAK/STAT immune pathway. Interestingly, JAK/STAT and RNAi immune pathways are targeted against viruses, while the TOLL pathway is targeted against bacteria (Evans et al., 2006), which may suggest that native and introduced populations are subject to different immune challenges. Previous studies showed changes in bacterial communities along the Argentine ant introduction pathway (Lester et al., 2017) with both acquisition and loss of bacteria, which is neither consistent nor contradictory with variation in TOLL genes we observe. It has been shown that viral loads can vary among Argentine ant populations, and that they acquired new viruses in their introduced range (Gruber et al., 2017; Sébastien et al., 2015). However, enemy release from viruses have been shown to occur in another invasive ant, the Red Imported Fire Ant (Yang et al., 2010). Our data indicate that mechanisms of defence against RNA viruses are downregulated in the introduced range, perhaps suggesting higher viral pressure in the native range.

Epigenetic regulation is likely to contribute to trait plasticity in invasive species, as it is a fast response to changing environmental conditions in a novel range (Pérez et al., 2006). We found two genes associated with epigenetic regulation to be differentially expressed across the ranges. In Argentina, a DNA-methyltransferase was upregulated compared to the introduced range, while a gene associated with msta protein was upregulated in Europe, Australia and New Zealand. In addition, another gene associated with epigenetic regulation was also consistently upregulated in Argentina – in both heads and bodies. Furthermore, DNA methylation levels correlated with recent introduction events have been recently reported in invertebrates (Ardura et al., 2017). While the targeted genomic regions subject to regulation by these genes remains unknown, these findings suggest that there may be a lot to learn in how epigenetic mechanisms can affect gene expression and differential ecological success of native and introduced populations.

A significant proportion of genes upregulated in the introduced range, compared to that of the native range, were surprisingly associated with vision. Some ant species heavily rely on vision to orientate themselves while foraging (Cheng et al., 2014), but Argentine ants have comparatively smaller eyes than the latter and are likely to have underdeveloped vision. Chemoreception appears to be the primary hallmark of Argentine ant's sensory biology, highlighted by the great number of associated genes in their genome (Smith et al., 2011). Nevertheless, our results may indicate that vision may have been overlooked in past studies.

We also found genes associated with muscular functions upregulated in Europe, Australia and New Zealand, as well as genes associated with metabolism upregulated in Australia and New Zealand. However, such functions are difficult to tie to behavioural processes, and their over-representation in our differential expression analysis remains unclear.

Here, we found that native and invasive Argentine ant populations were characterised by distinct transcriptomic signatures. Genes associated with neural pathways, chemoreception and immune pathways were differentially expressed across the ranges, suggesting that functional adaptations may contribute to the success of invasive Argentine ant populations.

General discussion

Summary

Behaviour is a key driver of biological invasions (Chapple et al., 2012; Holway and Suarez, 1999), especially in ant species that are amongst the most harmful invaders (Holway et al., 2002; Silverman and Buczkowski, 2016). The overall aim of this thesis was to study changes in key behaviours and their molecular basis associated with successive introductions of a globally invasive species, the Argentine ant. First, I investigated the molecular basis of behaviour in the Argentine ant, and tested if variation among morphological castes and behavioural worker castes was associated with variation in the expression of genes associated with neural pathways, chemoreception and immunity. I also used neurochemical manipulations to specifically investigate the role of octopamine in modulating behaviour. Second, I tested the hypothesis that behaviours that promote invasiveness may be selected for in introduced Argentine ant populations. I measured behavioural variation along the ant's introduction pathway, as well as variation in transcriptome-wide gene expression profiles that may underlie the invasion success of the Argentine ant.

In this section, I first summarise my key findings, and then discuss them in the context of the Argentine ant introductions. Second, I suggest how future research could address the limitations relative to my findings, and what the priorities are to further investigate how the introduction process may alter behavioural traits in invasive species.

Morphological castes and behavioural worker subcastes are characterised by distinct transcriptomic basis

In chapter I, I found that Argentine ant queens and workers were characterised by distinct head transcriptomic signatures. Workers of different age and behavioural subcastes also had distinct transcriptomic signatures. Specifically, I showed that workers – especially mature workers – exhibited higher expression of chemoreceptors than queens, and also showed differences in the expression of genes associated with specific neural pathways. Furthermore, worker behavioural subcastes (i.e. associated with foraging propensity) were also associated with differences in expression of genes related to olfactory reception and many neural pathways, including the octopaminergic system.

Foraging behaviour and aggression are in part modulated by octopamine

I specifically investigated the association of octopaminergic system with ant behaviour at the colony and individual levels using pharmacological manipulations in chapter II. I found that octopamine and an octopamine antagonist affected colony-level foraging activity, but the effects at the individual level were variable. I argue that octopamine aids in the modulation of division of labour in Argentine ant colonies.

Serial introductions are not associated with variation in foraging behaviour and behavioural plasticity

In chapter III, I tested the hypothesis that successive introductions were associated with changes in behaviour variation and plasticity, comparing foraging behaviour and aggression in the native and introduced ranges, and response to octopamine administration. I found that behaviour was highly variable among regions, sites and experimental colonies. The observed variation was not consistent with the selection for increased colony-level foraging activity. Furthermore, behavioural plasticity remained overall unchanged along the introduction pathway, suggesting that mechanisms that promote behavioural variation act in a similar fashion both in the native and invaded ranges.

Native and invaded Argentine ant population exhibit distinct transcriptomic signatures

I investigated changes associated with the introduction process at the transcriptomic level in chapter IV. I found that native and introduced populations were characterised by distinct transcriptomic signatures, including the overall higher expression of genes related to most neural pathways in the native populations, and differential regulation of specific immune pathways. These results may suggest that functional adaptations are associated with the introduction of the Argentine ant outside of its native range.

Synthesis

Biological invasions are a key factor contributing to the loss of biodiversity worldwide and human-caused environmental change (Vitousek et al., 1996), and behaviour is thought to be a key driver of invasions (Holway and Suarez, 1999). The introduction of exotic species outside of their native range could be associated with changes in behavioural traits that can subsequently promote their invasion success. I showed that key behaviours that likely contribute to invasiveness, i.e. foraging and aggressive behaviour, have molecular basis

in Argentine ants. Indeed, their foraging propensity was associated with distinct gene expression pattern in genes related to specific neural pathways and olfactory reception (Chapter I). Furthermore, I demonstrated that worker foraging behaviour and aggression could be manipulated by disrupting their octopaminergic system (Chapter II). These results are consistent with findings in other social insects, especially honeybees in which octopamine has been shown to modulate division of labour, and scouting shown to be associated with differential regulation of multiple genes associated with other neural pathways (Liang et al., 2014, 2012; Schulz et al., 2002). Once the molecular basis of behaviour is established, it is possible for the genes underpinning behavioural variation to be subjected to selection during the introduction process and in novel environments, potentially leading to behavioural differences among native and introduced populations.

Behaviour in terms of foraging activity was very variable within regions, but overall consistent across the introduction pathway (Chapter III). These findings provide grounds to reject the hypothesis of the introduction process selecting for behavioural traits that promote invasiveness. However, it is worth noting that the conclusions from my research can only be restricted to the very specific behaviours that were measured in the Argentine ant, i.e. colony-level foraging activity in a relatively small colony setting. Argentine ants form large-scale foraging networks, and foraging success may be more heavily influenced by other factors, such as trail network efficiency or loss of intraspecific aggression. Interestingly, we observed differences in expression of genes related to neural pathways across the native and invaded ranges (Chapter IV), which may suggest behavioural differences that we did not investigate in the present study. Indeed, biogenic amines and other compounds are associated with neuromodulation of many behaviours in animals (Hoyle, 1985; Libersat and Pflueger, 2004), including social behaviour in ants and other social insects (Kamhi and Traniello, 2013). For instance, the loss of intraspecific aggression over the large geographic scale of introduced populations is thought to be a key component in their success (Holway et al., 1998; Holway and Suarez, 2003; Tsutsui et al., 2000). Interestingly, genes related to neural pathways known to be associated with aggressive behaviour (e.g. dopaminergic and serotonergic pathways) were overall downregulated in the introduced range compared to the native range (Chapter IV). Furthermore, specific genes associated with olfactory reception – a key function in nestmate recognition – were also differentially regulated between native and invaded populations. Although more research dedicated to this specific question is required, it is possible that variation in specific neural pathways or sensory functions is associated with the

alteration of intraspecific aggression in the Argentine ant's introduced range, or other behaviours that may contribute to its invasion success.

The newest introduced population in this study was that from New Zealand, which was discovered in the 1990s. It is possible that after at least 25 years (and much more in the other introduced regions), evolutionary forces have acted to maintain behavioural variation and its underlying molecular basis in a similar fashion across the ranges. In introduced geckos for instance, it has been shown that invasive populations quickly reached genetic equilibrium, in a matter of just a few decades after introduction (Detwiler and Criscione, 2014). Detwiler and Criscione's findings may indicate that even if recently introduced populations may exhibit signatures of population bottlenecks, these signatures can rapidly disappear, especially in species with short generation times such as the Argentine ant. Therefore, I suggest extending this dataset to newer introductions, in order to perhaps detect some changes in behaviour more tightly associated with the introduction process. Interestingly, the largest transcriptome-wide differences in expression profiles I found were between Argentina and New Zealand, both at the extremes of the introduction pathway, which may suggest gradual changes with serial introductions. There were clearly distinct expression profiles between native and introduced ranges for genes related to specific functions such as neural and immune pathways. However, gene expression pattern for genes associated with such key functions showed no directional variation along the introduction pathway. Population genetics studies at neutral markers point to high genetic similarity among Argentine ant populations in the introduced regions included in the present study (Van Wilgenburg et al., 2010; Vogel et al., 2010). Therefore, perhaps only the first successful introduction event may have affected the ant's biology, allowing the spread of the transcontinental supercolony studied here.

The Argentine ant forms supercolonies in Argentina, but in introduced populations these supercolonies are typically much larger than in the native range (Pedersen et al., 2006; Suarez et al., 2008). The factors underlying the formation and maintenance of such large supercolonies in the introduced range remain unclear and debated. It has been attributed to reduced genetic diversity (Tsutsui et al., 2000), reduced interspecific competition (Vogel et al., 2010), or a genetic cleansing of less common recognition cues (Giraud et al., 2002). Nevertheless, unicoloniality is a key factor in both its ecological success in the invaded range, and also in its ability for long-distance dispersal (Holway, 1999; Holway and Suarez,

2003, 1999; Silverman and Buczkowski, 2016). Irrespective of the underlying mechanisms of large-scale unicoloniality in the introduced range, this population structure undeniably confers competitive advantages to introduced populations. Unicoloniality might largely explain the success of Argentine ants as invaders by facilitating both its spread in novel environments and also to novel regions. In these conditions, there may be only weak selective pressures on other behavioural traits during the introduction process, as suggested by the absence of directional variation in foraging activity that I found along the ant's introduction pathway.

Limitations and future avenues of research

Improvements to the annotation of the Argentine ant genome may allow more extensive knowledge of functional genomics in this species. While the majority of *Trinity*-assembled transcripts mapped to the Argentine ant genome, many of these were not annotated (i.e. “uncharacterised loci”). Furthermore, the gene annotations in the *Linepithema humile* official gene set are derived from homologies with known loci in other organisms, and the current state of functional ant genomics does not allow for a definite and accurate functional categorisation. Inaccurate predictions on the function of Argentine ant genes may undermine the validity of conclusions drawn from genomic studies in non-model organisms. In order to improve the quality of genomic and transcriptomic studies in ants, a priority is therefore to expand the current annotations of the various ant genomes. Furthermore, functional studies in ants are needed to understand better the role of specific genes, without having to rely on the function of homologue genes in model organisms. Gene-editing techniques such as RNA interference and CRISPR/CAS-9 have been successfully used in ants (Ratzka et al., 2013; Triple et al., 2017; Yan et al., 2017). Such techniques enable knockdown of specific genes to investigate their functions, and hold great potential for the future of functional genomics in ants.

An ideal context by which to understand selection is to be able to compare putatively selected genes with genes evolving in a neutral manner. RNA-sequencing is a powerful tool to investigate functional variation in terms of gene expression, but also allows for variant calling to investigate genomic variation (Piskol et al., 2013) and identify genomic regions under selection. The identification of single-nucleotide polymorphism (SNPs) across the genome, especially variations the rate of synonymous versus non-synonymous substitutions

will contribute to a better understanding of Argentine ant population genetic structure across its native and introduced ranges, but also to investigate evidence for adaptations that may be associated with its success in the introduced range.

Transcriptome sequencing of whole animals not only captures the target transcriptome, but can also capture symbiont and pathogen sequences. The RNA-sequencing dataset produced in this thesis would allow investigation of variation in microbial communities (Johansson et al., 2013, Gruber et al., 2017). Immune gene expression data presented in Chapter IV may indicate differences in pathogen pressure between native and introduced range, which needs to be confirmed by pathogen screening to make further inferences. Given the extent of our sampling along the Argentine ant's introduction pathway, this type of analysis can assist in better understanding how introduction process can affect endogenous microbial communities.

Conclusion

I hypothesised that the introduction process may select for behavioural traits that promote invasiveness in the Argentine ant. While I found clear differences among introduced and native populations. I did not find support for my primary hypothesis at the behavioural level. However, this does not reject the hypothesis for all behaviours in all invasive species. Indeed, the interaction between the introduction process and behavioural variation appears to be complex. For instance, the unique characteristics of invasive ants, and the tendency to unicoloniality is likely to largely facilitate secondary introductions, making selection on phenotypes that promote invasiveness weak. In addition, behavioural variation is likely to be extremely variable as it emerges from a multitude of factors, from genes to environmental factors. Interestingly, I found distinct transcriptomic signatures between native and introduced Argentine ant populations, and further investigations at the genomic level are needed to provide more data to discuss the validity of this hypothesis. Furthermore, non-social species or social insects that do not form supercolonies or that form populations characterised by multiple introductions from different origins, such as skinks or wasps, may be more tractable to evaluate how the introduction process influences behaviour in introduced populations. Further research is needed to improve our understanding of how the introduction process may influence the biology of invaders – both in the Argentine ant at the genomic level but also in other invasive species.

Here, I undertook an integrative study of the Argentine ant invasion, and investigated how the introduction process may influence its behaviour in the introduced range. I did not find behavioural differences among native and introduced populations, but discovered that they were characterised by distinct transcriptomic signatures, perhaps underlying their invasion success. My work also advanced our understanding of the molecular basis of social behaviour in the Argentine ant, which will contribute to further elucidate the underlying mechanisms of sociality – thought to be the hallmark of the ecological success of social insects, and invasion success in the ones that are considered invasive.

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Supplementary materials

Supplementary material for Chapter I

Table I.S1. Differentially expressed features among queens and workers, including retained BLAST hit

Table I.S2. Differentially expressed features among callow and mature workers, including retained BLAST hits

Table I.S3. List of genes used in the candidate gene approach in the queen, callow and mature workers comparison

Table I.S4. Output of the post-hoc pairwise comparisons after GLMs for neural pathways

Table I.S5. Output of the post-hoc pairwise comparisons after GLMs for chemoreceptors

Table I.S6. Output of the post-hoc pairwise comparisons after GLMs for immune pathways

Table I.S7. Differentially expressed features among insiders, foragers and scouts, including retained BLAST hit

Table I.S8. List of genes used in the candidate gene approach

Table I.S9. Output of the post-hoc pairwise comparisons after GLMs for neural pathways

Table I.S10. Output of the post-hoc pairwise comparisons after GLMs for chemoreceptors

Table I.S11. Output of the post-hoc pairwise comparisons after GLMs for immune pathways

Figure S1. Cluster plots defined for log2-transformed TMMs with $p < 0.001$ and fold change $FC > 4$ between queens and workers

Figure I.S2. Cluster plots defined for log2-transformed TMMs with $p < 0.001$ and fold change $FC > 2$ between callow and mature workers

Table I.S1. Best *BLAST* results for differentially expressed transcripts associated with morphological castes from *BLASTp* on the Argentine ant gene set, *BLASTp* on the Swissport database and *BLASTx* on the *nr/nt* database. Transcripts are presented in the same order as in Figure I.11. Cluster 1 is characterised by transcripts upregulated in workers, and cluster 2 by transcripts upregulated in queens.

Cluster	qseqid	sseqid	pidnt	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore	Gene name
1	TRINITY_GG_10484_c5_g2_i1	XP_012214812	100	240	0	0	105	824	1	240	7.00E-158	454	PREDICTED: uncharacterized LOC105667550
1	TRINITY_GG_2872_c0_g2_i1	XP_012214721	90.14	71	7	0	3	215	239	309	1.00E-23	102	PREDICTED: cytochrome P450 3A19-like
1	TRINITY_GG_5431_c17_g1_i2	XP_012230777	100	53	0	0	970	812	473	525	2.00E-23	111	PREDICTED: cytochrome P450 4C1-like
1	TRINITY_GG_4606_c0_g3_i1	XP_012214721	87.56	217	27	0	763	113	93	309	1.00E-123	367	PREDICTED: cytochrome P450 3A19-like
1	TRINITY_GG_8061_c1_g1_i2	BRM_DROME	76.17	1175	251	11	739	1893	365	1530	0	1745	[Drosophila melanogaster] brahma
1	TRINITY_GG_1889_c3_g1_i1	XP_012214721	90.32	124	12	0	611	240	186	309	9.00E-62	207	PREDICTED: cytochrome P450 3A19-like
1	TRINITY_GG_9666_c7_g4_i3	LH14425	75.1	241	49	2	72	310	2	233	2.00E-126	367	cytochrome P450 4C1-like
2	TRINITY_GG_10107_c2_g1_i1	LH16026	100	249	0	0	1	249	1	249	0	504	circadian clock-controlled protein
2	TRINITY_GG_1884_c1_g1_i1	LH23693	100	347	0	0	26	372	1	347	0	716	uncharacterized LOC105677856
2	TRINITY_GG_3423_c0_g1_i1	LH11757	98.46	259	1	1	1	256	1	259	0	520	farnesol dehydrogenase-like
2	TRINITY_GG_1353_c4_g1_i1	LH15385	60.42	288	113	1	1	288	98	384	1.00E-134	394	uncharacterized LOC105669905
2	TRINITY_GG_2674_c5_g1_i1	LH15531	87.01	154	0	1	1	154	1	134	1.00E-92	267	LIRP
2	TRINITY_GG_2631_c0_g2_i1	XP_012218638	100	201	0	0	864	262	299	499	1.00E-140	419	PREDICTED: myosinase 1-like
2	TRINITY_GG_5937_c0_g1_i1	LH14500	100	198	0	0	1	198	1	198	1.00E-145	406	protein THEM6-like
2	TRINITY_GG_8852_c8_g1_i1	XP_012220027	80.37	163	32	0	241	729	1	163	5.00E-78	254	PREDICTED: leucine-rich repeat-containing protein egg-6-like
2	TRINITY_GG_8852_c2_g1_i1	LH21085	29.55	132	91	2	56	185	63	194	2.00E-10	59.3	peroxidasin
2	TRINITY_GG_1425_c0_g1_i1	XP_012215636	100	244	0	0	1179	448	1	244	3.00E-166	479	PREDICTED: uncharacterized LOC105668041

2	TRINITY_GG_3232_c0_g1_i1	LH18353	100	123	0	0	1	123	1	123	2.00E-85	248	uncharacterized LOC105672700
2	TRINITY_GG_9920_c7_g1_i9	LH18522	99.73	372	1	0	1	372	1	372	0	765	dnaJ homolog subfamily C member 22
2	TRINITY_GG_2272_c20_g5_i4	LH20862	92.19	909	69	2	1	908	1	908	0	1743	patched domain-containing protein 3-like
2	TRINITY_GG_5937_c0_g1_i2	LH14500	100	198	0	0	1	198	1	198	1.00E-145	406	protein THEM6-like
2	TRINITY_GG_1349_c1_g1_i1	LH10244	100	433	0	0	6	438	1	433	0	900	lysosomal aspartic protease
2	TRINITY_GG_383_c1_g1_i1	XP_012222396	100	167	0	0	925	425	1	167	8.00E-113	340	uncharacterized LOC105672202
2	TRINITY_GG_10844_c1_g1_i5	LH16195	87.3	244	5	2	59	301	16	234	7.00E-149	421	cytochrome P450 4C1-like
2	TRINITY_GG_8597_c3_g1_i1	EZA50924	83.02	53	9	0	189	347	1	53	2.00E-08	58.5	[Ooceraea biro] hypothetical protein X777_10552
2	TRINITY_GG_4271_c0_g1_i1	XP_012225192	98.13	268	5	0	3	806	48	315	0	550	PREDICTED: acyl-CoA Delta(11) desaturase-like
2	TRINITY_GG_10635_c1_g1_i1	LH20180	55.17	58	25	1	1	57	1	58	2.00E-12	64.7	No hit
2	TRINITY_GG_4887_c0_g1_i1	XP_012228020	100	76	0	0	994	767	1	76	3.00E-36	138	PREDICTED: chymotrypsin inhibitor-like
2	TRINITY_GG_2631_c0_g2_i1	XP_012218638	100	201	0	0	864	262	299	499	1.00E-140	419	PREDICTED: myosinase 1-like
2	TRINITY_GG_3751_c1_g1_i1	LH17706	100	428	0	0	1	428	1	428	0	888	uncharacterized LOC105672080
2	TRINITY_GG_850_c2_g5_i1	LH16389	98.4	624	0	1	1	624	454	1067	0	1253	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like
2	TRINITY_GG_5968_c1_g4_i3	LH25729	97.67	516	0	2	11	518	10	521	0	1022	organic cation transporter protein-like
2	TRINITY_GG_5968_c1_g4_i2	LH25729	97.07	409	0	2	11	411	10	414	0	807	organic cation transporter protein-like
2	TRINITY_GG_39_c5_g1_i1	LH26013	100	620	0	0	1	620	1	620	0	1241	slit homolog 2 protein-like
2	TRINITY_GG_3751_c0_g1_i1	LH17703	28.98	245	135	8	1	216	161	395	1.00E-20	92	lipid storage droplets surface-binding protein 1
2	TRINITY_GG_1425_c1_g1_i1												No hit
2	TRINITY_GG_7039_c0_g1_i1	LH14237	99.71	695	0	1	1	695	1	693	0	1434	phenoloxidase 2-like
2	TRINITY_GG_5431_c17_g14_i3	LH22783	94.65	243	13	0	89	331	31	273	1.00E-167	476	cytochrome P450 4C1-like
2	TRINITY_GG_2621_c0_g1_i2	LH15475	99.62	526	2	0	56	581	8	533	0	1081	UDP-glucuronosyltransferase 2C1-like
2	TRINITY_GG_9753_c2_g1_i1	LH23532	100	382	0	0	1	382	1	382	0	790	venom acid phosphatase Acph-1-like
2	TRINITY_GG_2272_c20_g5_i1	LH20862	91.46	855	71	2	1	854	1	854	0	1626	patched domain-containing protein 3-like
2	TRINITY_GG_2871_c0_g1_i1	LH11418	99.69	323	1	0	1	323	1	323	0	664	uncharacterized LOC105677659
2	TRINITY_GG_151_c27_g5_i5	LH23348	97.57	783	0	2	32	795	1	783	0	1574	DDB1- and CUL4-associated factor 5
2	TRINITY_GG_10923_c3_g2_i3												No hit

2	TRINITY_GG_2899_c2_g1_i3	LH16524	100	692	0	0	1	692	1	692	0	1385	putative uncharacterized protein DDB_G0271606
2	TRINITY_GG_2637_c0_g1_i1	LH15510	98.03	507	0	1	1	497	601	1107	0	1008	uncharacterized LOC105670069
2	TRINITY_GG_2621_c0_g1_i3	LH15475	100	523	0	0	1	523	11	533	0	1078	UDP-glucuronosyltransferase 2C1-like
2	TRINITY_GG_6430_c0_g1_i1	LH22968	23.6	250	163	6	9	245	33	267	3.00E-10	59.3	psychosine receptor-like
2	TRINITY_GG_2620_c1_g1_i1	LH15507	100	122	0	0	49	170	5	126	2.00E-84	247	icarapin-like
2	TRINITY_GG_4897_c0_g1_i1	XP_012228022	100	76	0	0	637	410	1	76	6.00E-44	154	PREDICTED: chymotrypsin inhibitor-like
2	TRINITY_GG_8126_c0_g2_i1	LH16069	97.24	508	0	1	16	523	1	494	0	1011	zinc finger and SCAN domain-containing protein 23-like
2	TRINITY_GG_2936_c8_g1_i1	LH21846	100	227	0	0	26	252	1	227	4.00E-165	459	uncharacterized LOC105676041
2	TRINITY_GG_1353_c3_g1_i5	LH17269	67.36	288	93	1	100	387	2	288	2.00E-150	436	uncharacterized LOC105671728
2	TRINITY_GG_7408_c0_g2_i1	XP_012221559	100	860	0	0	61	2640	1	860	0	1661	PREDICTED: uncharacterized LOC105671724
2	TRINITY_GG_7814_c0_g2_i3	LH20862	86.06	538	74	1	11	548	1	537	0	977	patched domain-containing protein 3-like
2	TRINITY_GG_9183_c8_g2_i1	XP_012222954	99.12	228	1	1	1154	471	1	227	2.00E-153	446	PREDICTED: venom allergen 3-like
2	TRINITY_GG_5513_c0_g1_i2	LH22963	100	871	0	0	1	871	313	1183	0	1772	uncharacterized LOC105676976
2	TRINITY_GG_6725_c0_g1_i3	LH22677	84.7	183	26	1	1	181	1	183	1.00E-100	296	retinol dehydrogenase 11-like
2	TRINITY_GG_10182_c0_g4_i1	LH23796	98.89	452	0	1	1	447	1	452	0	913	ankyrin repeat and SAM domain-containing protein 3-like
2	TRINITY_GG_7191_c5_g1_i1	LH17459	100	445	0	0	1	445	1	445	0	938	uncharacterized LOC105671877
2	TRINITY_GG_1069_c6_g1_i1	XP_012232188	46.15	91	44	2	510	779	52	138	9.00E-06	56.2	PREDICTED: extensin-like
2	TRINITY_GG_383_c1_g1_i1	XP_012222396	100	167	0	0	925	425	1	167	8.00E-113	340	uncharacterized LOC105672202
2	TRINITY_GG_9920_c6_g1_i1	LH18523	100	336	0	0	1	336	1	336	0	702	clavesin-1-like
2	TRINITY_GG_3186_c2_g1_i1	LH23093	100	155	0	0	1	155	1	155	1.00E-110	314	ecdysteroid-regulated 16 kDa protein
2	TRINITY_GG_1885_c13_g1_i8	LH23697	100	262	0	0	1	262	1	262	0	542	tetraspanin-9
2	TRINITY_GG_9233_c1_g1_i4	LH18111	100	401	0	0	9	409	1	401	0	828	2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial
2	TRINITY_GG_9197_c1_g2_i1	XP_012222989	100	200	0	0	1872	1273	1	200	4.00E-119	366	PREDICTED: uncharacterized LOC105672550
2	TRINITY_GG_11044_c2_g1_i1	LH13463	100	195	0	0	19	213	1	195	3.00E-139	390	uncharacterized LOC105668188
2	TRINITY_GG_2272_c20_g5_i3	LH11612	95.63	366	13	1	1	366	469	831	0	721	patched domain-containing protein 3-like
2	TRINITY_GG_7880_c12_g2_i3	LH24453	96.48	341	0	1	1	329	18	358	0	670	alpha-tocopherol transfer protein-like
2	TRINITY_GG_2634_c0_g1_i2	LH15490	100	203	0	0	107	309	1	203	9.00E-147	420	dipeptidase 1-like

2	TRINITY_GG_7715_c0_g1_i1	KYN38567	50.88	57	25	1	127	297	30	83	1.00E-10	65.5	[Trachymyrmex septentrionalis] hypothetical protein ALC56_07050
2	TRINITY_GG_9281_c11_g7_i1	XP_012222900	97.83	92	2	0	278	3	38	129	1.00E-55	186	PREDICTED: alpha-tocopherol transfer protein-like
2	TRINITY_GG_7417_c10_g2_i2												No hit
2	TRINITY_GG_4397_c0_g1_i1	LH21908	100	309	0	0	1	309	1	309	0	637	short-chain dehydrogenase/reductase family 16C member 6-like
2	TRINITY_GG_4202_c0_g1_i1												No hit
2	TRINITY_GG_8581_c0_g1_i1	XP_012233899	98.84	172	2	0	517	2	547	718	1.00E-101	323	PREDICTED: uncharacterized LOC105678807
2	TRINITY_GG_1259_c8_g2_i1	LH17094	100	286	0	0	1	286	1	286	0	588	putative fatty acyl-CoA reductase CG5065
2	TRINITY_GG_1259_c8_g3_i1												No hit
2	TRINITY_GG_8632_c1_g1_i1												No hit
2	TRINITY_GG_1343_c1_g1_i3	LH11788	90.58	382	20	2	1	382	1	366	0	712	putative fatty acyl-CoA reductase CG5065
2	TRINITY_GG_1425_c0_g2_i1	XP_012215636	100	244	0	0	1177	446	1	244	3.00E-166	479	PREDICTED: uncharacterized LOC105668041
2	TRINITY_GG_8157_c4_g1_i1	LH12072	97.99	398	0	1	1	398	1	390	0	791	rhodopsin-like
2	TRINITY_GG_2631_c0_g3_i1	LH15477	100	306	0	0	1	306	1	306	0	642	myrosinase 1-like
2	TRINITY_GG_7870_c0_g1_i1	XP_012233110	100	100	0	0	1052	753	1	100	7.00E-63	209	PREDICTED: uncharacterized LOC105678395
2	TRINITY_GG_3060_c0_g1_i1	LH11521	100	296	0	0	18	313	1	296	0	605	uncharacterized LOC105678730
2	TRINITY_GG_3060_c0_g2_i1	LH11521	100	296	0	0	18	313	1	296	0	605	uncharacterized LOC105678730
2	TRINITY_GG_5968_c0_g1_i1												No hit
2	TRINITY_GG_174_c8_g1_i4	LH23369	99.15	354	3	0	38	391	10	363	0	735	SEC14-like protein 2
2	TRINITY_GG_1425_c5_g1_i1	LH16030	29.52	105	74	0	50	154	9	113	1.00E-12	62	uncharacterized LOC105670441
2	TRINITY_GG_8578_c0_g1_i1	XP_012233899	100	205	0	0	385	999	1	205	4.00E-127	396	PREDICTED: uncharacterized LOC105678807
2	TRINITY_GG_10927_c0_g1_i1	XP_012225930	93.75	80	5	0	633	394	403	482	4.00E-59	159	PREDICTED: nose resistant to fluoxetine protein 6-like
2	TRINITY_GG_10906_c0_g1_i1												No hit
2	TRINITY_GG_5575_c0_g1_i1	LH22968	25.12	207	137	6	11	208	35	232	1.00E-12	66.2	psychosine receptor-like
2	TRINITY_GG_1122_c6_g3_i1	XP_015375258	44.29	70	38	1	782	576	9	78	1.00E-08	52.8	PREDICTED: Diuraphis noxia uncharacterized LOC107169847
2	TRINITY_GG_628_c0_g1_i1	XP_012233899	97.34	263	7	0	586	1374	100	362	7.00E-165	499	PREDICTED: uncharacterized LOC105678807
2	TRINITY_GG_629_c0_g1_i1												No hit

2	TRINITY_GG_8987_c0_g1_i1	LH14281	100	105	0	0	1	105	486	590	1.00E-67	216	acyl-CoA synthetase short-chain family member 3, mitochondrial
2	TRINITY_GG_7188_c0_g4_i1	LH17498	100	92	0	0	93	184	1	92	1.00E-59	184	vitellogenin-2-like
2	TRINITY_GG_3104_c0_g1_i1	XP_012234876	100	79	0	0	358	122	163	241	2.00E-32	125	PREDICTED: protein takeout-like
2	TRINITY_GG_4959_c0_g1_i1	XP_012225930	100	151	0	0	3	455	567	717	3.00E-97	309	PREDICTED: nose resistant to fluoxetine protein 6-like
2	TRINITY_GG_2670_c3_g1_i1	XP_012218726	100	114	0	0	633	292	140	253	4.00E-74	237	PREDICTED: chymotrypsin-2-like
2	TRINITY_GG_9347_c0_g1_i7	LH10845	87.97	133	16	0	4	136	1	133	8.00E-87	253	alpha-tocopherol transfer protein-like
2	TRINITY_GG_8110_c0_g1_i1	XP_012219468	100	246	0	0	771	34	1	246	3.00E-178	507	PREDICTED: protein takeout-like
2	TRINITY_GG_7188_c2_g1_i1	LH17501	99.12	113	1	0	1	113	2029	2141	3.00E-73	238	vitellogenin-2-like
2	TRINITY_GG_7188_c1_g1_i1	XP_012221806	89.47	38	4	0	1	114	1698	1735	1.00E-11	70.9	PREDICTED: vitellogenin-2-like
2	TRINITY_GG_4033_c0_g1_i3	XP_012216106	100	148	0	0	553	110	36	183	2.00E-98	305	PREDICTED: uncharacterized LOC105668348
2	TRINITY_GG_4033_c0_g1_i1												No hit
2	TRINITY_GG_9881_c2_g1_i1	LH10845	89.1	156	17	0	1	156	9	164	1.00E-104	300	alpha-tocopherol transfer protein-like

Table I.S2. Best *BLAST* results for differentially expressed transcripts associated with worker age from *BLASTp* on the Argentine ant gene set, *BLASTp* on the Swissport database and *BLASTx* on the *nr/nt* database. Transcripts are presented in the same order as in Figure I.11. Cluster 1 is characterised by transcripts upregulated in mature workers, and cluster 2 by transcripts upregulated in callow workers.

Cluster	qseqid	sseqid	pident	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore	Gene name
1	TRINITY_GG_9757_c22_g2_i1	LH23517	99.2	376	3	0	1	376	1	376	0	780	actin, muscle
1	TRINITY_GG_7188_c0_g1_i1	LH17497	100	215	0	0	136	350	1	215	2.00E-160	451	vitellogenin-2-like
1	TRINITY_GG_7209_c0_g1_i1	LH17495	98.82	1436	7	1	1	1426	357	1792	0	2933	uncharacterized LOC105671873
1	TRINITY_GG_9791_c10_g3_i8	LH23480	93.54	944	1	2	131	1074	1	884	0	1738	FK506-binding protein 5
1	TRINITY_GG_8597_c3_g1_i1	XR_001101693.1	99.465	187	1	0	1	187	424	610	6.93E-93	340	PREDICTED: uncharacterized LOC105678313, ncRNA
1	TRINITY_GG_2241_c6_g3_i2	LH22183	99.61	516	2	0	1	516	179	694	0	1030	serine/arginine repetitive matrix protein 2
1	TRINITY_GG_1623_c19_g1_i1	LH20784	91.18	431	0	1	187	617	1	393	0	798	zinc transporter 9
1	TRINITY_GG_9791_c10_g3_i5	LH23480	92.6	946	10	2	131	1076	1	886	0	1719	FK506-binding protein 5
1	TRINITY_GG_4036_c20_g1_i4	LH13554	100	406	0	0	35	440	1	406	0	838	arrestin homolog
1	TRINITY_GG_4606_c0_g3_i2	LH12745	97.57	247	2	1	248	494	1	243	6.00E-174	492	cytochrome P450 3A19-like
1	TRINITY_GG_5431_c16_g6_i1	XM_012375356.1	99.827	577	1	0	742	1318	1325	1901	0	1061	PREDICTED: cytochrome P450 4C1-like
1	TRINITY_GG_5431_c16_g6_i2	LH10850	69.84	305	70	2	2	297	45	336	7.00E-147	443	cytochrome P450 4C1-like
1	TRINITY_GG_4036_c21_g1_i1	XM_012360427.1	99.494	395	1	1	20	413	9476	9082	0	717	PREDICTED: uncharacterized LOC105668197
1	TRINITY_GG_8966_c0_g1_i1	LH14369	100	448	0	0	16	463	2	449	0	922	probable citrate synthase 2, mitochondrial
1	TRINITY_GG_1153_c0_g1_i1	XM_012365715.1	99	NA	NA	NA	NA	NA	NA	NA	0	4717	PREDICTED: glutamate dehydrogenase, mitochondrial-like
1	TRINITY_GG_4664_c1_g1_i1	LH21300	100	300	0	0	1	300	1	300	0	612	ADP,ATP carrier protein 2
1	TRINITY_GG_4664_c1_g2_i1	LH21300	100	300	0	0	1	300	1	300	0	612	ADP,ATP carrier protein 2

1	TRINITY_GG_10107_c2_g1_i1	LH16026	100	249	0	0	1	249	1	249	0	504	circadian clock-controlled protein
1	TRINITY_GG_3894_c0_g1_i1	XM_012370129.1	98.724	1254	0	2	1	1252	1249	10	0	2213	PREDICTED: uncharacterized LOC105674057
1	TRINITY_GG_3785_c25_g2_i2	LH18885	96.85	1619	40	2	336	1954	31	1638	0	3113	myosin heavy chain, muscle
1	TRINITY_GG_7354_c5_g1_i1	LH11849	99.24	393	3	0	29	421	90	482	0	826	venom carboxylesterase-6-like
1	TRINITY_GG_2775_c0_g1_i1	KX146468.1	100	826	0	0	18	843	13118	13943	0	1526	Linepithema humile voucher LH20150510 mitochondrion
1	TRINITY_GG_5633_c5_g4_i1	LH12840	99.35	306	2	0	1	306	628	933	0	629	slit homolog 1 protein-like
1	TRINITY_GG_3851_c6_g1_i2	LH18812	99.83	578	1	0	1	578	377	954	0	1204	sarcalumenin
1	TRINITY_GG_3785_c9_g1_i1					0							No hit
1	TRINITY_GG_8245_c0_g1_i1	XM_012380129.1	99	NA	NA	NA	NA	NA	NA	NA	0.00E+00	2990	PREDICTED: uncharacterized LOC105679844
1	TRINITY_GG_26_c1_g3_i1	LH23163	100	446	0	0	1	446	1	446	0	909	transcriptional adapter 3-B
1	TRINITY_GG_4021_c16_g1_i1	LH13528	100	114	0	0	1	114	1	114	2.00E-77	229	PDZ and LIM domain protein 3
1	TRINITY_GG_4386_c4_g1_i2												No hit
1	TRINITY_GG_3785_c25_g2_i1	LH18885	96.71	1611	42	2	336	1946	31	1630	0	3090	myosin heavy chain, muscle
1	TRINITY_GG_9987_c14_g3_i2	LH19123	73.01	515	138	1	1	514	1	515	0	813	cytochrome P450 6k1-like
1	TRINITY_GG_1437_c0_g1_i1	XM_012376601.1	100	NA	NA	NA	NA	NA	NA	NA	6.00E-14	79	PREDICTED: dorsal-ventral patterning tolloid-like protein 1
2	TRINITY_GG_5798_c4_g1_i7	LH23208	69.03	155	1	1	55	209	134	241	2.00E-60	193	nuclear pore complex protein DDB_G0274915-like
2	TRINITY_GG_4036_c20_g1_i1	LH13554	100	406	0	0	35	440	1	406	0	838	arrestin homolog
2	TRINITY_GG_8290_c1_g5_i2	LH20998	100	137	0	0	56	192	344	480	2.00E-96	291	chitinase-3-like protein 1
2	TRINITY_GG_4623_c1_g1_i7	LH16285	100	679	0	0	1	679	1	679	0	822	uncharacterized transmembrane protein DDB_G0289901-like
2	TRINITY_GG_4084_c0_g1_i1	XR_001100115.1	100	379	0	0	1	379	257	635	0	701	PREDICTED: uncharacterized LOC105668501, ncRNA
2	TRINITY_GG_797_c0_g1_i1	LH21774	99.75	785	2	0	1	785	1335	2119	0	1625	fatty acid synthase-like
2	TRINITY_GG_1928_c0_g1_i2	LH10196	100	123	0	0	3	125	74	196	3.00E-75	235	probable serine/threonine-protein kinase DDB_G0283337
2	TRINITY_GG_9682_c1_g1_i1	XM_012368851.1	99.861	722	1	0	210	931	722	1	0	1328	PREDICTED: pheromone-binding protein Gp-9-like
2	TRINITY_GG_1343_c1_g1_i6	LH11788	90.84	382	19	2	1	382	1	366	0	713	putative fatty acyl-CoA reductase CG5065
2	TRINITY_GG_5693_c0_g1_i1	LH11825	100	357	0	0	1	357	1	357	0	743	acyl-CoA Delta(11) desaturase-like

2	TRINITY_GG_1675_c0_g1_i1	LH19123	34.26	505	313	9	7	499	13	510	4.00E-97	306	cytochrome P450 6k1-like
2	TRINITY_GG_8087_c7_g2_i2	LH11300	100	489	0	0	1	489	1	489	0	986	facilitated trehalose transporter Tret1
2	TRINITY_GG_5656_c7_g5_i5	XM_012359489.1	99.931	1443	1	0	244	1686	4921	3479	0	2660	PREDICTED: janus kinase and microtubule-interacting protein 3
2	TRINITY_GG_4623_c1_g1_i1	LH16285	98.19	332	6	0	118	449	348	679	1.00E-82	281	uncharacterized transmembrane protein DDB_G0289901-like
2	TRINITY_GG_1021_c1_g1_i1	LH20534	100	358	0	0	191	548	1	358	0	737	uncharacterized LOC105674921
2	TRINITY_GG_1066_c3_g1_i2	LH20658	96.26	401	1	1	1	387	139	539	0	785	fatty-acid amide hydrolase 2-like
2	TRINITY_GG_8998_c0_g2_i3	XM_012380393.1	99	NA	NA	NA	NA	NA	NA	NA	0	6861	PREDICTED: CD109 antigen-like
2	TRINITY_GG_6704_c8_g1_i1	LH15304	98.61	721	1	1	1	721	1	712	0	1469	uncharacterized LOC105669738
2	TRINITY_GG_3610_c15_g2_i1	LH19162	100	564	0	0	1	564	1	564	0	1180	alpha-glucosidase-like
2	TRINITY_GG_3762_c7_g3_i3	XM_012366802.1	95.444	1273	56	2	1	1272	1701	430	0	2028	PREDICTED: uncharacterized LOC105672092
2	TRINITY_GG_5512_c0_g1_i1	XR_001101494.1	100	325	0	0	3	327	325	1	2.88E-171	601	PREDICTED: uncharacterized LOC105676985, ncRNA
2	TRINITY_GG_2244_c1_g2_i2	LH22200	100	144	0	0	23	166	447	590	6.00E-96	291	mitogen-activated protein kinase kinase kinase 7-like
2	TRINITY_GG_5656_c7_g5_i4	LH12896	99.89	884	1	0	1	884	37	920	0	1770	janus kinase and microtubule-interacting protein 3
2	TRINITY_GG_1115_c8_g1_i7	LH10049	97.9	619	2	2	214	832	16	623	0	1243	twitchin
2	TRINITY_GG_2695_c1_g1_i13	LH26217	99.07	216	2	0	92	307	224	439	3.00E-157	450	serine protease nudel-like
2	TRINITY_GG_10542_c0_g2_i1	XM_012367245.1	92.912	522	35	2	209	729	1222	702	0	758	PREDICTED: cytochrome P450 4C1-like
2	TRINITY_GG_6538_c0_g1_i1	LH25847	87.18	117	2	1	3	119	233	336	1.00E-67	210	serine protease inhibitor dipetalogastin-like
2	TRINITY_GG_5313_c0_g1_i1	LH19991	100	209	0	0	1	209	1	209	7.00E-153	426	leucine-rich repeat-containing protein 40
2	TRINITY_GG_9791_c13_g1_i1	LH23477	97.75	489	0	1	1	489	1	478	0	980	probable cytochrome P450 6a14
2	TRINITY_GG_4606_c0_g3_i3	LH12745	92.31	247	15	1	117	363	1	243	4.00E-165	464	cytochrome P450 3A19-like
2	TRINITY_GG_9987_c14_g1_i4	XM_012369085.1	97	NA	NA	NA	NA	NA	NA	NA	0	2613	PREDICTED: cytochrome P450 6k1-like
2	TRINITY_GG_10228_c12_g1_i9	LH23498	33.05	351	231	2	5	352	6	355	6.00E-68	220	probable cytochrome P450 6a13
2	TRINITY_GG_6741_c9_g6_i1	LH15195	99.47	375	2	0	1	375	1	375	0	772	dihydropyrimidine dehydrogenase [NADP()]
2	TRINITY_GG_3762_c7_g3_i2	XM_012366802.1	100	852	0	0	1	852	1701	850	0	1574	PREDICTED: uncharacterized LOC105672092

2	TRINITY_GG_5798_c4_g1_i5	LH23208	92.09	278	0	1	223	500	33	288	2.00E-173	493	nuclear pore complex protein DDB_G0274915-like
2	TRINITY_GG_1970_c0_g1_i3	LH22324	100	331	0	0	1	331	1	331	0	686	cAMP-dependent protein kinase catalytic subunit PRKX
2	TRINITY_GG_3885_c3_g1_i2	LH19774	99.33	747	5	0	76	822	1	747	0	1539	uncharacterized LOC105674045
2	TRINITY_GG_8632_c1_g1_i1	XR_001101710.1	98.734	553	6	1	1	553	806	255	0	981	PREDICTED: uncharacterized LOC105678491, ncRNA
2	TRINITY_GG_6227_c5_g1_i1	LH16691	90.67	75	4	1	15	89	1	72	2.00E-43	143	ejaculatory bulb-specific protein 3-like
2	TRINITY_GG_10638_c5_g1_i2	LH17642	91.1	236	2	1	37	272	7	223	2.00E-151	426	microtubule-associated protein RP/EB family member 1-like
2	TRINITY_GG_742_c0_g1_i1	LH10529	100	121	0	0	24	144	1	121	1.00E-87	254	ejaculatory bulb-specific protein 3-like
2	TRINITY_GG_5798_c4_g1_i3	LH23208	92.09	278	0	1	223	500	33	288	2.00E-173	493	nuclear pore complex protein DDB_G0274915-like
2	TRINITY_GG_6227_c2_g1_i1	LH16674	100	735	0	0	1	735	1	735	0	1530	ATP-dependent RNA helicase SUV3 homolog, mitochondrial
2	TRINITY_GG_9987_c14_g1_i1	LH19121	94.53	512	28	0	1	512	1	512	0	994	cytochrome P450 6k1-like
2	TRINITY_GG_7155_c1_g1_i1	XM_012359035.1	100	942	0	0	7	948	1	942	0	1740	PREDICTED: uncharacterized LOC105667316
2	TRINITY_GG_1343_c1_g1_i2	LH11790	100	204	0	0	1	204	116	319	5.00E-149	420	putative fatty acyl-CoA reductase CG5065
2	TRINITY_GG_151_c24_g1_i2	LH23345	99.8	497	1	0	1	497	1	497	0	1022	cytochrome P450 4c3-like
2	TRINITY_GG_2819_c22_g1_i1	LH24002	99.82	545	1	0	7	551	1	545	0	1131	uncharacterized LOC105678090
2	TRINITY_GG_1413_c0_g2_i1	XM_012360199.1	99.072	431	0	4	1	427	8	438	0	771	PREDICTED: uncharacterized LOC105668029
2	TRINITY_GG_2819_c22_g1_i3	LH24002	94.96	476	24	0	7	482	1	476	0	953	uncharacterized LOC105678090
2	TRINITY_GG_2819_c22_g1_i2	LH24002	98.36	427	7	0	3	429	547	973	0	877	uncharacterized LOC105678090
2	TRINITY_GG_3762_c7_g1_i2	XM_012366800.1	94	NA	NA	NA	NA	NA	NA	NA	0	1616	PREDICTED: uncharacterized LOC105672090
2	TRINITY_GG_743_c6_g1_i1	LH16300	99.8	493	1	0	6	498	1	493	0	1031	alpha-amylase 2-like

Table I.S3. List of genes used in the candidate gene approach in the queen, callow and mature workers comparison. Details are shown for a) neural pathways, b) chemoreceptors and c) immune pathways.

a. Neural pathways

Accession number	Gene name	Neural pathway
LH12970	octopamine receptor beta-1R-like	OA
LH12975	octopamine receptor beta-3R-like	OA
LH12976	octopamine receptor beta-3R-like	OA
LH12978	octopamine receptor beta-3R-like	OA
LH15962	octopamine receptor 1	OA
LH20188	octopamine receptor beta-2R-like	OA
LH17274	dopamine D2-like receptor	DA
LH17278	dopamine D2-like receptor	DA
LH17279	dopamine D2-like receptor	DA
LH18257	dopamine receptor 1	DA
LH21516	dopamine N-acetyltransferase-like	DA
LH11105	5-hydroxytryptamine receptor 2A-like	SER
LH13146	5-hydroxytryptamine receptor 1	SER
LH13147	5-hydroxytryptamine receptor 1	SER
LH13259	5-hydroxytryptamine receptor 2C	SER
LH13265	5-hydroxytryptamine receptor 2C	SER
LH10390	tyramine beta-hydroxylase	TYR
LH20865	tyramine receptor 1	TYR
LH13102	sodium- and chloride-dependent GABA transporter 1-like	GABA
LH24213	sodium- and chloride-dependent GABA transporter 1	GABA
LH11173	sodium- and chloride-dependent GABA transporter 1-like	GABA
LH11543	glutamate receptor-interacting protein 2	glutamate
LH11544	glutamate receptor-interacting protein 2	glutamate
LH11545	glutamate receptor-interacting protein 2	glutamate
LH11817	glutamate receptor ionotropic, delta-2-like	glutamate
LH11960	glutamate receptor 1-like	glutamate
LH11961	glutamate receptor-like	glutamate
LH13089	metabotropic glutamate receptor 8-like	glutamate
LH14616	glutamate receptor ionotropic, kainate 2	glutamate
LH14618	glutamate receptor ionotropic, kainate 2	glutamate
LH15532	probable glutamate receptor	glutamate
LH15649	glutamate receptor ionotropic, kainate 2	glutamate
LH15650	glutamate receptor ionotropic, kainate 2	glutamate
LH15651	glutamate receptor ionotropic, kainate 2	glutamate

LH15717	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH15718	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH15719	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH19015	vesicular glutamate transporter 1	glutamate
LH20394	glutamate receptor ionotropic, kainate 2-like	glutamate
LH20866	glutamate receptor ionotropic, kainate 5	glutamate
LH22634	metabotropic glutamate receptor	glutamate
LH24038	glutamate receptor ionotropic, kainate 2	glutamate
LH24044	glutamate receptor ionotropic, kainate 2-like	glutamate
LH25312	glutamate receptor U1-like	glutamate
LH25313	glutamate receptor ionotropic, kainate 5-like	glutamate
LH25314	glutamate receptor ionotropic, delta-2-like	glutamate
LH25429	metabotropic glutamate receptor 8-like	glutamate
LH25903	glutamate receptor ionotropic, kainate 5	glutamate
LH25907	glutamate receptor ionotropic, kainate 5	glutamate
LH25937	glutamate receptor ionotropic, delta-2	glutamate
LH26073	glutamate receptor ionotropic, kainate 2	glutamate
LH10392	glutamate [NMDA] receptor subunit 1	glutamate

b. Chemoreceptors

Accession number	Gene name	Receptor type
LH11296	odorant receptor Or2-like	OR
LH12711	odorant receptor 4-like	OR
LH12712	odorant receptor 4-like	OR
LH12960	general odorant-binding protein 72-like	OR
LH12962	general odorant-binding protein lush	OR
LH13656	odorant receptor Or2-like	OR
LH15023	general odorant-binding protein 69a	OR
LH15184	odorant receptor Or1-like	OR
LH16356	odorant receptor 4-like	OR
LH16357	odorant receptor 4-like	OR
LH16358	odorant receptor 22c-like	OR
LH16359	odorant receptor 2a-like	OR
LH16360	odorant receptor 4/2a/22c-like	OR
LH16362	odorant receptor 4/22c-like	OR
LH16363	odorant receptor 13a-like	OR
LH16365	odorant receptor 13a-like	OR
LH16367	odorant receptor 13a-like	OR
LH17511	odorant receptor 43a-like	OR
LH18177	odorant receptor 67a-like	OR
LH18458	odorant receptor 13a-like	OR
LH18459	putative odorant receptor 85d	OR
LH18577	putative odorant-binding protein A10	OR

LH18671	odorant receptor 13a-like	OR
LH18714	odorant receptor 13a-like	OR
LH18722	odorant receptor 45b-like	OR
LH18761	general odorant-binding protein 71	OR
LH19031	putative odorant receptor 85d	OR
LH19170	odorant receptor 22c/67c/coreceptor-like	OR
LH20597	odorant receptor 67c-like	OR
LH21669	odorant receptor Or1-like	OR
LH21670	odorant receptor Or1-like	OR
LH22142	odorant receptor 46a, isoform B-like	OR
LH22492	odorant receptor Or2-like	OR
LH22493	odorant receptor 4/13a/22c-like	OR
LH22725	odorant receptor 13a-like	OR
LH23112	general odorant-binding protein 56d-like	OR
LH23506	odorant receptor 13a-like	OR
LH23509	odorant receptor 13a-like	OR
LH23511	odorant receptor 9a-like	OR
LH23858	odorant receptor 49a/83a-like	OR
LH25597	odorant receptor coreceptor-like	OR
LH25606	odorant receptor 22c-like	OR
LH25610	odorant receptor 85b-like	OR
LH25611	odorant receptor 4-like	OR
LH25633	odorant receptor 43a-like	OR
LH25636	odorant receptor 43a-like	OR
LH25648	odorant receptor 7a-like	OR
LH25713	odorant receptor 22c-like	OR
LH25733	odorant receptor 82a-like	OR
LH25955	odorant receptor Or1-like	OR
LH25976	odorant receptor 13a-like	OR
LH26038	odorant receptor 13a-like	OR
LH26046	odorant receptor 13a-like	OR
LH26048	odorant receptor 4-like	OR
LH26050	odorant receptor 4-like	OR
LH11242	putative gustatory receptor 28a	GR
LH12478	gustatory receptor for sugar taste 64f-like	GR
LH13821	putative gustatory receptor 23a, isoform B	GR
LH14675	gustatory receptor for sugar taste 64f-like	GR
LH14807	gustatory receptor for sugar taste 64f-like	GR
LH19341	gustatory receptor for sugar taste 43a	GR

c. Immune pathways

Accession number	Gene name	Immune pathway
LH15872	endoribonuclease Dicer	RNAi
LH26164	protein argonaute-2	RNAi
LH11634	protein argonaute-3	RNAi
LH15418	protein argonaute-2	RNAi
LH22601	evolutionarily conserved signaling intermediate in Toll pathway, mitochondrial	TOLL
LH19752	protein toll	TOLL
LH26252	protein toll-like	TOLL
LH26257	protein toll-like	TOLL
LH26213	protein toll	TOLL
LH24353	toll-interacting protein-like	TOLL
LH16665	serine protease persephone-like	TOLL
LH24134	protein spaetzle	TOLL
LH17362	serine/threonine-protein kinase pelle	TOLL
LH24529	NF-kappa-B inhibitor cactus-like	TOLL
LH26206	NF-kappa-B inhibitor cactus-like	TOLL
LH21592	defensin-2	TOLL
LH12637	sex-regulated protein janus-A-like	JAK_STAT
LH12652	sex-regulated protein janus-A-like	JAK_STAT
LH12896	janus kinase and microtubule-interacting protein 3	JAK_STAT
LH23786	tyrosine-protein kinase hopscotch	JAK_STAT
LH15767	stress-activated protein kinase JNK	JNK
LH18476	JNK1/MAPK8-associated membrane protein	JNK
LH22008	JNK-interacting protein 3	JNK
LH23953	JNK-interacting protein 1	JNK

Table I.S4. Output of the post-hoc pairwise comparisons after GLMs for neural pathways in queens, callow and mature workers. Data are shown for a) octopaminergic, b) dopaminergic, c) serotonergic, d) tyraminergetic, e) GABAergic, and f) glutamatergetic systems.

a. Octopaminergic system

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0 -0.08373    0.16490  -0.508    0.868
Queens - Callow\nworkers == 0         -0.25924    0.16490  -1.572    0.258
Queens - Mature\nworkers == 0         -0.17550    0.16490  -1.064    0.536
(Adjusted p values reported -- single-step method)

```

b. Dopaminergic system

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0 -0.07091    0.13760  -0.515 0.863851
Queens - Callow\nworkers == 0         -0.59918    0.13760  -4.355 < 1e-04 ***
Queens - Mature\nworkers == 0         -0.52827    0.13760  -3.839 0.000339 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

```

c. Serotonergic system

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0  0.1461    0.1584   0.923   0.626
Queens - Callow\nworkers == 0          -0.1745    0.1584  -1.102   0.513
Queens - Mature\nworkers == 0          -0.3207    0.1584  -2.025   0.106
(Adjusted p values reported -- single-step method)

```

d. Tyraminerbic system

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Mature\nworkers - Callow\nworkers == 0	-0.06705	0.14513	-0.462	0.889049
Queens - Callow\nworkers == 0	-0.65004	0.14513	-4.479	< 1e-04 ***
Queens - Mature\nworkers == 0	-0.58300	0.14513	-4.017	0.000192 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

e. GABAergic system

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Mature\nworkers - Callow\nworkers == 0	-0.4631	0.1744	-2.655	0.0217 *
Queens - Callow\nworkers == 0	-0.8815	0.1744	-5.053	<0.001 ***
Queens - Mature\nworkers == 0	-0.4184	0.1744	-2.399	0.0434 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

f. Glutamatergic system

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Mature\nworkers - Callow\nworkers == 0	0.02189	0.06917	0.316	0.946
Queens - Callow\nworkers == 0	-0.11360	0.06917	-1.642	0.228
Queens - Mature\nworkers == 0	-0.13548	0.06917	-1.959	0.123

(Adjusted p values reported -- single-step method)

Table I.S5. Output of the post-hoc pairwise comparisons after GLMs for chemoreceptors in queens, callow and mature workers. Data are shown for a) olfactory and b) gustatory receptors.

a. Olfactory receptors

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0  0.14860    0.05509   2.697   0.0192 *
Queens - Callow\nworkers == 0          -0.28133    0.05509  -5.107   <0.001 ***
Queens - Mature\nworkers == 0          -0.42993    0.05509  -7.804   <0.001 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

```

b. Gustatory receptors

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0  0.0003455  0.0999543   0.003     1
Queens - Callow\nworkers == 0          -0.3603193  0.0999543  -3.605   <0.001 ***
Queens - Mature\nworkers == 0          -0.3606648  0.0999543  -3.608   <0.001 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

```

Table I.S6. Output of the post-hoc pairwise comparisons after GLMs for immune pathways in queens, callow and mature workers. Data are shown for a) pooled immune genes, and genes associated with the b) TOLL, c) JAK/STAT and d) RNAi pathways.

a. Pooled immune genes

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0  0.091213    0.105593   0.864    0.663
Queens - Callow\nworkers == 0          0.096384    0.105593   0.913    0.632
Queens - Mature\nworkers == 0          0.005171    0.105593   0.049    0.999
(Adjusted p values reported -- single-step method)

```

b. TOLL pathway

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0 -0.12204     0.15700  -0.777    0.717
Queens - Callow\nworkers == 0          -0.20037     0.15700  -1.276    0.409
Queens - Mature\nworkers == 0          -0.07833     0.15700  -0.499    0.872
(Adjusted p values reported -- single-step method)

```

c. JAK/STAT pathway

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
Mature\nworkers - Callow\nworkers == 0  0.15091     0.11624   1.298    0.396
Queens - Callow\nworkers == 0          -0.05644     0.11624  -0.486    0.878
Queens - Mature\nworkers == 0          -0.20735     0.11624  -1.784    0.175
(Adjusted p values reported -- single-step method)

```


d. RNAi pathway

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_QWC)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
Mature\nworkers - Callow\nworkers == 0	0.5866	0.1079	5.438	<1e-04	***
Queens - Callow\nworkers == 0	0.7014	0.1079	6.502	<1e-04	***
Queens - Mature\nworkers == 0	0.1148	0.1079	1.064	0.537	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

Table I.S7. Best *BLAST* results for differentially expressed transcripts associated with foraging propensity from *BLASTp* on the Argentine ant gene set, *BLASTp* on the Swissport database and *BLASTx* on the *nr/nt* database. Transcripts are presented in the same order as in Figure I.11. Cluster 1 is characterised by transcripts upregulated in insiders, and cluster 2 by transcripts upregulated in scouts. Octothorps in the cluster column indicate a significant difference between insiders and foragers.

Cluster	qseqid	sseqid	pidet	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore	Gene name
1	TRINITY_GG_10383_c3_g1_i6	LH25091	91.51	801	5	3	181	918	60	860	0	1488	unconventional myosin-le-like
1	TRINITY_GG_2906_c2_g1_i1	LH21851	85.67	293	1	1	121	413	13	264	0	511	protein henna
1	TRINITY_GG_743_c2_g2_i1	No hit											No hit
1	TRINITY_GG_7195_c0_g2_i1	No hit											No hit
1	TRINITY_GG_10709_c2_g1_i2	LH15810	100	203	0	0	1	203	1	203	3.00E-147	411	DNA fragmentation factor subunit alpha-like
1 #	TRINITY_GG_162_c15_g1_i2	LH23345	99.8	497	1	0	1	497	1	497	0	1022	cytochrome P450 4c3-like
1	TRINITY_GG_7074_c0_g1_i2	XM_012361476.1	99.702	672	2	0	7	678	828	157	0	1232	PREDICTED: uncharacterized LOC105668857
1	TRINITY_GG_162_c10_g1_i1	No hit											No hit
1	TRINITY_GG_7074_c0_g1_i1	XM_012361476.1	99.758	828	2	0	7	834	828	1	0	1520	PREDICTED: uncharacterized LOC105668857
1	TRINITY_GG_2152_c0_g1_i2	LH22079	99.77	443	1	0	55	497	1	443	0	888	putative sodium-dependent multivitamin transporter
1	TRINITY_GG_2906_c2_g1_i2	LH21851	85.67	293	1	1	102	394	13	264	0	510	protein henna
1	TRINITY_GG_1404_c0_g3_i47	LH11378	100	263	0	0	1	263	69	331	0	549	peroxisomal hydratase-dehydrogenase-epimerase-like
1 #	TRINITY_GG_4758_c0_g1_i3	LH21054	98.42	507	0	1	10	508	1	507	0	1023	probable cytochrome P450 305a1
1 #	TRINITY_GG_1748_c0_g1_i1	XM_012366918.1	99.574	4456	2	4	1	4451	327	4770	0	8107	PREDICTED: uncharacterized LOC105672171
1 #	TRINITY_GG_7695_c2_g1_i1	LH21708	100	406	0	0	1	406	1	406	0	850	uncharacterized LOC105675913
1	TRINITY_GG_10078_c22_g1_i2	XM_012369085.1	99.253	1740	13	0	2066	3805	1740	1	0	3144	PREDICTED: cytochrome P450 6k1-like
1	TRINITY_GG_10078_c14_g1_i1	LH19123	100	516	0	0	1	516	1	516	0	1061	cytochrome P450 6k1-like

1 #	TRINITY_GG_8781_c0_g1_i1	LH25572	99.65	573	0	1	10	582	1	571	0	1181	zinc finger protein 358-like
1	TRINITY_GG_10450_c1_g1_i1	LH25149	100	503	0	0	1	503	512	1014	0	1018	solute carrier family 2, facilitated glucose transporter member 8
1 #	TRINITY_GG_5362_c4_g1_i1	LH22946	100	676	0	0	16	691	1	676	0	1418	NADPH--cytochrome P450 reductase
1	TRINITY_GG_3543_c13_g1_i2	LH19148	100	570	0	0	1	570	1	570	0	1191	venom metalloproteinase 3-like
1	TRINITY_GG_8049_c4_g1_i1	LH11300	100	489	0	0	1	489	1	489	0	986	facilitated trehalose transporter Tret1
1	TRINITY_GG_10974_c0_g1_i2	LH11093	99.39	493	3	0	52	544	3	495	0	1014	4-coumarate--CoA ligase 1-like
1	TRINITY_GG_396_c8_g1_i1	LH17833	92.15	344	10	1	54	380	21	364	0	649	probable cytochrome P450 6a14
1	TRINITY_GG_1061_c1_g1_i1	LH13302	72.27	119	32	1	5	122	150	268	4.00E-57	186	uncharacterized methyltransferase C1347.09-like
1	TRINITY_GG_1198_c1_g1_i1	No hit											No hit
1	TRINITY_GG_3671_c0_g2_i1	LH17706	100	304	0	0	1	304	125	428	0	633	uncharacterized LOC105672080
1	TRINITY_GG_9098_c1_g1_i1	LH12329	100	291	0	0	1	291	49	339	0	613	uncharacterized LOC105680018
1 #	TRINITY_GG_6697_c12_g1_i1	LH15195	98.09	209	4	0	1	209	740	948	1.00E-138	421	dihydropyrimidine dehydrogenase [NADP(+)]
1 #	TRINITY_GG_5737_c0_g1_i1	LH23210	100	188	0	0	1	188	328	515	7.00E-137	395	uncharacterized LOC105677350
1	TRINITY_GG_3119_c3_g1_i1	LH23078	97.28	441	4	1	112	544	150	590	0	887	glucose dehydrogenase [FAD, quinone]-like
1 #	TRINITY_GG_9737_c1_g1_i1	XM_012368851.1	99.861	722	1	0	322	1043	722	1	0	1328	PREDICTED: pheromone-binding protein Gp-9-like
1 #	TRINITY_GG_6032_c0_g1_i1	LH13999	99.3	143	1	0	11	153	146	288	2.00E-95	280	troponin C, isoform 1-like
1	TRINITY_GG_4729_c6_g1_i4	XR_001101261.1	100	618	0	0	37	654	1026	409	0	1142	PREDICTED: uncharacterized LOC105675344
1 #	TRINITY_GG_2780_c10_g1_i1	LH24002	93.28	476	32	0	1	476	551	1026	0	931	uncharacterized LOC105678090
1 #	TRINITY_GG_7973_c2_g1_i1	LH14969	100	295	0	0	1	295	1	295	0	622	inositol oxygenase
1 #	TRINITY_GG_2780_c10_g1_i3	LH24002	94.35	566	32	0	1	566	551	1116	0	1110	uncharacterized LOC105678090
1 #	TRINITY_GG_2780_c10_g1_i2	LH24002	99.82	545	1	0	1	545	1	545	0	1130	uncharacterized LOC105678090
1	TRINITY_GG_799_c0_g2_i1	No hit											No hit
1 #	TRINITY_GG_7195_c1_g1_i1	XM_012359035.1	100	942	0	0	754	1695	942	1	0	1740	PREDICTED: uncharacterized LOC105667316
1 #	TRINITY_GG_743_c4_g1_i1	XM_012364392.1	100	1642	0	0	45	1686	1	1642	0	3033	PREDICTED: alpha-amylase 2-like
1	TRINITY_GG_4541_c0_g1_i1	No hit											No hit

1 #	TRINITY_GG_11068_c2_g1_i3	LH10850	69.46	239	68	1	64	302	494	727	7.00E-113	353	cytochrome P450 4C1-like
1 #	TRINITY_GG_799_c1_g1_i1	XM_012373549.1	99.787	470	1	0	1	470	6347	6816	0	863	PREDICTED: fatty acid synthase-like
1	TRINITY_GG_2780_c7_g1_i1	No hit											No hit
1 #	TRINITY_GG_743_c6_g1_i1	No hit											No hit
1	TRINITY_GG_6187_c0_g1_i2	XM_012367245.1	92.65	449	31	2	66	513	1222	775	0	645	PREDICTED: cytochrome P450 4C1-like
1 #	TRINITY_GG_8710_c0_g1_i1	LH22784	83.05	118	20	0	35	152	1	118	2.00E-67	207	cytochrome P450 4C1-like
1	TRINITY_GG_4550_c0_g1_i1	LH10850	86.87	99	13	0	4	102	14	112	2.00E-55	186	cytochrome P450 4C1-like
2 #	TRINITY_GG_209_c1_g2_i1	LH23464	100	111	0	0	1	111	1	111	7.00E-77	225	uncharacterized LOC105677611
2	TRINITY_GG_318_c0_g1_i1	XM_012366973.1	100	1093	0	0	4	1096	1093	1	0	2019	PREDICTED: uncharacterized LOC105672202
2	TRINITY_GG_7826_c10_g4_i2	XM_012377687.1	100	871	0	0	1	871	35	905	0	1609	PREDICTED: uncharacterized LOC105678395
2	TRINITY_GG_3680_c0_g1_i1	No hit											No hit
2	TRINITY_GG_9328_c3_g1_i1	LH18209	94.24	295	7	1	1	295	1	285	0	573	alpha-tocopherol transfer protein-like
2	TRINITY_GG_9276_c2_g1_i1	XM_012367566.1	100	971	0	0	788	1758	971	1	0	1794	PREDICTED: uncharacterized LOC105672550
2	TRINITY_GG_2083_c2_g1_i4	LH22482	100	204	0	0	317	520	353	556	5.00E-135	422	uncharacterized LOC105676540
2	TRINITY_GG_7505_c1_g1_i5	XM_012366141.1	99.733	748	2	0	541	1288	1000	253	0	1371	PREDICTED: uncharacterized LOC105671728
2	TRINITY_GG_7505_c1_g1_i1	LH17269	100	249	0	0	1	249	39	287	0	526	uncharacterized LOC105671728
2	TRINITY_GG_1651_c8_g1_i7	LH20799	78.06	278	1	3	1	218	1	278	5.00E-134	381	troponin I-like
2	TRINITY_GG_6031_c0_g1_i1	LH14024	99.75	403	1	0	22	424	29	431	0	842	probable chitinase 2
2 #	TRINITY_GG_2263_c9_g3_i3	LH20880	100	192	0	0	1	192	125	316	7.00E-141	399	mitochondrial uncoupling protein 2-like
2	TRINITY_GG_1390_c1_g1_i1	LH19940	100	742	0	0	1	742	1	742	0	1518	neurochondrin homolog
2	TRINITY_GG_1271_c2_g2_i2	LH16986	100	418	0	0	1	418	345	762	0	882	adenylyl cyclase-associated protein 1
2	TRINITY_GG_1613_c0_g1_i1	XM_012371734.1	99.827	579	1	0	224	802	2523	1945	0	1064	PREDICTED: gonadotropin-releasing hormone II receptor
2	TRINITY_GG_1373_c0_g1_i1	LH10244	100	433	0	0	8	440	1	433	0	901	lysosomal aspartic protease
2 #	TRINITY_GG_10653_c0_g2_i1	LH16561	100	398	0	0	1	398	1121	1518	0	827	uncharacterized LOC105670974
2	TRINITY_GG_1532_c0_g1_i1	LH24114	100	143	0	0	40	182	1	143	6.00E-103	295	uncharacterized LOC105678174
2	TRINITY_GG_2929_c2_g1_i1	LH21832	100	515	0	0	1	515	1	515	0	989	paramyosin, short form-like

2	TRINITY_GG_5191_c0_g1_i4	XM_012369051.1	99.907	2147	1	1	1240	3386	5004	2859	0	3952	PREDICTED: alpha-actinin, sarcomeric
2	TRINITY_GG_5191_c0_g1_i6	XM_012369051.1	99.907	2147	1	1	1240	3386	5098	2953	0	3952	PREDICTED: alpha-actinin, sarcomeric
2	TRINITY_GG_5191_c0_g1_i2	XM_012369051.1	99.907	2147	1	1	1240	3386	5098	2953	0	3952	PREDICTED: alpha-actinin, sarcomeric
2	TRINITY_GG_2929_c2_g1_i2	LH21832	100	363	0	0	516	878	800	1162	0	701	paramyosin, long form-like
2	TRINITY_GG_5191_c0_g1_i1	XM_012369051.1	99.907	2147	1	1	1240	3386	5098	2953	0	3952	PREDICTED: alpha-actinin, sarcomeric
2	TRINITY_GG_2721_c2_g1_i1	XM_012375034.1	89.761	586	49	2	8	583	962	1546	0	739	PREDICTED: retinol dehydrogenase 11-like
2	TRINITY_GG_2721_c2_g2_i1	XM_012375034.1	89.761	586	49	2	8	583	962	1546	0	739	PREDICTED: retinol dehydrogenase 11-like
2	TRINITY_GG_7367_c5_g1_i1	LH11849	99.24	393	3	0	9	401	90	482	0	826	venom carboxylesterase-6-like
2	TRINITY_GG_4945_c0_g1_i2	LH12691	88.65	326	0	1	1	326	1	289	0	576	dnaJ homolog subfamily B member 6-B-like
2 #	TRINITY_GG_4014_c7_g1_i1	LH13543	100	832	0	0	198	1029	1	832	0	1747	2-oxoglutarate dehydrogenase, mitochondrial #
2	TRINITY_GG_10754_c5_g2_i4	XM_012366663.1	100	3320	0	0	12	3331	3646	327	0	6131	PREDICTED: microtubule-associated protein RP/EB family member 1-like
2	TRINITY_GG_8284_c6_g2_i1	XM_012375924.1	97.093	172	5	0	1405	1576	172	1	4.54E-74	291	PREDICTED: putative uncharacterized protein FLJ37770
2	TRINITY_GG_1163_c0_g1_i4	XM_012365202.1	99.945	1822	1	0	571	2392	1822	1	0	3360	PREDICTED: aldehyde dehydrogenase, dimeric NADP-preferring-like
2	TRINITY_GG_5409_c1_g1_i1	LH22881	99.83	583	1	0	1	583	405	987	0	1164	zinc finger CCCH domain-containing protein 13
2	TRINITY_GG_3851_c1_g1_i1	LH19757	99	3406	3	2	1	3384	1	3397	0	6857	apolipoporphins
2	TRINITY_GG_9253_c0_g1_i1	XM_012367531.1	99.641	835	0	1	34	868	1	832	0	1522	PREDICTED: venom allergen 3-like
2 #	TRINITY_GG_10078_c4_g1_i1	LH19123	76.81	138	32	0	1	138	286	423	3.00E-73	229	cytochrome P450 6k1-like
2 #	TRINITY_GG_2604_c1_g1_i1	LH15597	100	465	0	0	12	476	1	465	0	950	sialin-like
2	TRINITY_GG_6221_c4_g2_i1	LH13028	91.18	363	31	1	33	395	1	362	0	683	esterase B1-like
2	TRINITY_GG_10386_c0_g1_i1	XM_012378888.1	99.56	1817	4	4	2	1816	391	2205	0	3308	PREDICTED: protein msta, isoform B-like
2	TRINITY_GG_10175_c3_g1_i2	LH23471	57.19	299	93	2	4	302	3	266	1.00E-115	337	alpha-tocopherol transfer protein-like
2 #	TRINITY_GG_9832_c6_g1_i1	LH23517	99.2	376	3	0	1	376	1	376	0	780	actin, muscle
2	TRINITY_GG_8251_c3_g1_i1	XM_012380129.1	99.632	1629	2	1	13	1637	5046	3418	0	2972	PREDICTED: uncharacterized LOC105679844
2	TRINITY_GG_10201_c2_g1_i1	XM_012363981.1	99.585	241	1	0	109	349	2160	1920	4.26E-119	440	PREDICTED: circadian clock-controlled protein
2	TRINITY_GG_2356_c7_g1_i1	XM_012362711.1	99.708	342	1	0	45	386	2668	2327	3.05E-175	627	PREDICTED: putative fatty acyl-CoA reductase CG5065
2	TRINITY_GG_9808_c9_g1_i1	XM_012378617.1	93.878	49	3	0	245	293	156	204	4.98E-09	75	PREDICTED: uncharacterized LOC105678905

2	TRINITY_GG_9097_c4_g2_i1	No hit											No hit
2	TRINITY_GG_3827_c0_g1_i1	XM_012370129.1	100	479	0	0	1	479	1143	665	0	885	PREDICTED: uncharacterized LOC105674057
2 #	TRINITY_GG_4612_c0_g2_i4	LH21230	100	255	0	0	28	282	7	261	0	540	protein msta, isoform B-like
2	TRINITY_GG_10078_c16_g2_i1	XM_012369087.1	99.83	587	1	0	75	661	2062	1476	0	1079	PREDICTED: cytochrome P450 6k1-like
2 #	TRINITY_GG_4612_c0_g2_i1	LH21230	86.31	482	35	4	28	478	7	488	0	828	protein msta, isoform B-like
2	TRINITY_GG_10078_c33_g1_i1	XM_012369095.1	99.688	321	1	0	1	321	2665	2985	1.44E-163	588	PREDICTED: cytochrome P450 6k1-like
2	TRINITY_GG_10078_c13_g1_i1	LH19123	73.72	137	36	0	1	137	379	515	2.00E-73	230	cytochrome P450 6k1-like
2 #	TRINITY_GG_10175_c3_g1_i1	LH23471	57.19	299	93	2	4	302	3	266	1.00E-115	337	alpha-tocopherol transfer protein-like
2	TRINITY_GG_4670_c0_g1_i1	XM_012372597.1	100	329	0	0	1	329	25	353	1.10E-169	608	PREDICTED: chymotrypsin inhibitor-like
2 #	TRINITY_GG_8614_c0_g1_i1	XR_001101693.1	100	128	0	0	1	128	483	610	6.00E-58	237	PREDICTED: uncharacterized LOC105678313
2	TRINITY_GG_9832_c5_g2_i1	XR_001101588.1	100	329	0	0	825	1153	646	318	1.10E-169	608	PREDICTED: uncharacterized LOC105677673
2 #	TRINITY_GG_1988_c0_g1_i1	LH22251	99.46	558	3	0	1	558	127	684	0	1139	arylphorin subunit alpha-like
2 #	TRINITY_GG_4612_c0_g2_i3	LH21230	86.31	482	35	4	28	478	7	488	0	828	protein msta, isoform B-like
2	TRINITY_GG_4944_c1_g1_i1	LH12665	99.45	183	1	0	1	183	827	1009	9.00E-115	351	hexamerin-like
2	TRINITY_GG_5380_c2_g2_i1	XR_001101615.1	97.697	304	7	0	74	377	715	1018	4.11E-144	523	PREDICTED: uncharacterized LOC105677839
2	TRINITY_GG_8891_c0_g1_i1	XM_012364600.1	95.588	272	12	0	1	272	1577	1306	5.51E-118	436	PREDICTED: negative regulator of reactive oxygen species-like
2	TRINITY_GG_8891_c1_g2_i1	XM_012364600.1	97.153	281	8	0	1	281	1047	1327	1.17E-129	475	PREDICTED: negative regulator of reactive oxygen species-like
2	TRINITY_GG_4548_c6_g1_i2	LH12745	90.86	197	18	0	1	197	22	218	1.00E-131	372	cytochrome P450 3A19-like
2 #	TRINITY_GG_7222_c0_g1_i1	LH17495	100	254	0	0	1	254	1539	1792	2.00E-180	543	uncharacterized LOC105671873
2 #	TRINITY_GG_7222_c1_g1_i1	LH17495	100	150	0	0	1	150	1393	1542	3.00E-99	314	uncharacterized LOC105671873

Table I.S8. List of genes used in the candidate gene approach in the insiders, foragers and scouts comparison. Details are shown for a) neural pathways, b) chemoreceptors and c) immune pathways.

a. Neural pathways

Accession number	Gene name	Neural pathway
LH12970	octopamine receptor beta-1R-like	OA
LH12975	octopamine receptor beta-3R-like	OA
LH12976	octopamine receptor beta-3R-like	OA
LH20188	octopamine receptor beta-2R-like	OA
LH17274	dopamine D2-like receptor	DA
LH17278	dopamine D2-like receptor	DA
LH18257	dopamine receptor 1	DA
LH21516	dopamine N-acetyltransferase-like	DA
LH11105	5-hydroxytryptamine receptor 2A-like	SER
LH13146	5-hydroxytryptamine receptor 1	SER
LH13147	5-hydroxytryptamine receptor 1	SER
LH13265	5-hydroxytryptamine receptor 2C	SER
LH10390	tyramine beta-hydroxylase	TYR
LH20865	tyramine receptor 1	TYR
LH20883	tyramine receptor 1	TYR
LH13102	sodium- and chloride-dependent GABA transporter 1-like	GABA
LH24213	sodium- and chloride-dependent GABA transporter 1	GABA
LH11173	sodium- and chloride-dependent GABA transporter 1-like	GABA
LH11543	glutamate receptor-interacting protein 2	glutamate
LH11545	glutamate receptor-interacting protein 2	glutamate
LH11817	glutamate receptor ionotropic, delta-2-like	glutamate
LH11960	glutamate receptor 1-like	glutamate
LH13089	metabotropic glutamate receptor 8-like	glutamate
LH13090	metabotropic glutamate receptor 8-like	glutamate
LH14616	glutamate receptor ionotropic, kainate 2	glutamate
LH15532	probable glutamate receptor	glutamate
LH15649	glutamate receptor ionotropic, kainate 2	glutamate
LH15650	glutamate receptor ionotropic, kainate 2	glutamate
LH15654	glutamate receptor ionotropic, kainate 2	glutamate
LH15717	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH15718	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH15719	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH19015	vesicular glutamate transporter 1	glutamate
LH20394	glutamate receptor ionotropic, kainate 2-like	glutamate
LH22634	metabotropic glutamate receptor	glutamate
LH24044	glutamate receptor ionotropic, kainate 2-like	glutamate

LH25312	glutamate receptor U1-like	glutamate
LH25313	glutamate receptor ionotropic, kainate 5-like	glutamate
LH25314	glutamate receptor ionotropic, delta-2-like	glutamate
LH25429	metabotropic glutamate receptor 8-like	glutamate
LH25907	glutamate receptor ionotropic, kainate 5	glutamate
LH25937	glutamate receptor ionotropic, delta-2	glutamate
LH10392	glutamate [NMDA] receptor subunit 1	glutamate
LH10494	glutamate receptor ionotropic, kainate 2-like	glutamate

b. Chemoreceptors

Accession number	Gene name	Receptor type
LH10129	odorant receptor Or2/49b-like	OR
LH11296	odorant receptor Or2-like	OR
LH12711	odorant receptor 4-like	OR
LH12712	odorant receptor 4-like	OR
LH12960	general odorant-binding protein 72-like	OR
LH12962	general odorant-binding protein lush	OR
LH13656	odorant receptor Or2-like	OR
LH15023	general odorant-binding protein 69a	OR
LH15184	odorant receptor Or1-like	OR
LH16356	odorant receptor 4-like	OR
LH16357	odorant receptor 4-like	OR
LH16358	odorant receptor 22c/82a-like	OR
LH16359	odorant receptor 2a-like	OR
LH16362	odorant receptor 4/22c-like	OR
LH16363	odorant receptor 13a-like	OR
LH17511	odorant receptor 43a-like	OR
LH18177	odorant receptor 67a-like	OR
LH18291	putative odorant receptor 71a	OR
LH18458	odorant receptor 13a-like	OR
LH18459	putative odorant receptor 85d	OR
LH18671	odorant receptor 13a-like	OR
LH18714	odorant receptor 13a-like	OR
LH18722	odorant receptor 45b-like	OR
LH18727	odorant receptor 30a-like	OR
LH18761	general odorant-binding protein 71	OR
LH19031	putative odorant receptor 85d	OR
LH19170	odorant receptor 22c/67c/coreceptor-like	OR
LH20416	putative odorant receptor 85d	OR
LH20597	odorant receptor 67c-like	OR
LH20607	odorant receptor 63a-like	OR
LH21669	odorant receptor Or1-like	OR
LH22142	odorant receptor 46a, isoform B-like	OR

LH22143	odorant receptor 46a, isoform A-like	OR
LH22492	odorant receptor Or2-like	OR
LH22493	odorant receptor 4/13a/22c-like	OR
LH22725	odorant receptor 13a-like	OR
LH22780	odorant receptor 22c-like	OR
LH23112	general odorant-binding protein 56d-like	OR
LH23511	odorant receptor 9a-like	OR
LH23858	odorant receptor 83a-like	OR
LH24938	odorant receptor 49a-like	OR
LH25597	odorant receptor coreceptor-like	OR
LH25604	odorant receptor 13a-like	OR
LH25606	odorant receptor 22c-like	OR
LH25609	odorant receptor 4/45b-like	OR
LH25610	odorant receptor 85b-like	OR
LH25633	odorant receptor 43a-like	OR
LH25649	putative odorant receptor 85d	OR
LH25651	odorant receptor 13a-like	OR
LH25713	odorant receptor 22c-like	OR
LH25714	odorant receptor 22c-like	OR
LH25730	odorant receptor 46a, isoform A-like	OR
LH25733	odorant receptor 82a-like	OR
LH25885	odorant receptor 67a-like	OR
LH25976	odorant receptor 13a-like	OR
LH26038	odorant receptor 13a-like	OR
LH26050	odorant receptor 4-like	OR
LH10133	gustatory and pheromone receptor 32a-like	GR
LH11242	putative gustatory receptor 28a	GR
LH12478	gustatory receptor for sugar taste 64f-like	GR
LH12479	gustatory receptor for sugar taste 64f-like	GR
LH12480	gustatory receptor for sugar taste 64f-like	GR
LH13821	putative gustatory receptor 23a, isoform B	GR
LH14675	gustatory receptor for sugar taste 64f-like	GR
LH14676	gustatory receptor for sugar taste 64f-like	GR
LH14802	gustatory receptor for sugar taste 64f-like	GR
LH14807	gustatory receptor for sugar taste 64f-like	GR
LH19341	gustatory receptor for sugar taste 43a	GR
LH24990	gustatory receptor for sugar taste 64f-like	GR

c. Immune pathways

Accession number	Gene name	Immune pathway
LH15872	endoribonuclease Dicer	RNAi
LH26164	protein argonaute-2	RNAi
LH11634	protein argonaute-3	RNAi
LH15418	protein argonaute-2	RNAi
LH22601	evolutionarily conserved signaling intermediate in Toll pathway, mitochondrial	TOLL
LH19752	protein toll	TOLL
LH26252	protein toll-like	TOLL
LH26257	protein toll-like	TOLL
LH26213	protein toll	TOLL
LH24353	toll-interacting protein-like	TOLL
LH16665	serine protease persephone-like	TOLL
LH24134	protein spaetzle	TOLL
LH17362	serine/threonine-protein kinase pelle	TOLL
LH24529	NF-kappa-B inhibitor cactus-like	TOLL
LH26206	NF-kappa-B inhibitor cactus-like	TOLL
LH21592	defensin-2	TOLL
LH12652	sex-regulated protein janus-A-like	JAK_STAT
LH23786	tyrosine-protein kinase hopscotch	JAK_STAT
LH15767	stress-activated protein kinase JNK	JNK
LH18476	JNK1/MAPK8-associated membrane protein	JNK
LH22008	JNK-interacting protein 3	JNK
LH23953	JNK-interacting protein 1	JNK

Table I.S9. Output of the post-hoc pairwise comparisons after GLMs for neural pathways in insiders, foragers, and scouts. Data are shown for a) octopaminergic, b) dopaminergic, c) serotonergic, d) tyraminergetic, e) GABAergic and f) glutamatergetic systems.

a. Octopaminergic system

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	0.04534	0.10877	0.417	0.9087
Scouts - Insiders == 0	0.28093	0.10877	2.583	0.0264 *
Scouts - Foragers == 0	0.23558	0.10877	2.166	0.0772 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

b. Dopaminergic system

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	0.3146	0.1580	1.990	0.1146
Scouts - Insiders == 0	0.5066	0.1580	3.205	0.0038 **
Scouts - Foragers == 0	0.1920	0.1580	1.215	0.4443

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

c. Serotoninergetic system

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	-0.04065	0.07253	-0.560	0.841
Scouts - Insiders == 0	0.40246	0.07253	5.549	<1e-04 ***
Scouts - Foragers == 0	0.44311	0.07253	6.109	<1e-04 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

d. Tyraminergetic system

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	0.06890	0.06663	1.034	0.5553
Scouts - Insiders == 0	-0.07479	0.06663	-1.122	0.5002
Scouts - Foragers == 0	-0.14370	0.06663	-2.157	0.0789 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

e. GABAergic system

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	0.27127	0.05472	4.957	<1e-05 ***
Scouts - Insiders == 0	0.30068	0.05472	5.495	<1e-05 ***
Scouts - Foragers == 0	0.02941	0.05472	0.538	0.853

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

f. Glutamatergic system

Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	0.11259	0.05658	1.99	0.115
Scouts - Insiders == 0	0.22066	0.05658	3.90	<0.001 ***
Scouts - Foragers == 0	0.10807	0.05658	1.91	0.136

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Table I.S10. Output of the post-hoc pairwise comparisons after GLMs for chemoreceptors in insiders, foragers, and scouts. Data are shown for a) olfactory and b) gustatory receptors.

a. Olfactory receptors

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Foragers - Insiders == 0  0.44739    0.02948  15.175 < 1e-04 ***
Scouts - Insiders == 0   0.53740    0.02948  18.228 < 1e-04 ***
Scouts - Foragers == 0   0.09001    0.02948   3.053 0.00634 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

```

b. Gustatory receptors

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
Foragers - Insiders == 0  0.12358    0.09037   1.368  0.358
Scouts - Insiders == 0   0.08764    0.09037   0.970  0.596
Scouts - Foragers == 0  -0.03594    0.09037  -0.398  0.917
(Adjusted p values reported -- single-step method)

```

Table LS11. Output of the post-hoc pairwise comparisons after GLMs for immune pathways in insiders, foragers, and scouts. Data are shown for a) pooled immune genes, and genes associated with the b) TOLL, c) JAK/STAT and d) RNAi pathways.

a. Pooled immune genes

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
Foragers - Insiders == 0  0.05654    0.04188   1.350    0.368
Scouts - Insiders == 0   0.01918    0.04188   0.458    0.891
Scouts - Foragers == 0  -0.03736    0.04188  -0.892    0.645
(Adjusted p values reported -- single-step method)

```

b. TOLL pathway

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
Foragers - Insiders == 0  0.05305    0.06561   0.809    0.698
Scouts - Insiders == 0  -0.02752    0.06561  -0.420    0.908
Scouts - Foragers == 0  -0.08057    0.06561  -1.228    0.437
(Adjusted p values reported -- single-step method)

```

c. JAK/STAT pathway

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
Foragers - Insiders == 0  -0.4901    0.3331  -1.471    0.305
Scouts - Insiders == 0   -0.3597    0.3331  -1.080    0.527
Scouts - Foragers == 0    0.1304    0.3331   0.391    0.919
(Adjusted p values reported -- single-step method)

```

d. RNAi pathway

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = value ~ Group.2, data = mean_cand_WWW)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)
Foragers - Insiders == 0	0.02777	0.05064	0.548	0.847
Scouts - Insiders == 0	0.09051	0.05064	1.787	0.174
Scouts - Foragers == 0	0.06274	0.05064	1.239	0.430

(Adjusted p values reported -- single-step method)

Figure I.S1. Detail of expression pattern of transcripts assigned to the a) worker-specific and b) queen-specific clusters. Individuals are indicated on the X-axis. Log-centred TMM-normalised TPMs are shown on the Y-axis. The blue line indicates the mean expression level for the cluster and the grey lines indicates individual values.

Figure I.S2. Detail of expression pattern of transcripts assigned to the a) callow worker-specific and b) mature worker-specific clusters. Individuals are indicated on the X-axis. Log-centred TMM-normalised TPMs are shown on the Y-axis. The blue line indicates the mean expression level for the cluster and the grey lines indicates individual values.

Supplementary material for Chapter II

Table II.S1. R GLM output: effect of experimental treatments on ant survival

Table II.S2a. R GLM output: effect of experimental treatments on OA head titres

Table II.S2b. R GLM output: effect of experimental treatments on 5-HT head titres

Table II.S3a. R GLM output: effect of experimental treatments on absolute colony foraging (experiment 1)

Table II.S3b. R GLM output: effect of experimental treatments on the proportion of the colony foraging (experiment 1)

Table II.S4a. R survival regression model output: effect of experimental treatments on the time to emerge from the shelter (experiment 2)

Table II.S4b. R output of post-hoc comparisons after survival regression model: effect of experimental treatments on the time to emerge from the shelter (experiment 2)

Table II.S5. R GLM output: effect of experimental treatments on the proportion of time spent outside the shelter (experiment 2)

Table II.S6. R GLM output: effect of experimental treatments on the mean distance from the shelter (experiment 2)

Table II.S7. R GLM output: effect of experimental treatments on the mean walking speed (experiment 2)

Table II.S8. R cumulative link model output: effect of experimental treatments on the aggression

Table II.S9a. R cumulative link model output: effect of experimental treatments on the damage in Argentine ants (experiment 3)

Table II.S9b. R cumulative link model output: effect of experimental treatments on the damage in *Prolasius advenus* (experiment 3)

Table II.S10a. R output of post-hoc comparisons after GLM: link between foraging propensity and OA head titres (experiment 4)

Table II.S10b. R output of post-hoc comparisons after GLM: link between foraging propensity and 5-HT head titres (experiment 4)

Figure II.S1. Effect of experimental treatments differentially on ant survival

Figure II.S2. Effect of experimental treatments significantly on OA

Figure II.S2. Effect of experimental treatments significantly on 5-HT levels

Figure II.S2a. Snapshot of a set of boldness assays (experiment 2)

Figure II.S2b. Tracked trajectories used in downstream analysis (experiment 2)

Figure II.S3. Snapshot of a set of aggression assays (experiment 3)

Table II.S1. R GLM output: effect of experimental treatments on ant survival

```
glm(formula = ants ~ treatment, data = survival)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-62.500  -13.125   -2.562    8.969   121.750

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    319.875     11.875   26.937 < 2e-16 ***
treatmentEPN     -9.875     16.794   -0.588    0.560
treatmentOA_2   -22.625     16.794   -1.347    0.187
treatmentOA_4   -80.375     16.794   -4.786 3.06e-05 ***
treatmentOA_8  -122.750     16.794   -7.309 1.53e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 1128.093)

Null deviance: 127750  on 39  degrees of freedom
Residual deviance:  39483  on 35  degrees of freedom
AIC: 401.31

Number of Fisher Scoring iterations: 2
```

Table II.S2a. R GLM output: effect of experimental treatments on OA head titres

```
glm(formula = log(amount_OA) ~ Treatment, data = exp_samples_OA_doses)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.4794  -0.4916  -0.1513   0.3482   3.4855

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.6753     0.1759  15.207  <2e-16 ***
TreatmentEPN   -0.5677     0.2488   -2.282   0.0244 *
TreatmentOA_2    3.3137     0.2488  13.319  <2e-16 ***
TreatmentOA_4    3.7643     0.2544  14.798  <2e-16 ***
TreatmentOA_8    4.1163     0.2515  16.368  <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.7427884)

Null deviance: 549.579  on 116  degrees of freedom
Residual deviance: 83.192  on 112  degrees of freedom
AIC: 304.13

Number of Fisher Scoring iterations: 2
```

Table II.S2b. R GLM output: effect of experimental treatments on 5-HT head titres

```
glm(formula = log(amount_SER) ~ Treatment, data = exp_samples_SER_doses)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.08354  -0.08832   0.15587   0.29869   1.47807

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    3.69339     0.13313  27.743  <2e-16 ***
TreatmentEPN   -0.01580     0.18827   -0.084   0.933
TreatmentOA_2    0.04790     0.18827    0.254   0.800
TreatmentOA_4    0.08805     0.19488    0.452   0.652
TreatmentOA_8    0.19890     0.19031    1.045   0.298
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.4253548)

Null deviance: 47.902  on 115  degrees of freedom
Residual deviance: 47.214  on 111  degrees of freedom
AIC: 236.92

Number of Fisher Scoring iterations: 2
```

Table II.S3a. R GLM output: effect of experimental treatments on absolute colony foraging (experiment 1)

```
glm(formula = early_explo ~ treatment * phase, data = both_phases)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-24.550   -7.975   -1.525    6.475   49.900

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)         34.400      1.879  18.304 < 2e-16 ***
treatmentEPN        -7.425      2.658  -2.794  0.00547 **
treatmentOA_2       -5.000      2.658  -1.881  0.06069 .
treatmentOA_4       -3.350      2.658  -1.260  0.20827
treatmentOA_8       -0.100      2.658  -0.038  0.97001
phasetreatment      -3.850      2.658  -1.449  0.14827
treatmentEPN:phasetreatment -9.600      3.759  -2.554  0.01103 *
treatmentOA_2:phasetreatment  3.275      3.759   0.871  0.38413
treatmentOA_4:phasetreatment  1.475      3.759   0.392  0.69497
treatmentOA_8:phasetreatment -0.350      3.759  -0.093  0.92586
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 141.2833)

Null deviance: 67439  on 399  degrees of freedom
Residual deviance: 55100  on 390  degrees of freedom
AIC: 3127.3
Number of Fisher Scoring iterations: 2
```

Table II.S3b. R GLM output: effect of experimental treatments on the proportion of the colony foraging (experiment 1)

```
glm(formula = as.numeric(prop_for) ~ treatment * phase, data = both_phases)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.120980	-0.023312	-0.004786	0.019000	0.248319

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	0.086000	0.006741	12.758	< 2e-16	***
treatmentEPN	-0.018562	0.009533	-1.947	0.0522	.
treatmentOA_2	-0.012500	0.009533	-1.311	0.1906	
treatmentOA_4	-0.008375	0.009533	-0.879	0.3802	
treatmentOA_8	-0.000250	0.009533	-0.026	0.9791	
phasetreatment	0.009531	0.009533	1.000	0.3180	
treatmentEPN:phasetreatment	-0.033422	0.013482	-2.479	0.0136	*
treatmentOA_2:phasetreatment	0.015168	0.013482	1.125	0.2613	
treatmentOA_4:phasetreatment	0.035999	0.013482	2.670	0.0079	**
treatmentOA_8:phasetreatment	0.058409	0.013482	4.332	1.88e-05	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.001817674)

Null deviance: 1.04401 on 399 degrees of freedom

Residual deviance: 0.70889 on 390 degrees of freedom

AIC: -1377.1

Number of Fisher Scoring iterations: 2

Table II.S4a. R survival regression model output: effect of experimental treatments on the time to emerge from the shelter (experiment 2)

```
survreg(formula = Surv(Emergence, Em_Status) ~ Treatment, data =
shelter_data)

Coefficients:
(Intercept) TreatmentEPN TreatmentOA2 TreatmentOA4 TreatmentOA8
  2.9546529    0.1788156   -0.1423688    0.6663520    0.7825752

Scale= 1.980175

Loglik(model)= -2525.1   Loglik(intercept only)= -2535
    Chisq= 19.79 on 4 degrees of freedom, p= 0.00055
n= 637
```

Table II.S4b. R output of post-hoc comparisons after survival regression model: effect of experimental treatments on the time to emerge from the shelter (experiment 2)

```
Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: survreg(formula = Surv(Emergence, Em_Status) ~ Treatment, data =
shelter_data)

Linear Hypotheses:
              Estimate Std. Error z value Pr(>|z|)
EPN - CTR == 0    0.1788    0.2567   0.696  0.95732
OA2 - CTR == 0   -0.1424    0.2556  -0.557  0.98111
OA4 - CTR == 0    0.6664    0.2573   2.590  0.07221 .
OA8 - CTR == 0    0.7826    0.2585   3.028  0.02071 *
OA2 - EPN == 0   -0.3212    0.2578  -1.246  0.72436
OA4 - EPN == 0    0.4875    0.2595   1.879  0.32864
OA8 - EPN == 0    0.6038    0.2606   2.317  0.13947
OA4 - OA2 == 0    0.8087    0.2584   3.130  0.01496 *
OA8 - OA2 == 0    0.9249    0.2595   3.564  0.00335 **
OA8 - OA4 == 0    0.1162    0.2611   0.445  0.99189
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```


Table II.S5. R GLM output: effect of experimental treatments on the proportion of time spent outside the shelter (experiment 2)

```
glm(formula = Proportion_out ~ Treatment, data = shelter_data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.6973  -0.1495   0.1057   0.2235   0.3814

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   0.697279   0.026690  26.125  <2e-16 ***
TreatmentEPN -0.006832   0.037671  -0.181   0.8561
TreatmentOA2 -0.027198   0.037745  -0.721   0.4714
TreatmentOA4 -0.063210   0.037745  -1.675   0.0945 .
TreatmentOA8 -0.078667   0.037671  -2.088   0.0372 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.09046773)

Null deviance: 57.786  on 636  degrees of freedom
Residual deviance: 57.176  on 632  degrees of freedom
AIC: 284.15

Number of Fisher Scoring iterations: 2
```

Table II.S6. *R* GLM output: effect of experimental treatments on the mean distance from the shelter (experiment 2)

```
glm(formula = Distance_shelter ~ Treatment, data = shelter_data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-24.8078  -0.6358   2.1843   4.2047  11.9285

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  24.8078     0.7067  35.105  <2e-16 ***
TreatmentEPN -2.5314     0.9974  -2.538   0.0114 *
TreatmentOA2 -0.2706     0.9994  -0.271   0.7867
TreatmentOA4 -0.8775     0.9994  -0.878   0.3802
TreatmentOA8 -1.6189     0.9974  -1.623   0.1051
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 63.42149)

Null deviance: 40626  on 636  degrees of freedom
Residual deviance: 40082  on 632  degrees of freedom
AIC: 4458.1

Number of Fisher Scoring iterations: 2
```

Table II.S7. *R* GLM output: effect of experimental treatments on the mean walking speed (experiment 2)

```
glm(formula = Segment ~ Treatment, data = shelter_data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-20.989   -4.762   -0.010    4.803   34.456

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   36.5790     0.7053  51.860 < 2e-16 ***
TreatmentEPN  -5.2202     1.0060  -5.189 2.92e-07 ***
TreatmentOA2    0.4599     1.0017   0.459  0.6463
TreatmentOA4  -2.1083     1.0082  -2.091  0.0369 *
TreatmentOA8  -2.1092     1.0127  -2.083  0.0377 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 60.19729)

Null deviance: 37425  on 586  degrees of freedom
Residual deviance: 35035  on 582  degrees of freedom
(50 observations deleted due to missingness)
AIC: 4078.1

Number of Fisher Scoring iterations: 2
```

Table II.S8. *R* cumulative link model output: effect of experimental treatments on the aggression

```

formula: as.factor(aggression_full$agg_full) ~ treatment
data:    aggression_full

link threshold nobs logLik   AIC      niter max.grad cond.H
logit flexible  2665 -3775.76 7567.52 5(2)  4.58e-07 5.6e+02

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
treatmentEPN -0.41425    0.11369  -3.644 0.000269 ***
treatmentOA2  0.19217    0.11029   1.742 0.081434 .
treatmentOA4 -0.07482    0.10816  -0.692 0.489063
treatmentOA8  0.02636    0.11276   0.234 0.815159
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Threshold coefficients:
      Estimate Std. Error z value
0|1 -0.48518    0.08084  -6.001
1|2 -0.35639    0.08058  -4.423
2|3  0.36740    0.08082   4.546
3|4  1.37653    0.08581  16.041

```

Table II.S9a. R cumulative link model output: effect of experimental treatments on the damage in Argentine ants (experiment 3)

```

formula: as.factor(damage_data$aa_damage_rev) ~ treatment
data:    damage_data

link threshold nobs logLik AIC      niter max.grad cond.H
logit flexible  609  -622.10 1258.20 7(1)   3.24e-10 3.6e+02

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
treatmentEPN  -0.8889    0.2572  -3.456 0.000549 ***
treatmentOA2  -0.2518    0.2697  -0.934 0.350418
treatmentOA4  -0.4772    0.2619  -1.822 0.068444 .
treatmentOA8  -0.5514    0.2643  -2.087 0.036932 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Threshold coefficients:
      Estimate Std. Error z value
0|1  -1.5165    0.2012  -7.539
1|2  -1.1373    0.1966  -5.784
2|3  -0.8474    0.1940  -4.367

```

Table II.S9b. R cumulative link model output: effect of experimental treatments on the damage in *Prolasius advenus* (experiment 3)

```

formula: as.factor(damage_data$p_damage_rev) ~ treatment
data:    damage_data

link threshold nobs logLik AIC      niter max.grad cond.H
logit flexible  609  -824.80 1663.61 5(0)   2.32e-12 1.0e+02

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
treatmentEPN  -0.56108    0.23357  -2.402 0.01630 *
treatmentOA2   0.02559    0.23160   0.110 0.91203
treatmentOA4  -0.58032    0.22521  -2.577 0.00997 **
treatmentOA8  -0.63559    0.23527  -2.702 0.00690 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Threshold coefficients:
      Estimate Std. Error z value
0|1  -1.4120    0.1767  -7.989
1|2  -0.3255    0.1670  -1.949
2|3   0.4511    0.1674   2.694

```

Table II.S10a. R output of post-hoc comparisons after GLM: link between foraging propensity and OA head titres (experiment 4)

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = log(amount_OA) ~ Treatment, data =
exp_samples_OA_castes)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
insider - forager == 0 -0.40377    0.05758  -7.013  <1e-06 ***
scout - forager == 0  -0.39245    0.06284  -6.245  <1e-06 ***
scout - insider == 0   0.01132    0.05629   0.201    0.978
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

```

Table II.S10b. R output of post-hoc comparisons after GLM: link between foraging propensity and 5-HT head titres (experiment 4)

```

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glm(formula = log(amount_SER) ~ Treatment, data =
exp_samples_SER_castes)

Linear Hypotheses:

              Estimate Std. Error z value Pr(>|z|)
insider - forager == 0  0.34490    0.18922   1.823    0.162
scout - forager == 0   0.28185    0.20835   1.353    0.365
scout - insider == 0  -0.06305    0.18704  -0.337    0.939
(Adjusted p values reported -- single-step method)

```

Figure II.S1. Experimental treatments differentially affected ant survival within colonies. Octopamine (OA) oral administration appears toxic when delivered at 4 and 8 mg.mL⁻¹. Dashed line represent the number of ants per colony at the start of the experiment. Only the p-values from the GLM outputs are shown, all groups compared to control, with full outputs in Table II.S1.

Figure II.S2. Experimental treatments significantly modified octopamine (OA) head titres but not serotonin (5-HT) levels. Data are shown for a) OA and b) 5-HT. The mean value in each group is represented by red dots. Only the p-values from the GLM outputs are shown, all groups compared to control, with full outputs in Table II.S2.

Figure II.S2a. Snapshot of a set of boldness assays (experiment 2) showing the individual arenas, each containing a shelter in the center (in black). For reference, we circled an ant in red.

Figure II.S2b. Tracked trajectories from movie shown in Fig. S2a, used in downstream analysis (experiment 2). Each black dot represents manual tracking of the ants' position every second, and red segments connect two consecutive positions. Shelters are represented in black.

Figure II.S3. Snapshot of a set of eight aggression assays showing an Argentine ant and a NZ bush ant interacting in each arena. The set of assays were recorded as shown, and the behaviour scored from the movies. Red arrows show ants fighting (experiment 3).

Supplementary material for Chapter III

Method S1. HPLC analysis

Table III.S1. Description of the collection sites

Table III.S2. Output of the GLMM on foraging activity during the exploration phase

Table III.S3. Output of the GLMM on foraging activity during the exploitation phase

Table III.S4. Output of the survival analysis on the time to reach the top of the tree

Table III.S5. Output of the GLMM on foraging performance associated with resource discovery

Table III.S6. Post-hoc pairwise Fligner-Killeen tests on variance of foraging activity

Table III.S7. Output of the CLM on aggression

Figure III.S1. picture of the experimental set-up

Figure III.S2. HPLC quantification of octopamine (OA)

Figure III.S3. Foraging performance associated with resource discovery

Method S1. HPLC analysis.

Samples were prepared under a stereomicroscope on a glass plate chilled with dry ice. Heads were detached from the body with two fine forceps (Drummond #5, Switzerland) and the antennae were discarded. Extracts were prepared on the same day as the HPLC runs. For each sample, 5 ant heads were homogenised in an Eppendorf tube in 60 μL of ice-cold 0.2 M perchloric acid containing 10 $\text{pg} \cdot \mu\text{L}^{-1}$ of 2,3-Dihydroxybenzoic acid (DHBA) as an internal standard. The tubes were centrifuged for 15 minutes at 15,000 rpm at 0°C, after which 50 μL of the supernatant was transferred into a vial glass insert of an amber-tinted vial chilled on ice. We used a C18 column (Hypersil™ BDS, 100 x 4.6 mm, 5 μm particle size) with an ESA Coulochem® III detector (guard cell 5020 and analytical cell 5011). The mobile phase was composed of 15% v/v methanol, 15% v/v acetonitrile, 75 mM monobasic sodium phosphate, 5 mM sodium citrate and 1.5 mM sodium dodecyl sulfate. The final pH was adjusted to 5.6 with phosphoric acid. We set up the flow rate to 1.2 $\text{mL} \cdot \text{min}^{-1}$, the column temperature at 28°C, guard cell voltage at 920 mV and analytical cell at -200 mV and 850 mV for channel 1 and 2, respectively. Series of external standards with known concentrations of OA were run before and after each set of samples, and calibration was performed in R using raw peak areas. The sample injection volume was 40 μL , and we ran 16 samples per experimental treatment. Difference among treatments was tested with a GLM using log-transformed amounts of OA per worker head, using treatment as a fixed effect.

Table III.S1. Details on the collection sites and environmental characteristics in the four study regions. Collections occurred in summer (January 2015 in Argentina, March 2015 in Australia, September 2015 in California and February 2016 in New Zealand). Ants were mostly collected in human-modified environments under stones, logs, potting, or debris. As soon as brood or areas with a high density of workers were identified, colonies were shovelled together with soil material in plastic containers lined with Fluon[®] (BioQuip products, USA) to prevent ants from escaping. The ants were kept in these soil-free containers for less than a week before the start of the assays, with test tubes half-filled with water and plugged with cotton as nesting sites.

Region	Site	North	East
Argentina (AR)	Otamendi, Campana (OTA) <i>Wet grassland on the edge of a natural reserve</i>	-34.22243	-58.89613
	Camping Municipal, Rosario (ROS) <i>Lawn in riparian public space</i>	-32.91053	-60.67563
	Velatropa, Buenos Aires (UBA) <i>Sand mount in a woodland</i>	-34.54013	-58.44703
	Vicente López, Buenos Aires (VLO) <i>Garden in residential area</i>	-34.54408	-58.49317
	Albany Bulb, Albany (BLB) <i>Former coastal landfill</i>	37.889064	-122.323311
	UC Berkeley Campus, Berkeley (CAL) <i>Debris and litter in derelict corner</i>	37.872321	-122.247681
California (CA)	Ghost town, Oakland (OAK) <i>Public lawn in residential area</i>	37.825258	-122.269217
	West Ohio Avenue, Richmond (RCH) <i>Roadside shrub in residential area</i>	37.931301	-122.373211
	Clifton Hill, Yarra (CLI) <i>Garden in residential area</i>	-37.7942	144.99783
Australia (AU)	Monash University, Clayton (MON) <i>Woodchip mulch in natural reserve</i>	-37.909674	145.140635
	Cowes, Phillip Island (PHI) <i>Lawn in residential area</i>	-38.45901	145.23868
	St Andrews beach, Rye (STA) <i>Coastal shrub</i>	-38.41909	144.83226
	Roy's Hill reserve, Hastings (HAS) <i>Woodchip mulch in wineries</i>	-39.60086	176.75056
New Zealand (NZ)	Kena Kena, Paraparaumu (PAR) <i>Woodchip mulch, sport field</i>	-40.88185	174.99499
	Petone, Lower Hutt (PET) <i>Sand mount on waterfront</i>	-41.22393	174.8659
	Oakura, New Plymouth (TAR) <i>Garden in residential area</i>	-39.119488	173.939672

Table III.S2. R output of the generalised mixed model on exploration activity.

```

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [
glmerMod]
Family: poisson ( log )
Formula: early_explo ~ treatment * region + (1 | site/subcolony) + (1 | day)
Data: T_phase

      AIC      BIC    logLik deviance df.resid
 9634.8   9728.5  -4798.4   9596.8     1005

Scaled residuals:
    Min       1Q   Median       3Q      Max
-7.197 -1.319 -0.120   1.045   8.146

Random effects:
Groups                Name      Variance Std.Dev.
subcolony:site (Intercept) 0.126846 0.35615
site (Intercept) 0.083037 0.28816
day (Intercept) 0.008871 0.09419
Number of obs: 1024, groups: subcolony:site, 128; site, 16; day, 8

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)    2.682846   0.197168  13.607 < 2e-16 ***
treatmentlow_sugar 0.285919   0.183968   1.554  0.1201
treatmentOA      0.799798   0.182716   4.377 1.20e-05 ***
treatmentOA_low_sugar 1.282161   0.182146   7.039 1.93e-12 ***
regionAU        -0.596759   0.276704  -2.157  0.0310 *
regionCA         0.109731   0.274687   0.399  0.6895
regionNZ         0.094337   0.274595   0.344  0.7312
treatmentlow_sugar:regionAU -0.044265   0.263430  -0.168  0.8666
treatmentOA:regionAU  0.406582   0.260527   1.561  0.1186
treatmentOA_low_sugar:regionAU 0.147356   0.259937   0.567  0.5708
treatmentlow_sugar:regionCA -0.588781   0.261340  -2.253  0.0243 *
treatmentOA:regionCA  0.007672   0.258113   0.030  0.9763
treatmentOA_low_sugar:regionCA 0.064244   0.257357   0.250  0.8029
treatmentlow_sugar:regionNZ  0.081754   0.259412   0.315  0.7526
treatmentOA:regionNZ  -0.274865   0.258281  -1.064  0.2872
treatmentOA_low_sugar:regionNZ 0.042744   0.257254   0.166  0.8680
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
      (Intr) trtmn_ trtmOA trOA__ regnAU regnCA regnNZ tr_:AU tOA:AU tOA__:A
trtmntlw_sg -0.469
treatmentOA -0.472  0.506
trtmntOA_l_ -0.474  0.507  0.511
regionAU    -0.692  0.334  0.336  0.337
regionCA    -0.697  0.336  0.339  0.340  0.497
regionNZ    -0.697  0.337  0.339  0.340  0.497  0.501
trtmntl_:AU  0.327 -0.698 -0.353 -0.354 -0.481 -0.235 -0.235
trtmntOA:AU  0.331 -0.355 -0.701 -0.358 -0.486 -0.238 -0.238  0.511
trtmOA__:AU  0.332 -0.356 -0.358 -0.701 -0.487 -0.238 -0.238  0.512  0.518
trtmntl_:CA  0.330 -0.704 -0.356 -0.357 -0.235 -0.473 -0.237  0.491  0.250  0.250
trtmntOA:CA  0.334 -0.358 -0.708 -0.362 -0.238 -0.479 -0.240  0.250  0.496  0.254
trtmOA__:CA  0.335 -0.359 -0.362 -0.708 -0.239 -0.480 -0.241  0.251  0.254  0.496
trtmntl_:NZ  0.332 -0.709 -0.359 -0.360 -0.237 -0.239 -0.476  0.495  0.252  0.252
trtmntOA:NZ  0.334 -0.358 -0.707 -0.361 -0.238 -0.240 -0.478  0.250  0.496  0.253
trtmOA__:NZ  0.335 -0.359 -0.362 -0.708 -0.239 -0.241 -0.480  0.251  0.254  0.496
tr_:CA tOA:CA tOA__:C tr_:NZ tOA:NZ
trtmntlw_sg
treatmentOA
trtmntOA_l_
regionAU
regionCA
regionNZ
trtmntl_:AU
trtmntOA:AU
trtmOA__:AU
trtmntl_:CA
trtmntOA:CA  0.503
trtmOA__:CA  0.504  0.511
trtmntl_:NZ  0.499  0.254  0.255
trtmntOA:NZ  0.252  0.501  0.256  0.506
trtmOA__:NZ  0.253  0.256  0.501  0.508  0.510

```

Table III.S3. R output of the generalised mixed model on exploitation activity.

```

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [
glmerMod]
Family: poisson ( log )
Formula: early_explo ~ treatment * region + (1 | site/subcolony) + (1 | day)
Data: TR_phase

      AIC      BIC    logLik deviance df.resid
 8225.8   8310.6  -4093.9   8187.8      621

Scaled residuals:
    Min       1Q   Median       3Q      Max
-6.7876 -1.5256  0.0051  1.3334  8.6264

Random effects:
Groups                Name      Variance Std.Dev.
subcolony:site (Intercept) 0.12269   0.3503
site (Intercept) 0.06610   0.2571
day (Intercept) 0.01152   0.1073
Number of obs: 640, groups: subcolony:site, 128; site, 16; day, 5

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)      3.69991    0.18666  19.822 < 2e-16 ***
treatmentlow_sugar 0.67910    0.17798   3.815 0.000136 ***
treatmentOA        0.87270    0.17783   4.907 9.23e-07 ***
treatmentOA_low_sugar 1.11481    0.17764   6.276 3.48e-10 ***
regionAU          -0.98529    0.25781  -3.822 0.000133 ***
regionCA           0.03616    0.25524   0.142 0.887343
regionNZ           0.13023    0.25497   0.511 0.609521
treatmentlow_sugar:regionAU 0.34838    0.25510   1.366 0.172051
treatmentOA:regionAU 0.61398    0.25444   2.413 0.015819 *
treatmentOA_low_sugar:regionAU 0.73609    0.25408   2.897 0.003767 **
treatmentlow_sugar:regionCA -0.30922    0.25223  -1.226 0.220225
treatmentOA:regionCA -0.08650    0.25165  -0.344 0.731051
treatmentOA_low_sugar:regionCA 0.20330    0.25127   0.809 0.418467
treatmentlow_sugar:regionNZ -0.02274    0.25151  -0.090 0.927949
treatmentOA:regionNZ -0.04617    0.25129  -0.184 0.854214
treatmentOA_low_sugar:regionNZ 0.09065    0.25101   0.361 0.717998
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
      (Intr) trtmn_ trtmOA trOA__ regnAU regnCA regnNZ tr_:AU tOA:AU tOA__:A
trtmntlw_sg -0.482
treatmentOA -0.482 0.506
trtmntOA_l_ -0.483 0.506 0.507
regionAU    -0.676 0.349 0.349 0.350
regionCA    -0.683 0.352 0.353 0.353 0.495
regionNZ    -0.684 0.353 0.353 0.353 0.495 0.500
trtmntl_:AU 0.336 -0.698 -0.353 -0.353 -0.508 -0.246 -0.246
trtmntOA:AU 0.337 -0.353 -0.699 -0.354 -0.509 -0.247 -0.247 0.515
trtmOA__:AU 0.337 -0.354 -0.354 -0.699 -0.510 -0.247 -0.247 0.515 0.517
trtmntl_:CA 0.340 -0.706 -0.357 -0.357 -0.246 -0.498 -0.249 0.492 0.249 0.250
trtmntOA:CA 0.341 -0.357 -0.707 -0.358 -0.247 -0.500 -0.250 0.250 0.494 0.251
trtmOA__:CA 0.341 -0.358 -0.358 -0.707 -0.247 -0.500 -0.250 0.250 0.251 0.494
trtmntl_:NZ 0.341 -0.708 -0.358 -0.358 -0.247 -0.249 -0.498 0.494 0.250 0.251
trtmntOA:NZ 0.341 -0.358 -0.708 -0.359 -0.247 -0.250 -0.499 0.250 0.495 0.251
trtmOA__:NZ 0.342 -0.358 -0.359 -0.708 -0.247 -0.250 -0.499 0.250 0.251 0.495
tr_:CA tOA:CA tOA__:C tr_:NZ tOA:NZ
trtmntlw_sg
treatmentOA
trtmntOA_l_
regionAU
regionCA
regionNZ
trtmntl_:AU
trtmntOA:AU
trtmOA__:AU
trtmntl_:CA
trtmntOA:CA 0.506
trtmOA__:CA 0.506 0.508
trtmntl_:NZ 0.499 0.253 0.253
trtmntOA:NZ 0.253 0.500 0.254 0.506
trtmOA__:NZ 0.253 0.253 0.500 0.506 0.507

```

Table III.S4. R output of the Cox-proportional hazard model on the time for the first ant to reach and be counted at the top of the tree.

```

Call:
coxph(formula = Surv(time_top2, status) ~ treatment * region,
      data = T_phase)

n= 1024, number of events= 958

              coef exp(coef) se(coef)      z Pr(>|z|)
treatmentlow_sugar      0.405641  1.500265  0.182862  2.218 0.026535 *
treatmentOA              0.448798  1.566428  0.182266  2.462 0.013804 *
treatmentOA_low_sugar    0.877254  2.404288  0.182839  4.798 1.6e-06 ***
regionAU                 -0.410337  0.663427  0.192154 -2.135 0.032723 *
regionCA                 0.103067  1.108566  0.184945  0.557 0.577333
regionNZ                 0.003173  1.003178  0.184971  0.017 0.986314
treatmentlow_sugar:regionAU -0.344556  0.708535  0.267452 -1.288 0.197644
treatmentOA:regionAU      0.400238  1.492180  0.261623  1.530 0.126059
treatmentOA_low_sugar:regionAU 0.130943  1.139902  0.261039  0.502 0.615933
treatmentlow_sugar:regionCA -0.986012  0.373061  0.268176 -3.677 0.000236 ***
treatmentOA:regionCA      -0.093975  0.910305  0.257294 -0.365 0.714929
treatmentOA_low_sugar:regionCA -0.225754  0.797914  0.256471 -0.880 0.378734
treatmentlow_sugar:regionNZ -0.049124  0.952063  0.258384 -0.190 0.849216
treatmentOA:regionNZ      0.303873  1.355097  0.257158  1.182 0.237342
treatmentOA_low_sugar:regionNZ 0.322529  1.380614  0.256241  1.259 0.208141
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
treatmentlow_sugar      1.5003      0.6665      1.0484      2.1469
treatmentOA              1.5664      0.6384      1.0959      2.2390
treatmentOA_low_sugar    2.4043      0.4159      1.6802      3.4405
regionAU                 0.6634      1.5073      0.4552      0.9668
regionCA                 1.1086      0.9021      0.7715      1.5929
regionNZ                 1.0032      0.9968      0.6981      1.4415
treatmentlow_sugar:regionAU 0.7085      1.4114      0.4195      1.1968
treatmentOA:regionAU      1.4922      0.6702      0.8936      2.4918
treatmentOA_low_sugar:regionAU 1.1399      0.8773      0.6834      1.9014
treatmentlow_sugar:regionCA 0.3731      2.6805      0.2206      0.6310
treatmentOA:regionCA      0.9103      1.0985      0.5498      1.5073
treatmentOA_low_sugar:regionCA 0.7979      1.2533      0.4827      1.3191
treatmentlow_sugar:regionNZ 0.9521      1.0504      0.5738      1.5798
treatmentOA:regionNZ      1.3551      0.7380      0.8186      2.2432
treatmentOA_low_sugar:regionNZ 1.3806      0.7243      0.8355      2.2813

Concordance= 0.659 (se = 0.017 )
Rsquare= 0.163 (max possible= 1 )
Likelihood ratio test= 182.1 on 15 df, p=0
Wald test              = 176.6 on 15 df, p=0
Score (logrank) test = 189.7 on 15 df, p=0

```

Table III.S5. R output of the generalised linear mixed-effects models on foraging performance associated with resource discovery.

```

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [
glmerMod]
Family: poisson ( log )
Formula: sug_10 ~ treatment + region + (1 | site/subcolony) + (1 | day)
Data: TR_phase

      AIC      BIC    logLik deviance df.resid
5195.3   5239.9  -2587.6   5175.3     630

Scaled residuals:
    Min       1Q   Median       3Q      Max
-5.3875 -1.2332 -0.0755  0.9863  5.4676

Random effects:
Groups             Name      Variance Std.Dev.
subcolony:site (Intercept) 0.191623 0.43775
site (Intercept) 0.062336 0.24967
day (Intercept) 0.002891 0.05377
Number of obs: 640, groups: subcolony:site, 128; site, 16; day, 5

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)      1.3865     0.1701   8.153 3.55e-16 ***
treatmentlow_sugar 0.8526     0.1199   7.111 1.15e-12 ***
treatmentOA       1.2615     0.1190  10.603 < 2e-16 ***
treatmentOA_low_sugar 1.8249     0.1182  15.434 < 2e-16 ***
regionAU          -0.3183     0.2120  -1.501  0.1334
regionCA           0.1123     0.2112   0.531  0.5951
regionNZ           0.4768     0.2106   2.265  0.0235 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
      (Intr) trtmn_ trtmOA trOA__ regnAU regnCA
trtmntlw_sg -0.377
treatmentOA -0.381 0.539
trtmntOA_l_ -0.384 0.542 0.550
regionAU    -0.614 -0.005 -0.010 -0.009
regionCA    -0.621 0.001 -0.002 -0.001 0.499
regionNZ    -0.626 0.003 0.005 0.006 0.500 0.502

```

Table III.S6. Fligner-Killeen post-hoc pairwise comparisons for the control groups among regions in the exploration and the exploitation phases. Bonferroni-corrected significance threshold is lowered to $\alpha \approx 0.00833$, with a degree of freedom of 1 in all cases.

	Exploration	Exploitation
California vs Argentina	$\chi^2 = 2.3444$, df = 1, p = 0.1257	$\chi^2 = 2.4299$, df = 1, p = 0.119
Australia vs Argentina	$\chi^2 = 2.8617$, df = 1, p = 0.09071	$\chi^2 = 2.707$, df = 1, p = 0.09991
New Zealand vs Argentina	$\chi^2 = 0.0507$, df = 1, p = 0.8218	$\chi^2 = 0.712$, df = 1, p = 0.3988
California vs Australia	$\chi^2 = 12.7501$, df = 1, p = 0.000356	$\chi^2 = 7.5175$, df = 1, p = 0.00611
New Zealand vs Australia	$\chi^2 = 2.9296$, df = 1, p = 0.08697	$\chi^2 = 3.0904$, df = 1, p = 0.07876
New Zealand vs California	$\chi^2 = 3.9513$, df = 1, p = 0.04683	$\chi^2 = 0.0465$, df = 1, p = 0.8292

Table III.S7. R output of the cumulative link model on aggression.

```

formula: as.factor(aggression_full$agg_full) ~ treatment + region
data:    aggression_full

link threshold nobs logLik   AIC      niter max.grad cond.H
logit flexible  6002 -8557.14 17130.28 6(1)  2.06e-07 2.0e+02

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
treatmentlow_sugar  -0.17107    0.06756  -2.532  0.01133 *
treatmentOA         0.12638    0.06726   1.879  0.06023 .
treatmentOA_low_sugar 0.17616    0.06816   2.584  0.00976 **
regionOZ            0.24550    0.04718   5.204  1.95e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Threshold coefficients:
      Estimate Std. Error z value
0|1 -0.17160    0.05551  -3.091
1|2  0.15104    0.05549   2.722
2|3  1.20731    0.05786  20.865
3|4  2.40730    0.06666  36.111

```

Figure III.S1. Experimental set-up showing the experimental colonies during foraging assays. Experimental colonies were kept in 30x30x12 cm plastic boxes, with inner walls coated with Fluon® (BioQuip Products, USA) to prevent ant escape. Colonies were maintained at a 10/14 dark/light cycle and an average temperature of $23.5 \pm 0.4^{\circ}\text{C}$ and $58 \pm 9\%$ humidity. Each colony was provided with a single 15x2 cm glass test tube darkened with red cellophane and equipped with a water reserve blocked with a cotton ball at 1/3 of the tube. Surface area for ants inside the test tube was increased by adding a strip of crumpled paper that also reduced the entry hole size. The foraging assay started when the exploration trees was placed in the middle of the box, and foragers were counted on the tree. During the exploitation phase, a sugar reward was placed at the top of the tree, by soaking a small cotton piece in sugar 20% w/v.

Figure III.S2. Octopamine (OA) brain content per head (in µg) showing more variable but potentially higher exposure to OA in low sugar + OA colonies compared to high sugar + OA colonies. Symbolic results of the GLMMs are shown (i.e. * < 0.001 < ** < 0.01 < * < 0.05 < NS). There was no difference in OA titration in the sugar-deprived colonies compared to control colonies ($b = -0.226$, $t = -0.638$, $p = 0.526$) but we found more OA in the worker heads from both OA-treated colonies (high sugar + OA: $b = 1.418$, $t = 4.136$, $p < 0.001$; low sugar + OA: $b = 1.558$, $t = 4.136$, $p < 0.001$).**

Figure III.S3. Number of workers counted at the food source after 10 minutes during the exploitation phase, as a proxy of resource discovery performance. Data are shown for the native range in Argentina (AR), and three invaded regions in California (CA), Australia (AU) and New Zealand (NZ). Symbolic results of the GLMMs are shown (i.e. $* < 0.001 < ** < 0.01 < * < 0.05 < \text{NS}$). Same letters indicate the absence of significant difference in the effect of treatment. If significant, the interaction between treatment and region is written in italics. Outliers (i.e. beyond 1.5 interquartile lower and upper ranges) are indicated with black dots.**

Supplementary material for Chapter IV

Table IV.S1. Sample description (sampling date, location, processing time)

Table IV.S2. Output of BLAST searches on the most differentially expressed genes in heads across the ranges

Table IV.S3. Output of BLAST searches on the most differentially expressed genes among body parts across the ranges

Table IV.S4. List of genes included in the candidate gene approach

Table IV.S5. Output of the GLMs for neural pathways

Table IV.S6. Output of the GLMs for chemoreceptors

Table IV.S7. Output of the GLMs for immune pathways

Figure IV.S1. WGCNA – outlier removal

Figure IV.S2. WGCNA – sample dendrogram and trait heatmap

Figure IV.S3. WGCNA – choice of soft thresholding power

Figure IV.S4. WGCNA - Eigengene correlation heatmap

Figure IV.S5. Cluster plot body-part genes

Table IV.S1. Sampling sites for libraries included in the study. Each library included either pooled heads or bodies from 20 individuals. Treatment refers to experimental treatments applied in chapter III. Region codes refer to Argentina (AR), California (CA), Europe (EU), Australia (AU) and New Zealand (NZ). For site codes, refer to Chapter III, i.e. Table III.S1.

Region	Sample ID	Library type	Experimental colony number	Site	GPS coordinates	Sampling date	Treatment	Seq. lane
AR	2	heads	AR20	OTA	-34.22243, -58.89613	January 2015	Control	1
AR	4	heads	AR24	UBA	-34.54013, -58.44703	January 2015	Control	1
AR	22	heads	AR28	VLO	-34.54408, -58.49317	January 2015	Control	1
AR	24	heads	AR32	ROS	-32.91053, -60.67563	January 2015	Control	1
AR	42	heads	AR15	OTA	-34.22243, -58.89613	January 2015	Low sugar	2
AR	62	bodies	AR20	OTA	-34.22243, -58.89613	January 2015	Control	2
AR	63	bodies	AR22	VLO	-34.54408, -58.49317	January 2015	Control	2
AR	64	bodies	AR24	UBA	-34.54013, -58.44703	January 2015	Control	2
CA	6	heads	CA4	BLB	37.889064, -122.323311	September 2015	Control	1
CA	7	heads	CA7	RCH	37.931301, -122.373211	September 2015	Control	1
CA	26	heads	CA13	BLB	37.889064, -122.323311	September 2015	Control	1
CA	27	heads	CA16	CAL	37.872321, -122.247681	September 2015	Control	1
CA	28	heads	CA28	OAK	37.825258, -122.269217	September 2015	Control	1
CA	50	heads	CA23	RCH	37.931301, -122.373211	September 2015	Low sugar	2
CA	72	bodies	CA28	OAK	37.825258, -122.269217	September 2015	Control	2
EU	18	heads	EU1	PLE	42.866444, 3.043996	August 2016	Control	1
EU	20	heads	EU2	TLS	43.559337, 1.472283	August 2016	Control	1
EU	40	heads	EU2	TLS	43.559337, 1.472283	August 2016	Control	1
EU	84	bodies	EU2	TLS	43.559337, 1.472283	August 2016	Control	2
AU	10	heads	AU8	CLI	-37.7942, 144.99783	March 2015	Control	1
AU	11	heads	AU10	PHI	-38.45901, 145.23868	March 2015	Control	1
AU	12	heads	AU11	MON	-37.909674, 145.140635	March 2015	Control	1

AU	30	heads	AU22	MON	-37.909674, 145.140635	March 2015	Control	1
AU	54	heads	AU24	STA	-38.41909, 144.83226	March 2015	Low sugar	2
AU	73	bodies	AU1	STA	-38.41909, 144.83226	March 2015	Control	2
NZ	13	heads	NZ1	HAS	-39.60086, 176.75056	February 2016	Control	1
NZ	14	heads	NZ5	PET	-41.22393, 174.8659	February 2016	Control	1
NZ	16	heads	NZ13	TAR	-39.119488, 173.939672	February 2016	Control	1
NZ	34	heads	NZ21	PET	-41.22393, 174.8659	February 2016	Control	1
NZ	36	heads	NZ30	HAS	-39.60086, 176.75056	February 2016	Control	1
NZ	57	heads	NZ14	HAS	-39.60086, 176.75056	February 2016	Low sugar	2
NZ	58	heads	NZ25	HAS	-39.60086, 176.75056	February 2016	Low sugar	2
NZ	78	bodies	NZ30	HAS	-39.60086, 176.75056	February 2016	Control	2
NZ	100	lane control	-	PAR	-40.88185, 174.99499	February 2016	Lane control	1
NZ	102	lane control	-	PAR	-40.88185, 174.99499	February 2016	Lane control	2

Table IV.S2. Output of BLAST searches on the most differentially expressed genes in heads across the ranges. “Cluster” refers to the gene co-expression clusters represented on Fig. IV.3.

Cluster	qseqid	sseqid	pident	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore	Gene name
purple	TRINITY_GG_10162_c2_g1	LH18261	95.81	453	2	1	1	436	371	823	0	905	peroxidase
purple	TRINITY_GG_10255_c5_g1	LH18209	83.73	295	38	1	1	295	1	285	0	508	alpha-tocopherol transfer protein-like
purple	TRINITY_GG_10260_c0_g1	LH18213	99.74	387	1	0	1	387	36	422	0	795	phosrestin-2
purple	TRINITY_GG_10726_c1_g1	LH19059	96.89	161	5	0	13	173	1	161	4.00E-112	319	pheromone-binding protein Gp-9-like
purple	TRINITY_GG_10879_c4_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_11066_c11_g1	LH19123	99.81	516	1	0	1	516	1	516	0	1060	cytochrome P450 6k1-like
purple	TRINITY_GG_11066_c3_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_11192_c0_g2	LH16033	98.43	127	2	0	1	127	113	239	1.00E-84	250	protein takeout-like
purple	TRINITY_GG_11292_c0_g1	LH18691	71.43	98	0	1	1	98	1252	1321	3.00E-35	129	myosin-IIIb-like
purple	TRINITY_GG_11302_c7_g1	LH19123	33.59	512	316	9	4	500	8	510	1.00E-97	307	cytochrome P450 6k1-like
purple	TRINITY_GG_1179_c0_g1	XM_012371478	99.81	1551	2	1	13	1563	1550	1	0	2846	PREDICTED: odorant receptor 63a
purple	TRINITY_GG_12205_c0_g2	LH13463	100	195	0	0	13	207	1	195	3.00E-139	390	uncharacterized LOC105668188
purple	TRINITY_GG_12280_c0_g1	LH12093	43.06	281	119	4	23	302	657	897	8.00E-72	240	fibroblast growth factor receptor homolog 1-like
purple	TRINITY_GG_1374_c0_g1	LH16805	100	151	0	0	2	152	1	151	8.00E-108	306	60S ribosomal protein L14
purple	TRINITY_GG_1658_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_1662_c0_g1	LH16030	34.02	97	64	0	47	143	17	113	1.00E-13	65.1	uncharacterized LOC105670441
purple	TRINITY_GG_1668_c2_g1	XM_012360199	99.29	423	0	3	1	420	423	1	0	761	PREDICTED: uncharacterized LOC105668029
purple	TRINITY_GG_1668_c3_g1	LH16030	31.46	89	61	0	2	90	25	113	5.00E-12	59.3	uncharacterized LOC105670441
purple	TRINITY_GG_1739_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_1739_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_1854_c0_g1	LH20785	95.12	574	1	1	1	547	477	1050	0	1148	transient-receptor-potential-like protein
purple	TRINITY_GG_1930_c3_g1	LH10614	100	150	0	0	1	150	1	150	2.00E-108	308	uncharacterized LOC105670278
purple	TRINITY_GG_1995_c0_g1	LH16486	100	180	0	0	17	196	1	180	4.00E-131	374	leucine-rich repeat transmembrane neuronal protein 2-

													like
purple	TRINITY_GG_2120_c0_g1	LH10196	99.23	130	1	0	2	131	73	202	4.00E-84	248	probable serine/threonine-protein kinase
purple	TRINITY_GG_2153_c1_g1	LH22108	88.78	196	21	1	304	499	20	214	2.00E-122	360	diacylglycerol O-acyltransferase 1
purple	TRINITY_GG_2280_c5_g2	LH22456	99.33	450	3	0	1	450	96	545	0	924	MAM and LDL-receptor class A domain-containing protein 2-like
purple	TRINITY_GG_2288_c0_g14	XM_011140463	81.82	176	20	10	4	174	3401	3569	4.00E-28	137	PREDICTED: Harpegnathos saltator kelch-like protein
purple	TRINITY_GG_2324_c4_g2	XM_012374110	99.84	1262	2	0	31	1292	1	1262	0	2320	PREDICTED: insulin-like growth factor-binding protein complex
purple	TRINITY_GG_2466_c3_g2	XM_012373981	100	314	0	0	255	568	1777	1464	1.00E-161	580	PREDICTED: uncharacterized LOC105676247
purple	TRINITY_GG_2468_c1_g1	LH22142	85.85	212	30	0	121	332	1	212	4.00E-123	370	odorant receptor 46a, isoform B-like
purple	TRINITY_GG_2470_c0_g1	LH22147	99.8	495	1	0	45	539	17	511	0	1019	uncharacterized LOC105676304
purple	TRINITY_GG_265_c0_g1	LH23469	99.41	338	2	0	1	338	1	338	0	672	uncharacterized LOC105677611
purple	TRINITY_GG_267_c0_g1	LH23423	100	132	0	0	24	155	1	132	2.00E-95	275	ejaculatory bulb-specific protein 3-like
purple	TRINITY_GG_2710_c1_g1	LH20514	100	107	0	0	9	115	1	107	3.00E-74	218	uncharacterized LOC105674777
purple	TRINITY_GG_2712_c0_g1	LH20507	99.21	378	0	1	1	378	1	375	0	769	opsin, blue-sensitive
purple	TRINITY_GG_2808_c3_g1	LH14886	98.91	457	5	0	114	570	1	457	0	933	poly(U)-specific endoribonuclease homolog
purple	TRINITY_GG_2812_c3_g1	LH15403	78.57	154	33	0	1	154	175	328	3.00E-88	263	pyruvate carboxylase, mitochondrial
purple	TRINITY_GG_2824_c0_g2	XM_012363534	99.74	1562	2	1	8	1569	4516	2957	0	2861	PREDICTED: bicaudal D-related protein homolog
purple	TRINITY_GG_2840_c1_g1	LH15597	100	465	0	0	12	476	1	465	0	950	sialin-like
purple	TRINITY_GG_3125_c0_g1	LH11406	100	308	0	0	1	308	1	308	0	635	uncharacterized LOC105677769
purple	TRINITY_GG_33_c4_g1	LH26013	99.84	620	1	0	1	620	1	620	0	1238	slit homolog 2 protein-like
purple	TRINITY_GG_3320_c0_g1	LH11521	100	296	0	0	6	301	1	296	0	605	uncharacterized LOC105678730
purple	TRINITY_GG_3425_c4_g1	LH23093	100	155	0	0	1	155	1	155	1.00E-110	314	ecdysteroid-regulated 16 kDa protein
purple	TRINITY_GG_3448_c4_g2	LH26165	97.71	306	7	0	4	309	82	387	0	627	transmembrane protease serine 9-like
purple	TRINITY_GG_3449_c3_g2	LH23054	100	170	0	0	1	170	1	170	5.00E-125	352	hemoglobin subunit beta
purple	TRINITY_GG_35_c1_g1	LH11383	39.69	257	143	3	1	255	1	247	1.00E-63	202	peroxisomal hydratase-dehydrogenase-epimerase-like
purple	TRINITY_GG_3599_c0_g1	LH15414	100	119	0	0	1	119	1	119	8.00E-85	246	ejaculatory bulb-specific protein 3-like
purple	TRINITY_GG_388_c0_g1	LH17943	100	293	0	0	1	293	146	438	0	594	leucine-rich repeat-containing protein 4-like
purple	TRINITY_GG_3930_c0_g1	LH19301	100	437	0	0	1	437	1	437	0	908	lipase 3-like

purple	TRINITY_GG_3986_c0_g1	XM_012380278	99.84	2491	4	0	63	2553	2504	14	0	4578	PREDICTED: short-chain dehydrogenase/reductase family
purple	TRINITY_GG_4034_c0_g2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_4034_c1_g3	LH17703	98.16	163	3	0	38	200	1	163	4.00E-111	332	lipid storage droplets surface-binding
purple	TRINITY_GG_409_c0_g1	XM_012366973	99.82	1123	2	0	1	1123	21	1143	0	2063	PREDICTED: uncharacterized LOC105672202
purple	TRINITY_GG_4356_c0_g1	XM_012360740	99.72	1450	4	0	11	1460	455	1904	0	2658	PREDICTED: plancitoxin-1
purple	TRINITY_GG_4434_c2_g1	LH13554	100	406	0	0	34	439	1	406	0	838	arrestin homolog
purple	TRINITY_GG_4512_c0_g1	LH13767	99.63	546	2	0	1	546	2	547	0	1130	esterase FE4-like
purple	TRINITY_GG_4518_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_4518_c2_g1	LH13639	99.66	293	1	0	1	293	1	293	0	589	uncharacterized LOC105668269
purple	TRINITY_GG_4520_c0_g1	XM_012024149	84.56	298	27	9	583	866	4004	3712	3.00E-70	278	PREDICTED: Vollenhovia emeryi putative mediator of RNA polymerase II
purple	TRINITY_GG_4786_c0_g1	LH21907	87.54	313	3	2	1	307	1	283	0	546	short-chain dehydrogenase/reductase family 16C member 6-like
purple	TRINITY_GG_4816_c3_g1	XM_012373873	99.3	1986	14	0	151	2136	1	1986	0	3592	PREDICTED: catalase-like
purple	TRINITY_GG_5087_c0_g1	LH21370	100	157	0	0	1	157	1	157	2.00E-113	321	lysozyme-like
purple	TRINITY_GG_5138_c0_g1	LH21140	94.57	608	4	2	1	597	1	590	0	1165	ATP-binding cassette sub-family G member 4-like
purple	TRINITY_GG_5163_c2_g1	XR_001101258	99.8	504	1	0	155	658	507	4	0	926	PREDICTED: uncharacterized LOC105675341
purple	TRINITY_GG_5163_c5_g5	XR_001101260	100	114	0	0	1	114	295	408	3.00E-50	211	PREDICTED: uncharacterized LOC105675343
purple	TRINITY_GG_5474_c1_g1	XM_012363431	99.56	1358	5	1	791	2147	1928	3285	0	2473	PREDICTED: sialin
purple	TRINITY_GG_5484_c3_g1	LH10036	99.34	152	1	0	25	176	1	152	4.00E-112	319	retinol dehydrogenase 11-like
purple	TRINITY_GG_5619_c2_g1	LH19414	75.18	713	146	11	9	696	378	1084	0	1032	endothelin-converting enzyme 1-like
purple	TRINITY_GG_565_c13_g12	XM_012365965	95.52	357	16	0	629	985	4679	5035	1.00E-158	571	PREDICTED: ankycorbin
purple	TRINITY_GG_565_c13_g19	LH19353	100	325	0	0	1	325	45	369	0	669	opsin, ultraviolet-sensitive
purple	TRINITY_GG_565_c13_g2	XM_012369538	100	421	0	0	1	421	1036	1456	0	778	PREDICTED: opsin, ultraviolet-sensitive
purple	TRINITY_GG_5690_c1_g1	LH10979	98.86	264	3	0	1	264	773	1036	1.00E-180	527	uncharacterized LOC105673750
purple	TRINITY_GG_5806_c0_g1	LH19986	100	576	0	0	1	576	688	1263	0	1184	chaoptin
purple	TRINITY_GG_5925_c1_g1	LH22822	97.11	484	6	1	28	503	363	846	0	953	aminopeptidase N-like
purple	TRINITY_GG_5929_c1_g1	LH22819	100	149	0	0	1	149	417	565	1.00E-101	305	organic cation transporter protein
purple	TRINITY_GG_5997_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit

purple	TRINITY_GG_6601_c1_g1	LH14045	100	325	0	0	1	325	389	713	0	671	C-1-tetrahydrofolate synthase, cytoplasmic
purple	TRINITY_GG_6618_c0_g1	LH14024	99.67	302	1	0	1	302	130	431	0	627	probable chitinase 2
purple	TRINITY_GG_6664_c2_g1	LH14190	99.68	314	1	0	625	938	8	321	0	671	membrane metallo-endopeptidase-like 1
purple	TRINITY_GG_6748_c15_g1	LH16674	99.86	735	1	0	1	735	1	735	0	1528	ATP-dependent RNA helicase SUV3 homolog, mitochondrial
purple	TRINITY_GG_6826_c3_g1	XM_012359809	99.67	2404	6	1	1	2404	4076	6477	0	4394	PREDICTED: laminin subunit beta-1
purple	TRINITY_GG_6831_c5_g1	XM_012359919	99.87	748	1	0	148	895	1	748	0	1376	PREDICTED: protein NPC2 homolog
purple	TRINITY_GG_685_c0_g1	XM_012369909	99.96	2303	1	0	1	2303	6173	8475	0	4248	PREDICTED: ATP-binding cassette sub-family C member 9
purple	TRINITY_GG_6868_c7_g1	LH12961	100	156	0	0	1	156	1	156	7.00E-112	317	pheromone-binding protein Gp-9-like
purple	TRINITY_GG_6978_c0_g1	LH22968	23.6	250	163	6	9	245	33	267	3.00E-10	59.3	psychosine receptor-like
purple	TRINITY_GG_7054_c7_g1	LH20255	100	309	0	0	1	309	1	309	0	636	3'(2'),5'-bisphosphate nucleotidase 1
purple	TRINITY_GG_7054_c8_g1	LH26239	100	125	0	0	1	125	1	125	1.00E-90	261	beta-1,3-glucan-binding protein-like
purple	TRINITY_GG_7054_c9_g1	LH26240	100	394	0	0	1	394	70	463	0	819	beta-1,3-glucan-binding protein-like
purple	TRINITY_GG_7316_c2_g1	LH15306	93.63	424	1	2	1	398	190	613	0	780	solute carrier family 28 member 3
purple	TRINITY_GG_7370_c2_g1	LH15196	100	399	0	0	1	399	1	399	0	808	3-ketoacyl-CoA thiolase, mitochondrial
purple	TRINITY_GG_7557_c0_g1	LH26257	98.5	533	7	1	1	532	554	1086	0	1093	protein toll-like
purple	TRINITY_GG_7597_c3_g1	LH24645	100	641	0	0	1	641	1	641	0	1352	carotenoid isomeroxygenase
purple	TRINITY_GG_7656_c0_g1	XR_001101758	98.78	987	8	2	101	1083	3164	2178	0	1753	PREDICTED: uncharacterized LOC105678893
purple	TRINITY_GG_772_c0_g1	LH17385	100	241	0	0	37	277	1	241	0	502	uncharacterized LOC105671857
purple	TRINITY_GG_7762_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_7762_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
purple	TRINITY_GG_7762_c2_g1	XM_012672086	92.42	198	11	3	1	198	50	243	2.00E-70	279	PREDICTED: Monomorium pharaonis uncharacterized LOC105831728
purple	TRINITY_GG_7778_c0_g1	LH14237	99.71	695	0	1	1	695	1	693	0	1434	phenoloxidase 2-like
purple	TRINITY_GG_8005_c0_g1	XM_012377049	100	451	0	0	1	451	19	469	0	833	PREDICTED: uncharacterized LOC105678050
purple	TRINITY_GG_8090_c3_g1	LH11849	99.24	393	3	0	18	410	90	482	0	827	venom carboxylesterase-6-like
purple	TRINITY_GG_8144_c1_g2	XM_012366136	99.63	2980	5	1	1	2980	3326	353	0	5437	PREDICTED: uncharacterized LOC105671724
purple	TRINITY_GG_8596_c1_g1	LH24354	100	115	0	0	1	115	1	115	1.00E-79	232	eukaryotic translation initiation factor 4E-binding protein 1

purple	TRINITY_GG_8642_c1_g1	LH24447	100	286	0	0	1	286	1	286	0	594	clavesin-1
purple	TRINITY_GG_8651_c0_g1	LH24442	98.57	350	1	1	1	346	1	350	0	709	guanine nucleotide-binding protein subunit beta-2
purple	TRINITY_GG_8780_c2_g1	LH15061	100	399	0	0	9	407	9	407	0	828	zinc transporter 2-like
purple	TRINITY_GG_8926_c0_g2	LH12165	100	189	0	0	201	389	1	189	7.00E-139	397	secretin receptor-like
purple	TRINITY_GG_9057_c0_g2	LH11956	100	725	0	0	1	725	3527	4251	0	1534	uncharacterized LOC105679739
purple	TRINITY_GG_9098_c8_g1	LH12077	96.38	387	1	1	1	374	1	387	0	761	rhodopsin
purple	TRINITY_GG_9117_c1_g1	XM_012379880	99.59	981	2	1	2	980	2463	1483	0	1788	PREDICTED: ras-related protein Rab-32
purple	TRINITY_GG_9180_c1_g4	LH20999	98.99	99	1	0	37	135	1	99	2.00E-66	199	39S ribosomal protein L54, mitochondrial
purple	TRINITY_GG_9503_c1_g1	LH11441	99.46	372	2	0	1	372	1	372	0	767	uncharacterized LOC105678183
purple	TRINITY_GG_955_c1_g1	LH16389	98.86	875	0	1	1	875	203	1067	0	1786	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like
purple	TRINITY_GG_9641_c0_g1	LH15864	100	259	0	0	12	270	1	259	0	545	uncharacterized LOC105670253
purple	TRINITY_GG_9848_c3_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_1505_c0_g2	LH17041	98.69	458	1	1	1	453	1	458	0	921	26S proteasome non-ATPase regulatory subunit 12
blue	TRINITY_GG_1564_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_1564_c0_g3	XM_012376763	91.12	338	27	2	293	630	1583	1917	7.00E-124	455	PREDICTED: uncharacterized LOC105677878
blue	TRINITY_GG_1564_c0_g6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_1570_c0_g1	XR_001100128	93.39	257	14	2	41	294	431	687	9.00E-101	377	PREDICTED: uncharacterized LOC105668565
blue	TRINITY_GG_2393_c0_g6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_3436_c12_g2	XM_011154272	79.17	240	35	14	44	276	3444	3213	7.00E-33	152	PREDICTED: Harpegnathos saltator synaptosomal- associated protein
blue	TRINITY_GG_3436_c7_g3	XM_011631640	90.71	183	11	3	278	458	3591	3413	6.00E-59	239	PREDICTED: Pogonomyrmex barbatus synaptosomal- associated protein
blue	TRINITY_GG_5853_c7_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_6223_c0_g1	XM_012372811	98.11	106	2	0	3	108	3028	3133	5.00E-43	185	PREDICTED: protein fem-1 homolog A
blue	TRINITY_GG_6224_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_6293_c0_g1	XM_012379275	98.46	65	1	0	2	66	258	322	1.00E-21	115	PREDICTED: THAP domain-containing protein 4-like
blue	TRINITY_GG_6304_c2_g1	XM_011345915	85.51	207	12	6	269	467	7823	8019	3.00E-47	200	PREDICTED: Cerapachys biroi ATP-dependent RNA helicase bel

blue	TRINITY_GG_679_c1_g15	XM_012370037	95.47	309	5	1	1	300	2329	2637	4.00E-133	484	PREDICTED: sensory neuron membrane protein 1-like
blue	TRINITY_GG_679_c1_g20	XM_012370037	95.79	309	4	1	1	300	2329	2637	9.00E-135	490	PREDICTED: sensory neuron membrane protein 1-like
blue	TRINITY_GG_6796_c1_g1	LH13175	99.45	181	1	0	9	189	1	181	6.00E-134	376	methyalted-DNA--protein-cysteine methyltransferase
blue	TRINITY_GG_8441_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_8476_c7_g8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_8803_c0_g1	XM_012380609	95.33	214	9	1	32	245	27	239	3.00E-89	339	PREDICTED: myb-like protein X
blue	TRINITY_GG_93_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
blue	TRINITY_GG_9696_c3_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
lightgreen	TRINITY_GG_3465_c0_g1	XM_012378978	97.17	283	6	1	2	284	2382	2102	8.00E-131	477	PREDICTED: ras-related protein Rab-37-like
lightgreen	TRINITY_GG_3465_c1_g1	XM_012365097	99.19	123	0	1	36	157	531	653	1.00E-53	220	PREDICTED: L-xylulose reductase-like
lightgreen	TRINITY_GG_6043_c1_g1	XM_012378978	97.01	335	10	0	188	522	2668	3002	9.00E-157	564	PREDICTED: ras-related protein Rab-37-like
lightgreen	TRINITY_GG_9269_c0_g1	XM_012378978	93.7	349	9	6	2	349	2205	2541	8.00E-141	510	PREDICTED: ras-related protein Rab-37-like
pink	TRINITY_GG_10206_c3_g2	XM_012367566	100	971	0	0	309	1279	1	971	0	1794	PREDICTED: uncharacterized LOC105672550
pink	TRINITY_GG_11003_c2_g1	LH18522	100	372	0	0	1	372	1	372	0	767	dnaJ homolog subfamily C member 22
pink	TRINITY_GG_11551_c0_g1	LH24937	100	294	0	0	1	294	1	294	0	610	carbonic anhydrase 2-like
pink	TRINITY_GG_11798_c9_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
pink	TRINITY_GG_1578_c1_g1	LH10244	100	433	0	0	24	456	1	433	0	901	lysosomal aspartic protease
pink	TRINITY_GG_1668_c0_g1	XM_012360215	99.74	778	2	0	1	778	202	979	0	1426	PREDICTED: uncharacterized LOC105668042
pink	TRINITY_GG_232_c8_g1	LH23362	98.79	414	5	0	1	414	1	414	0	855	lipase 3-like
pink	TRINITY_GG_3070_c0_g1	LH24002	94.84	446	23	0	1	446	1	446	0	892	uncharacterized LOC105678090
pink	TRINITY_GG_3070_c2_g1	XM_012377129	99.1	222	2	0	1	222	5059	5280	1.00E-107	399	PREDICTED: uncharacterized LOC105678090
pink	TRINITY_GG_3208_c2_g1	LH21846	100	227	0	0	29	255	1	227	1.00E-165	461	uncharacterized LOC105676041
pink	TRINITY_GG_3416_c2_g2	LH23078	97.28	441	4	1	112	544	150	590	0	887	glucose dehydrogenase [FAD, quinone]-like
pink	TRINITY_GG_3416_c3_g1	LH23078	99.84	617	1	0	2	618	686	1302	0	1290	glucose dehydrogenase [FAD, quinone]-like
pink	TRINITY_GG_3449_c2_g1	LH23052	75.71	210	1	1	3	162	121	330	5.00E-104	304	protein dimmed-like
pink	TRINITY_GG_3513_c1_g1	XM_012359850	99.79	2350	5	0	1450	3799	2380	31	0	4313	PREDICTED: uncharacterized family 31 glucosidase
pink	TRINITY_GG_3899_c7_g1	LH19148	99.82	570	1	0	1	570	1	570	0	1189	venom metalloproteinase 3-like
pink	TRINITY_GG_4913_c0_g1	XM_012367949	96.17	209	7	1	14	222	588	795	8.00E-90	340	PREDICTED: uncharacterized LOC105672789
pink	TRINITY_GG_6327_c1_g1	LH23210	100	188	0	0	1	188	328	515	7.00E-137	395	uncharacterized LOC105677350

pink	TRINITY_GG_6334_c0_g1	XM_012375861	99.09	661	6	0	3	663	663	3	0	1188	PREDICTED: uncharacterized LOC105677317
pink	TRINITY_GG_6988_c7_g1	LH20209	99.73	372	1	0	1	372	1	372	0	770	aldose 1-epimerase-like
pink	TRINITY_GG_7354_c10_g2	LH15188	70.67	150	39	1	63	212	48	192	6.00E-72	219	phospholipase A2-like
pink	TRINITY_GG_7357_c1_g1	LH15204	100	310	0	0	27	336	3	312	0	637	regucalcin-like
pink	TRINITY_GG_7478_c0_g2	LH21621	100	406	0	0	1	406	36	441	0	813	major facilitator superfamily domain-containing protein 12-like
pink	TRINITY_GG_8383_c2_g1	XM_012374675	100	997	0	0	1	997	3411	4407	0	1842	PREDICTED: uncharacterized LOC105676627
pink	TRINITY_GG_862_c0_g1	XM_012364392	99.94	1642	1	0	1	1642	1	1642	0	3027	PREDICTED: alpha-amylase 2-like
pink	TRINITY_GG_865_c0_g1	XM_012364393	99.82	1628	3	0	7	1634	1	1628	0	2990	PREDICTED: alpha-amylase A
pink	TRINITY_GG_866_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
pink	TRINITY_GG_8987_c6_g1	LH12019	97.41	347	4	1	56	397	28	374	0	703	EH domain-containing protein 3
pink	TRINITY_GG_9320_c2_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
pink	TRINITY_GG_9511_c1_g1	XR_001101710	98.74	554	6	1	2	555	807	255	0	983	PREDICTED: uncharacterized LOC105678491
pink	TRINITY_GG_9782_c0_g1	XM_012364601	98.36	244	4	0	1	244	1198	1441	1.00E-116	429	PREDICTED: leucine-rich repeat-containing protein egg-6
cyan	TRINITY_GG_11182_c1_g1	LH16026	100	249	0	0	1	249	1	249	0	504	circadian clock-controlled protein
cyan	TRINITY_GG_11194_c0_g1	LH16034	100	246	0	0	1	246	1	246	0	504	uncharacterized LOC105670444
cyan	TRINITY_GG_11402_c3_g1	LH25025	100	288	0	0	1	288	62	349	0	604	protein msta, isoform B-like
cyan	TRINITY_GG_1441_c0_g3	LH16960	100	193	0	0	1	193	1	193	3.00E-143	400	sarcoplasmic calcium-binding protein 1
cyan	TRINITY_GG_232_c9_g2	LH26033	71.88	224	63	0	1	224	536	759	5.00E-113	343	lipase 3-like
cyan	TRINITY_GG_3063_c4_g1	XM_012377215	99.68	3405	7	2	182	3586	3414	14	0	6224	PREDICTED: muscle LIM protein Mlp84B-like
cyan	TRINITY_GG_3658_c0_g1	XM_018455561	89.92	129	11	2	771	899	2877	3003	3.00E-36	165	PREDICTED: Trachymyrmex zeteki polyphosphoinositide phosphatase
cyan	TRINITY_GG_4150_c1_g1	XM_012368458	99.13	918	0	5	1	916	6111	7022	0	1644	PREDICTED: myosin heavy chain, muscle
cyan	TRINITY_GG_4394_c2_g2	LH13547	94.8	404	2	1	36	420	2	405	0	758	uncharacterized LOC105668197
cyan	TRINITY_GG_514_c0_g1	LH17847	97.87	329	7	0	14	342	15	343	0	670	putative uncharacterized protein DDB_G0282499
cyan	TRINITY_GG_7063_c0_g1	LH20257	96.88	352	11	0	30	381	1	352	0	713	venom serine protease-like
cyan	TRINITY_GG_7153_c1_g1	XR_001101118	100	245	0	0	1098	1342	247	3	5.00E-123	453	PREDICTED: uncharacterized LOC105674471
cyan	TRINITY_GG_7897_c0_g1	LH17495	100	536	0	0	1	536	1257	1792	0	1125	uncharacterized LOC105671873
cyan	TRINITY_GG_8200_c7_g1	LH17158	97.97	148	3	0	1	148	1	148	4.00E-104	298	myosin light chain alkali

cyan	TRINITY_GG_8913_c0_g1	LH12186	100	389	0	0	1	389	1	389	0	805	uncharacterized oxidoreductase C26H5.09c-like
cyan	TRINITY_GG_8939_c0_g1	XM_012380129	99.63	1629	2	1	233	1857	3418	5046	0	2972	PREDICTED: uncharacterized LOC105679844
cyan	TRINITY_GG_9927_c0_g1	LH14366	100	88	0	0	26	113	1	88	9.00E-60	181	uncharacterized LOC105668946
darkgreen	TRINITY_GG_1335_c0_g1	LH16815	99.37	159	1	0	33	191	29	187	1.00E-111	322	uncharacterized LOC105671203
darkgreen	TRINITY_GG_2991_c0_g1	XM_012370687	95.93	246	10	0	1	246	5847	6092	4.00E-107	399	PREDICTED: protein hu-li tai shao
brown	TRINITY_GG_5404_c1_g1	XM_012359108	99.29	709	2	1	9	717	2833	2128	0	1279	PREDICTED: uncharacterized LOC105667360
orange	TRINITY_GG_2280_c7_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit

Table IV.S3. Output of BLAST searches on the most differentially expressed genes among body parts across the ranges

Gene ID	sseqid	pident	length	mismatch	gapopen	qstart	qend	sstart	send	evalue	bitscore	Gene name
TRINITY_GG_6223_c0_g1	XM_012372811	98.11	106	2	0	3	108	3028	3133	5.00E-43	185	PREDICTED: Linepithema humile protein fem-1 homolog A
TRINITY_GG_12197_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_1909_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_93_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_2801_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_9801_c0_g1	XM_012367292	99.05	1891	0	2	105	1985	1121	3003	0	3376	PREDICTED: Linepithema humile metallophosphoesterase 1-like
TRINITY_GG_10885_c1_g3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_3554_c0_g1	XM_012370372	90.38	52	5	0	2	53	67	118	4.00E-08	69.4	PREDICTED: Linepithema humile guanylate kinase
TRINITY_GG_2393_c0_g6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_2096_c0_g9	XM_011346446	88.2	178	13	3	142	319	3671	3502	5.00E-49	206	PREDICTED: Cerapachys biroi uncharacterized LOC105283582
TRINITY_GG_9638_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_4962_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_2593_c1_g1	XR_001100470	95.56	45	2	0	758	802	1939	1983	1.00E-08	73.1	PREDICTED: Linepithema humile uncharacterized LOC105670700
TRINITY_GG_8803_c0_g1	XM_012380609	95.33	214	9	1	32	245	27	239	3.00E-89	339	PREDICTED: Linepithema humile myb-like protein X
TRINITY_GG_4196_c0_g2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_70_c1_g3	XM_012375784	98.53	68	1	0	306	373	3423	3356	2.00E-23	121	PREDICTED: Linepithema humile NADP-dependent malic enzyme
TRINITY_GG_12197_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_8441_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_12299_c1_g3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_6155_c2_g19	XM_012359492	94.96	278	0	1	1	278	3220	3483	8.00E-115	424	PREDICTED: Linepithema humile janus kinase and microtubule-interacting protein 3
TRINITY_GG_1564_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_10897_c0_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_8056_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit

TRINITY_GG_12299_c1_g1	XM_012362615	73.46	878	186	32	912	1769	3271	2421	1.00E-72	287	PREDICTED: Linepithema humile uncharacterized LOC105669575
TRINITY_GG_5771_c2_g1	XM_012364188	99.32	148	1	0	68	215	3027	2880	3.00E-68	268	PREDICTED: Linepithema humile vacuolar protein sorting-associated protein 18 homolog
TRINITY_GG_8833_c0_g1	XR_001100249	99.69	324	1	0	125	448	1108	785	1.00E-165	593	PREDICTED: Linepithema humile uncharacterized LOC105669372
TRINITY_GG_1526_c0_g1	XR_001099907	99.44	1065	3	3	43	1107	444	1505	0	1930	PREDICTED: Linepithema humile uncharacterized LOC105667321
TRINITY_GG_679_c1_g15	XM_012370037	95.47	309	5	1	1	300	2329	2637	4.00E-133	484	PREDICTED: Linepithema humile sensory neuron membrane protein 1-like
TRINITY_GG_4655_c5_g1	XM_012374893	100	233	0	0	29	261	644	412	4.00E-117	431	PREDICTED: Linepithema humile uncharacterized LOC105676754
TRINITY_GG_2801_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_5394_c1_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_1573_c7_g1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	no hit
TRINITY_GG_11200_c0_g1	XR_001101077	98.18	55	0	1	1	54	740	794	7.00E-16	95.3	PREDICTED: Linepithema humile tumor necrosis factor receptor superfamily member 3-like
TRINITY_GG_679_c1_g20	XM_012370037	95.79	309	4	1	1	300	2329	2637	9.00E-135	490	PREDICTED: Linepithema humile sensory neuron membrane protein 1-like
TRINITY_GG_1472_c0_g3	XM_012365392	99.73	1127	2	1	1	1126	85	1211	0	2063	PREDICTED: Linepithema humile uncharacterized LOC105671332
TRINITY_GG_5704_c0_g1	XM_012018679	87.04	216	14	8	39	246	6195	6404	3.00E-56	231	PREDICTED: Vollenhovia emeryi histone deacetylase 4
TRINITY_GG_3436_c7_g3	XM_011631640	90.71	183	11	3	278	458	3591	3413	6.00E-59	239	PREDICTED: Pogonomyrmex barbatus synaptosomal-associated protein 25
TRINITY_GG_3436_c12_g2	XM_011154272	79.17	240	35	14	44	276	3444	3213	7.00E-33	152	PREDICTED: Harpegnathos saltator synaptosomal-associated protein 25

Table IV.S4a. Candidate genes for neural pathways.

Accession number	Gene name	Neural pathway
LH10390	tyramine beta-hydroxylase	TYR
LH20865	tyramine receptor 1	TYR
LH13102	sodium- and chloride-dependent GABA transporter 1-like	GABA
LH24213	sodium- and chloride-dependent GABA transporter 1	GABA
LH12970	octopamine receptor beta-1R-like	OA
LH12972	octopamine receptor beta-1R-like	OA
LH12975	octopamine receptor beta-3R-like	OA
LH12976	octopamine receptor beta-3R-like	OA
LH20188	octopamine receptor beta-2R-like	OA
LH17274	dopamine D2-like receptor	DA
LH17278	dopamine D2-like receptor	DA
LH17279	dopamine D2-like receptor	DA
LH18257	dopamine receptor 1	DA
LH21516	dopamine N-acetyltransferase-like	DA
LH11105	5-hydroxytryptamine receptor 2A-like	SER
LH13259	5-hydroxytryptamine receptor 2C	SER
LH13265	5-hydroxytryptamine receptor 2C	SER
LH11544	glutamate receptor-interacting protein 2	glutamate
LH11545	glutamate receptor-interacting protein 2	glutamate
LH11817	glutamate receptor ionotropic, delta-2-like	glutamate
LH11960	glutamate receptor 1-like	glutamate
LH13089	metabotropic glutamate receptor 8-like	glutamate
LH13090	metabotropic glutamate receptor 8-like	glutamate
LH14616	glutamate receptor ionotropic, kainate 2	glutamate
LH15532	probable glutamate receptor	glutamate
LH15718	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH15719	glutamate receptor ionotropic, NMDA 2B-like	glutamate
LH19015	vesicular glutamate transporter 1	glutamate
LH20394	glutamate receptor ionotropic, kainate 2-like	glutamate
LH20866	glutamate receptor ionotropic, kainate 5	glutamate
LH22634	metabotropic glutamate receptor	glutamate
LH24041	glutamate receptor ionotropic, kainate 2	glutamate
LH24044	glutamate receptor ionotropic, kainate 2-like	glutamate
LH25312	glutamate receptor U1-like	glutamate
LH25313	glutamate receptor ionotropic, kainate 5-like	glutamate

LH25314	glutamate receptor ionotropic, delta-2-like	glutamate
LH25368	glutamate receptor 3-like	glutamate
LH25937	glutamate receptor ionotropic, delta-2	glutamate
LH10392	glutamate [NMDA] receptor subunit 1	glutamate
LH10494	glutamate receptor ionotropic, kainate 2-like	glutamate

Table IV.S4b. Candidate genes for chemoreception.

Accession number	Gene name	Receptor type
LH11096	odorant receptor Or2-like	OR
LH11296	odorant receptor Or2-like	OR
LH12711	odorant receptor 4-like	OR
LH12712	odorant receptor 4-like	OR
LH12960	general odorant-binding protein 72-like	OR
LH12962	general odorant-binding protein lush	OR
LH13656	odorant receptor Or2-like	OR
LH15023	general odorant-binding protein 69a	OR
LH16353	odorant receptor 4-like	OR
LH16356	odorant receptor 4-like	OR
LH16357	odorant receptor 4-like	OR
LH16358	odorant receptor 22c/82a-like	OR
LH16359	odorant receptor 2a-like	OR
LH16360	odorant receptor 2a/4/22c-like	OR
LH16363	odorant receptor 13a-like	OR
LH16365	odorant receptor 13a-like	OR
LH16367	odorant receptor 13a-like	OR
LH17511	odorant receptor 43a-like	OR
LH18177	odorant receptor 67a-like	OR
LH18291	putative odorant receptor 71a	OR
LH18458	odorant receptor 13a-like	OR
LH18464	odorant receptor Or2/46a-like	OR
LH18671	odorant receptor 13a-like	OR
LH18714	odorant receptor 13a-like	OR
LH18722	odorant receptor 45b-like	OR
LH18727	odorant receptor 30a-like	OR
LH18761	general odorant-binding protein 71	OR
LH19031	putative odorant receptor 85d	OR
LH19170	odorant receptor 22c/67c/coreceptor-like	OR
LH20597	odorant receptor 67c-like	OR

LH20607	odorant receptor 63a-like	OR
LH21669	odorant receptor Or1-like	OR
LH22142	odorant receptor 46a, isoform B-like	OR
LH22142	odorant receptor 46a, isoform B-like	OR
LH22143	odorant receptor 46a, isoform A-like	OR
LH22492	odorant receptor Or2-like	OR
LH22493	odorant receptor 4/13a-like	OR
LH22725	odorant receptor 13a-like	OR
LH22780	odorant receptor 22c-like	OR

Table IV.S4b. Candidate genes for chemoreception (continued).

Accession number	Gene name	Receptor type
LH23112	general odorant-binding protein 56d-like	OR
LH23506	odorant receptor 13a-like	OR
LH23511	odorant receptor 9a-like	OR
LH23858	odorant receptor 83a-like	OR
LH24139	odorant receptor Or2-like	OR
LH24938	odorant receptor 49a-like	OR
LH25597	odorant receptor coreceptor-like	OR
LH25603	odorant receptor 4-like	OR
LH25609	odorant receptor 4/45b-like	OR
LH25610	odorant receptor 85b-like	OR
LH25633	odorant receptor 43a-like	OR
LH25648	odorant receptor 7a-like	OR
LH25713	odorant receptor 22c-like	OR
LH25714	odorant receptor 22c-like	OR
LH25730	odorant receptor 46a, isoform A-like	OR
LH25733	odorant receptor 82a-like	OR
LH25733	odorant receptor 82a-like	OR
LH25885	odorant receptor 67a-like	OR
LH25976	odorant receptor 13a-like	OR
LH26038	odorant receptor 13a-like	OR
LH26046	odorant receptor 13a-like	OR
LH26048	odorant receptor 4-like	OR
LH26050	odorant receptor 4-like	OR
LH10133	gustatory and pheromone receptor 32a-like	GR
LH11242	putative gustatory receptor 28a	GR
LH12478	gustatory receptor for sugar taste 64f-like	GR

LH13821	"putative gustatory receptor 23a, isoform B"	GR
LH14675	gustatory receptor for sugar taste 64f-like	GR
LH19341	gustatory receptor for sugar taste 43a	GR

Table IV.S4c. Candidate genes for immune pathways.

Accession number	Gene name	Immune pathway
LH15872	endoribonuclease Dicer	RNAi
LH26164	protein argonaute-2	RNAi
LH11634	protein argonaute-3	RNAi
LH15418	protein argonaute-2	RNAi
LH22601	signaling intermediate in Toll pathway, mitochondrial	TOLL
LH19752	protein toll	TOLL
LH26252	protein toll-like	TOLL
LH26256	protein toll-like	TOLL
LH26257	protein toll-like	TOLL
LH26213	protein toll	TOLL
LH24353	toll-interacting protein-like	TOLL
LH16665	serine protease persephone-like	TOLL
LH24134	protein spaetzle	TOLL
LH17362	serine/threonine-protein kinase pelle	TOLL
LH24529	NF-kappa-B inhibitor cactus-like	TOLL
LH26206	NF-kappa-B inhibitor cactus-like	TOLL
LH21592	defensin-2	TOLL
LH12652	sex-regulated protein janus-A-like	JAK_STAT
LH23786	tyrosine-protein kinase hopscotch	JAK_STAT
LH15767	stress-activated protein kinase JNK	JNK
LH18476	JNK1/MAPK8-associated membrane protein	JNK
LH22008	JNK-interacting protein 3	JNK
LH23953	JNK-interacting protein 1	JNK

Table IV.S5. Output of the GLMs for neural pathways. All introduced regions (California, Europe, Australia, New Zealand) are compared with Argentina. Data are shown for a) octopaminergic, b) dopaminergic, c) serotonergic, d) tyraminerbic, e) GABAergic and f) glutamaterbic systems.

a. Octopaminergic system

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.07973  -0.08056  -0.00642   0.10400   0.38886

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.3543      0.1434   2.471  0.02259 *
regionAU     -0.3478      0.2028  -1.716  0.10169
regionCA     -0.3391      0.2028  -1.672  0.11004
regionEU     -0.7431      0.2028  -3.665  0.00154 **
regionNZ     -0.4583      0.2028  -2.260  0.03512 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.1027734)

    Null deviance: 3.4808  on 24  degrees of freedom
Residual deviance: 2.0555  on 20  degrees of freedom
AIC: 20.488

Number of Fisher Scoring iterations: 2
```

b. Dopaminergic system

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.60600  -0.16609  -0.03663   0.11917   0.66204

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.3355      0.1336   2.511  0.0207 *
regionAU     -0.3795      0.1890  -2.008  0.0583 .
regionCA     -0.3178      0.1890  -1.682  0.1081
regionEU     -0.5069      0.1890  -2.682  0.0143 *
regionNZ     -0.4856      0.1890  -2.570  0.0183 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.0892712)

    Null deviance: 2.6188  on 24  degrees of freedom
Residual deviance: 1.7854  on 20  degrees of freedom
AIC: 16.966

Number of Fisher Scoring iterations: 2
```

c. Serotonergic system

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.26104  -0.04610  -0.01205   0.07025   0.17294

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.32261    0.07628   4.229 0.001746 **
regionAU    -0.38642    0.10788  -3.582 0.004995 **
regionCA    -0.26299    0.10788  -2.438 0.034979 *
regionEU    -0.46306    0.10788  -4.292 0.001580 **
regionNZ    -0.49839    0.10788  -4.620 0.000951 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.01745613)

Null deviance: 0.66155  on 14  degrees of freedom
Residual deviance: 0.17456  on 10  degrees of freedom
AIC: -12.235

Number of Fisher Scoring iterations: 2
```

d. Tyraminergetic system

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    1     2     3     4     5     6     7     8     9    10
-0.291339  0.291339  0.015962 -0.015962 -0.001123  0.001123  0.053981 -0.053981  0.174526 -0.174526

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.2527    0.1540   1.642   0.162
regionAU    -0.2918    0.2177  -1.340   0.238
regionCA    -0.3184    0.2177  -1.462   0.203
regionEU    -0.2306    0.2177  -1.059   0.338
regionNZ    -0.3586    0.2177  -1.647   0.160

(Dispersion parameter for gaussian family taken to be 0.04740319)

Null deviance: 0.39819  on 9  degrees of freedom
Residual deviance: 0.23702  on 5  degrees of freedom
AIC: 2.9566

Number of Fisher Scoring iterations: 2
```

e. GABAergic system

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.23115  -0.03320   0.02172   0.07630   0.13800

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.15553    0.06699   2.322  0.0427 *
regionAU     -0.18642    0.09474  -1.968  0.0774 .
regionCA     -0.19949    0.09474  -2.106  0.0615 .
regionEU     -0.08441    0.09474  -0.891  0.3939
regionNZ     -0.23737    0.09474  -2.505  0.0312 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.01346489)

    Null deviance: 0.24821  on 14  degrees of freedom
Residual deviance: 0.13465  on 10  degrees of freedom
AIC: -16.129

Number of Fisher Scoring iterations: 2
```

f. Glutamatergic system

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.54566  -0.11501   0.00139   0.09563   0.65423

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.22696    0.04331   5.241 7.79e-07 ***
regionAU     -0.28082    0.06124  -4.585 1.21e-05 ***
regionCA     -0.33431    0.06124  -5.459 3.00e-07 ***
regionEU     -0.19229    0.06124  -3.140 0.00217 **
regionNZ     -0.27345    0.06124  -4.465 1.95e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.04313507)

    Null deviance: 6.3254  on 114  degrees of freedom
Residual deviance: 4.7449  on 110  degrees of freedom
AIC: -28.249

Number of Fisher Scoring iterations: 2
```

Table IV.S6. Output of the GLMs for chemoreceptors. All introduced regions (California, Europe, Australia, New Zealand) are compared with Argentina. Data are shown for a) olfactory and b) gustatory receptors.

a. Olfactory receptors

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.72678 -0.10572  0.00644  0.10205  1.24257

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.01123    0.02403   0.467  0.6407
regionAU     -0.06188    0.03398  -1.821  0.0696 .
regionCA     -0.18037    0.03398  -5.308 2.18e-07 ***
regionEU     -0.01206    0.03398  -0.355  0.7229
regionNZ      0.16227    0.03398   4.775 2.83e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.03464186)

Null deviance: 13.888  on 299  degrees of freedom
Residual deviance: 10.219  on 295  degrees of freedom
AIC: -150.49

Number of Fisher Scoring iterations: 2
```

b. Gustatory receptors

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.32453 -0.12249 -0.00227  0.13000  0.33284

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.15744    0.07344   2.144  0.0420 *
regionAU     -0.13661    0.10385  -1.315  0.2003
regionCA     -0.28262    0.10385  -2.721  0.0117 *
regionEU     -0.15210    0.10385  -1.465  0.1555
regionNZ     -0.17978    0.10385  -1.731  0.0958 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.03235685)

Null deviance: 1.05587  on 29  degrees of freedom
Residual deviance: 0.80892  on 25  degrees of freedom
AIC: -11.261

Number of Fisher Scoring iterations: 2
```

Table IV.S7. Output of the GLMs for immune pathways. All introduced regions (California, Europe, Australia, New Zealand) are compared with Argentina. Data are shown for a) pooled immune genes, and genes associated with the b) TOLL, c) JAK/STAT and d) RNAi pathways.

a. Pooled immune genes

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.83571  -0.08267  -0.00357   0.12036   0.69001

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.051829   0.045458  -1.140   0.257
regionAU     0.045812   0.064287   0.713   0.478
regionCA     0.130956   0.064287   2.037   0.044 *
regionEU     0.005779   0.064287   0.090   0.929
regionNZ     0.045060   0.064287   0.701   0.485
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.04752695)

Null deviance: 5.4798  on 114  degrees of freedom
Residual deviance: 5.2280  on 110  degrees of freedom
AIC: -17.099

Number of Fisher Scoring iterations: 2
```

b. TOLL pathway

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.70235  -0.12390   0.00603   0.14722   0.59993

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.18519   0.06411  -2.889   0.00538 **
regionAU     0.18800   0.09067   2.074   0.04242 *
regionCA     0.29823   0.09067   3.289   0.00168 **
regionEU     0.18163   0.09067   2.003   0.04967 *
regionNZ     0.22009   0.09067   2.428   0.01822 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.05343237)

Null deviance: 3.8302  on 64  degrees of freedom
Residual deviance: 3.2059  on 60  degrees of freedom
AIC: 0.85222

Number of Fisher Scoring iterations: 2
```


c. JAK/STAT pathway

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    1      2      3      4      5      6      7      8 
-0.22206  0.22206 -0.00009  0.00009 -0.02232  0.02232  0.33621 -0.33621 
 0.03372 -0.03372 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.08111    0.18110  -0.448   0.673
regionAU      0.09754    0.25611   0.381   0.719
regionCA      0.15683    0.25611   0.612   0.567
regionEU      0.14260    0.25611   0.557   0.602
regionNZ      0.03606    0.25611   0.141   0.894

(Dispersion parameter for gaussian family taken to be 0.06559284)

Null deviance: 0.36445  on 9  degrees of freedom
Residual deviance: 0.32796  on 5  degrees of freedom
AIC: 6.2044

Number of Fisher Scoring iterations: 2
```

d. RNAi pathway

```
Call:
glm(formula = value ~ region, data = mean_candidates)

Deviance Residuals:
    Min       1Q   Median       3Q      Max 
-0.35018 -0.08959  0.00634  0.07434  0.35601 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.28218    0.09978   2.828  0.01272 *
regionAU     -0.31781    0.14112  -2.252  0.03972 *
regionCA     -0.26882    0.14112  -1.905  0.07614 .
regionEU     -0.50149    0.14112  -3.554  0.00289 **
regionNZ     -0.37574    0.14112  -2.663  0.01774 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.0398269)

Null deviance: 1.1469  on 19  degrees of freedom
Residual deviance: 0.5974  on 15  degrees of freedom
AIC: -1.4604

Number of Fisher Scoring iterations: 2
```

Figure IV.S1. WGCNA – Outlier removal

Figure IV.S2. WGCNA – Sample dendrogram showing range (native in white, invaded in red).

Figure IV.S3. WGCNA - Scale free topology criterion with a R^2 threshold of 0.9. A soft threshold power of 8 was chosen.

Figure IV.S4. WGCNA – Eigengene correlation heatmap. Modules on the bottom right of the heatmap tend to be positively correlated with invasion status, and modules in the center of the heatmap tend to be negatively correlated with range.

Figure IV.S5. Detail of expression pattern of genes assigned to the a) head-specific, b) body-specific and c) Argentina-specific clusters shown in Figure IV.4. Individuals are indicated on the X-axis. Log-centred TMM-normalised TPMs are shown on the Y-axis. The blue line indicates the mean expression level for the cluster and the grey lines indicates individual values. Note the unusual pattern in the Argentina-specific cluster, where genes are expressed or upregulated in Argentina only, regardless of the bodypart.