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The genomics of mutualistic dependence in the plant-associated ant genus *Philidris*

Laura C.E. Campbell

Thesis abstract

Mutualisms are ubiquitous, ecologically and evolutionary important and highly diverse. One element of mutualism diversity that is relatively poorly studied is mutualistic dependence, the level to which a species relies on its mutualistic partner. Mutualistic dependence is important to many aspects of mutualism evolution, particularly stability and has even been found to affect the evolution of traits not involved in the mutualism. The effect of mutualism on genome evolution is also understudied with the exception of bacterial endosymbionts. Here, I investigated genome evolution in the ant genus *Philidris* (Dolichoderinae). *Philidris* is most well-known for the Fijian farming ant *Philidris nagasau*, which has evolved an obligate, highly specialised farming mutualism with six species of the epiphytic plant genus *Squamellaria* (Hydnophytinae, Rubiaceae). In addition to the highly specialised mutualism, several *Philidris* species engage in a facultative generalist “ant-garden” mutualism. Additionally, there are some *Philidris* populations that do not engage with plant mutualism at all. This variability in mutualism strategy makes *Philidris* an ideal system to study mutualistic dependence.

First, I establish the extent of plant-cultivation by ants by performing a systematic review and creating a database of ant-farmed plants and plant-farming ants. I assess the diversity of these mutualisms in relation to taxonomic diversity, presence of domatia and food rewards, specificity, and dependence and finally assess how these systems compare with the conceptualisation of “true” agriculture. I conclude that this framework is overly restrictive and propose a new inclusive eco-evolutionary framework encompassing all farming systems.

Next in Chapter 3, I assemble and annotate two genomes (of the species *P. nagasau* and *P. laevigata*) as well as 38 genomes from high coverage Illumina resequencing data representing 9 operational taxonomic units (OTUs). I use these genomes to construct a genome-wide phylogeny from 2,100 genes. This comparative genomic and phylogenomic framework is the foundation of the next two chapters.

In Chapter 4 I investigate gene family expansion and contractions within *Philidris* and find that *P. nagasau* has the most significantly expanding and contracting gene families. Within these expanding and contracting gene families are olfactory receptors (ORs), gustatory receptors (GRs) and genes relating to the nervous system, especially the neurotransmitter glutamate. I investigate signatures of selection within the OR repertoire of *P. nagasau* and find that 11% have significant evidence for diversifying selection.

In Chapter 5, I investigate signatures of selection within *Philidris* single copy orthologues to investigate genome-wide signatures evolution relating to the shift to obligate specialist mutualism. *Philidris nagasau* has a higher average ratio of non-synonymous to synonymous substitutions (dN/dS) which appears to be a result of both positive selection on some genes and relaxed purifying selection on others. *Philidris nagasau* has a higher nucleotide substitution rate compared to a sister clade, indicating that the shift to obligate specialist mutualism evolves with Red Queen dynamics.

These results suggest that the obligate specialist mutualism between *P. nagasau* and *Squamellaria* is likely mediated by olfactory cues. We detect signatures relaxation and intensification in the genome, mirroring the losses and gains in behaviours in *P. nagasau*.

**The genomics of mutualistic dependence in
the plant-associated ant genus *Philidris***



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Declaration

The material contained within this thesis has not previously been submitted for a degree at Durham University or any other university. The research reported within this thesis was conducted by the author unless indicated otherwise.

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Chapter 1: General introduction



1. Mutualisms

Mutualism, interactions between two or more species which are beneficial for all species involved, are ubiquitous in nature, with virtually all species are involved in some kind of mutualism (Bronstein et al. 2006). Most plants rely on insects or other animal pollinators for reproduction (78% in Temperate regions and 94% in the Tropics (Ollerton et al. 2011)). Mycorrhizal fungi play a significant role in plant nutrition in over 80% of land plant species (van der Heijden et al. 2015). Coral-zooxanthellae mutualisms foster 25% of all marine species in reef communities (Bronstein 2015). A fifth of known fungi engage in mutualistic relationships with algae, known as lichens (Lumbsch and Rikkinen 2017). Microbes play a significant role in the digestion in many animals (Nikoh et al. 2014, Kaszyca-Taszakowska and Depa 2022, Mahmood et al. 2023, Yao 2023) including humans (Gilbert et al. 2018). Mutualisms modulate biodiversity across both ecological and evolutionary scale, by influencing processes such as species coexistence within communities and species diversification (Chomicki et al. 2019). As well as being pivotal to many significant shifts in the history of life have been mediated by mutualisms, including the colonisation of land by plants and even the origin of eukaryotes (Thompson 2005).

1.1 Defining and classifying mutualism

The term “mutualism” was first introduced to biology by Pierre van Beneden in 1873 to mean “mutual aid” among species (Bronstein 2015). There is often the conflation between the terms “mutualism” and “symbiosis”. The term symbiosis was originally coined by Anton de Bary in 1879 (Bary 1879, Bronstein 2015) referring to “unlike organisms living together”. While De Bary clearly specified that symbiosis refers to mutualistic, commensal, and parasitic interactions, confusion between the terms remains till now, with many scholars often using the word symbiosis to refer to mutualism.

Species interactions can be classified based on the effects on fitness, seen in the interaction grid in Figure 1.1. For an interspecific interaction to be considered mutualistic the net fitness benefit has to be positive for both of the interacting species, in contrast to a commensalism which is positive for one species and neutral for the other, or predation/parasitism which are positive for one and negative for the other (Figure 1.1).

Mutualisms are highly diverse. Symbiosis is one important type of mutualism diversity, a symbiosis describes a system wherein species live closely together in contrast some mutualisms are mediated by periods of brief exchange such as most pollinator mutualisms and seed dispersal (Leigh 2010).

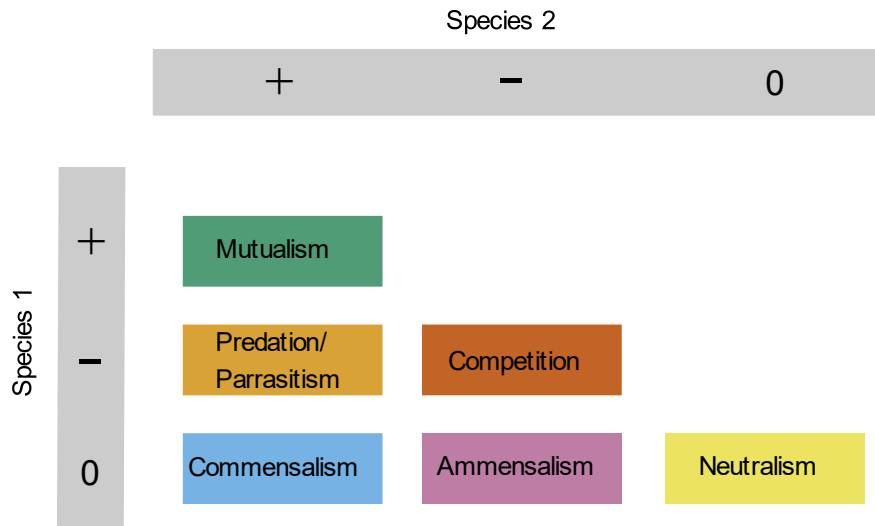


Figure 1.1 Species interactions grid. Reproduced from Bronstein (2015).

Another way to classify mutualism diversity is through the kinds of services provided, which can loosely be split into (i) transportation (ii) protection and (iii) nutrition (Bronstein 2015). Transportation involves the movement of the mutualist such as in pollination and seed dispersal. Protection mutualisms involve one mutualist providing defence, either against biotic threats such as predation as seen in the *Acacia* tree which provides nesting space in the form of domatia in return for protection from herbivores thanks to the ants highly aggressive behaviour (Ward and Downie 2005, Orón-Tamayo et al. 2013, Chomicki et al. 2015, Palmer 2023) or from abiotic threats, for example the provisioning of domatia allows ant brood to be sheltered from the elements. Finally, nutritional mutualisms include mycorrhizal fungi (West et al. 2002, van der Heijden et al. 2015) or *Rhizobium* bacteria involved in nitrogen-fixing symbioses with legumes and allies (Klinger et al. 2016). Usually, mutualist partners provide different services, for example in a typical plant/insect pollination mutualism the plant is providing nutrition in return for the insect transporting plant gametes. Some mutualist partners provide each other with the same type of service, for example nutrition, but the actual nutrients being exchanged are different such as the case with *Rhizobium* which provides plants with nitrogen in return for the plant providing sugars (Kiers and Denison 2008). While most mutualisms do involve the exchange of services, some mutualisms do not involve any direct exchange of resources or services, such as the

simultaneous fruiting, also known as gregarious fruiting, of many tree species in the dipterocarp forests, a strategy that reduces the amount of seeds lost to granivores (Sun et al. 2007, Leigh 2010) as well as Mullerian mimics wherein species benefit from their partner having a similar warning signal with an accompanying deterrent, such as noxious chemicals or a sting (Mallet 1999, Sherratt 2008, Hill 2021).

Mutualisms are often used as model systems for the study of coevolution (Thompson 1994, 1999, 2005). Many people intuit mutualisms, particularly highly specialised ones, are coevolved, however, this is not necessarily true, as famously argued by Janzen (1980). Coevolution involves reciprocal evolutionary change in interacting species, driving by natural selection. It requires that an evolutionary change occurs in one population in response to a trait in individuals in a second population followed by an evolutionary change in the secondary population in a response to a trait in the individuals in the first population (Janzen 1980).

1.2 Cheaters, exploiters, and the stability of mutualisms

“Natural selection cannot possibly produce any modification in any one species exclusively for the good of another species”, page 200 (Darwin 1859).

Natural selection is selfish, only traits that benefit the individual are expected to evolve and be maintained (Sachs et al., 2004). Therefore, the evolution of cooperation between species (mutualism) had to overcome an initial hurdle that the mutualistic trait, that is benefiting the mutualist partner, must also be of benefit to the individual that evolved that trait. Evolutionary theory has long predicted that mutualisms are inherently unstable, and liable to become parasitic (Trivers, 1971; Axelrod and Hamilton, 1981). Yet mutualisms are ubiquitous and there is little evidence for widespread shifts to parasitism from mutualistic ancestors (Sachs & Simms, 2006). There are several threats to the stability of mutualisms, including cheaters, exploiters, conflict over reproduction, and symbiont competition. Cheaters refer to species that are evolutionary derived from true mutualists and are obtaining rewards or services without reciprocating, increasing their own fitness at the cost of the mutualist partner. Exploiters also obtain resources or services from mutualists, but in contrast are not evolutionarily derived from mutualists (Bronstein 2001a, Yu 2001).

Reproductive conflict is also predicted to be problematic for mutualists, for example in mutualisms between ants and plants wherein the plant provides nesting space in the form of domatia in return for protection from herbivores by the presence of aggressive ants, resources allocated to reproduction are in conflict with the ants need for domatia. In response to this several species of ant have evolved to sterilise plants by removing their flowers, thus redirecting carbon towards vegetative parts, including domatia (Stanton et al.

1999, Frederickson 2009). Reproductive conflict can be overcome via vertical transmission wherein symbionts are transferred between parent and child compared to horizontal transfer of symbionts, where symbionts are obtained from the environment (Sachs and Wilcox 2006). Virulence theory, initially developed to study the evolution of pathogens, predicts that horizontal transmission of symbionts allows for the evolution of harmful symbionts in two ways, (i) by allowing symbionts to adopt selfish strategies extracting resources from their host before moving on and (ii) many unrelated symbionts can infect a host at once, leading to competition between symbionts and causing harm to the host in the process (Frank 1996). The algae *Symbiodinium microadriaticum* was experimentally manipulated to infect the jellyfish *Cassiopea xamachana*. Half of the population were manipulated so that the algae would infect the jellyfish horizontally and the other half were infected vertically, as predicted by virulence theory, the algae that were experimentally manipulated to be horizontally transmitted began to shift towards parasitism (Sachs and Wilcox 2006).

However, despite these findings, most mutualisms are horizontally transferred and persist without becoming parasitic, how do these interactions persist?

1.3 Mechanisms for stabilisation of mutualism

There are three main types of stabilising mechanisms that allow mutualisms to persist despite the inherent interspecific conflict they create: (i) by-product mutualism, (ii) partner-fidelity feedback and (iii) partner choice.

By-product mutualisms, sometimes called pseudoreciprocity (Leimar and Connor 2003) refer to mutualisms wherein there is no cost for a mutualist to engage in the mutualistic interaction and the mutualistic service (Bronstein, 2001b; Chomicki, Kiers, et al., 2020). For example, seed dispersal via the offer a food reward seen in frugivorous primates (Lambert and Garber 1998), there is no selection pressure towards cheating as dispersing the seed comes at no extra cost to the gain of fitness of consuming the fruit.

Partner-fidelity feedback occurs when the fitness of one partner is inextricably linked to that of the other, thus exploiting the partner would have a negative effect on the fitness of the exploitative partner (Bull and Rice 1991). Selection therefore favours cooperative partners in this scenario. Partner-fidelity feedback can be seen in a system of four sponge species (Wulff 2008), three of the species (*Lotrochota birotulata*, *Amphimedon compressa* and *Aplysina fulva*) which have similar life histories, all benefit from growing adhered to a heterospecific sponge whereas a fourth species (*Desmapsamma anchorata*) benefits from other sponges but its interactions are short lived. *Desmapsamma anchorata* was most likely to exploit the other three species, whereas the mutualist species do not cheat as this would reduce overall fitness (Wulff 2008). Other examples include ant/plant symbioses in which the

growth of the plant increase the ant nesting space (and sometimes food reward production), and in return this increase the amount of nutrients brought to the plant and so on and so forth (Chomicki and Renner, 2019).

Partner choice mechanisms – where a mutualist partner gives rewards exclusively to specific partners – incentivises cooperation (Sachs et al. 2004). An example of partner choice stabilisation is the obligate mutualism between the ant *Philidris nagasau* and the ant-plant *Squamellaria*; five *Squamellaria* species produce a sugary food reward concealed by a thick epidermis that only *P. nagasau* can access, allowing *Squamellaria* to invest resources in the mutualism without exploitation (Chomicki et al. 2016). Similarly, sanctioning behaviour stabilises mutualism but instead of rewarding beneficial partners sanctioning behaviour acts as a “punishment” to cheating partners. For example rhizobia experimentally forced into “cheating” were found to have 50% lower reproductive success than their cooperative counterparts (Kiers et al. 2003).

These three stabilising mechanisms do not act in isolation, for example in the termite-fungus mutualism it has been found that the recurrent process of partner choice (termites selecting fungi) leads to lifelong commitment between the fungal crop and termites, and therefore partner fidelity feedback (Aanen et al. 2009). The Fijian farming mutualism between *P. nagasau* and *Squamellaria* has all three, nutrients are provided to *Squamellaria* via defecation, which has no cost to *P. nagasau* (a by-product mutualism). The fitness of *Squamellaria* and *P. nagasau* are tightly linked, as exemplified by the tight correlation between the number of ants and the size of the domatia, which are in a positive feedback loop (Chomicki and Renner, 2019). *Squamellaria* also uses partner choice via concealed food rewards, where the sugary rewards are concealed under a thick epidermis that reduces attraction to opportunists (Chomicki et al., 2016). *Philidris nagasau* is also highly specific in the species seeds it collects (Chomicki and Renner 2016a), suggesting that partner choice in this case occurs for both partners.

1.4 Mutualistic dependence

The degree of mutualistic dependence appears to have a great effect on many aspects of the evolution of mutualisms (Chomicki et al. 2020a), even affecting the evolution of traits unrelated to the mutualistic interaction (Burin et al. 2024). However, this aspect of mutualism diversity remains relatively poorly studied. The study of mutualistic dependence is hindered by lack of consistent terminology, similar to what we see with the terms symbiosis and coevolution. The main issue with descriptions of mutualistic dependence is the conflation of dependence with specialisation, which are best understood as being two separate axes (Chomicki et al. 2020a), see Figure 1.2. The first, dependence describes the extent of fitness

loss that occurs as a result of the removal of the mutualist partner. Dependence varies from obligate, wherein the loss of mutualism results in death or loss of ability to reproduce to facultative which vary in levels of fitness lost. The second axis, specialisation, describes how many species a mutualist engages with, varying from specialists which engage with one or a few species and generalists which engage with many. Within this framework we can understand mutualisms are occurring in four categories: (i) facultative generalists, (ii) facultative specialists, (iii) obligate generalists and (iv) obligate specialists. Specialisation and dependence are correlated, but there are exceptions seen in the facultative specialists and obligate generalists. In addition to the degree of dependence, dependence can also vary in relation to the “mode” as proposed by Lewis (Lewis 1985) distinguishing between the different mechanisms that cause a species to become dependent on another species, for example a species may be obligately dependent due to a biochemical change, for example the loss of the arginine biosynthesis pathway in obligate specialist Leafcutter ants (Nygaard et al. 2011, 2016, Suen et al. 2011) but are still can separate beings, compared to endosymbionts that cannot survive, even briefly, outside of a host, due to extreme genome streamlining (Moran and Bennett 2014).

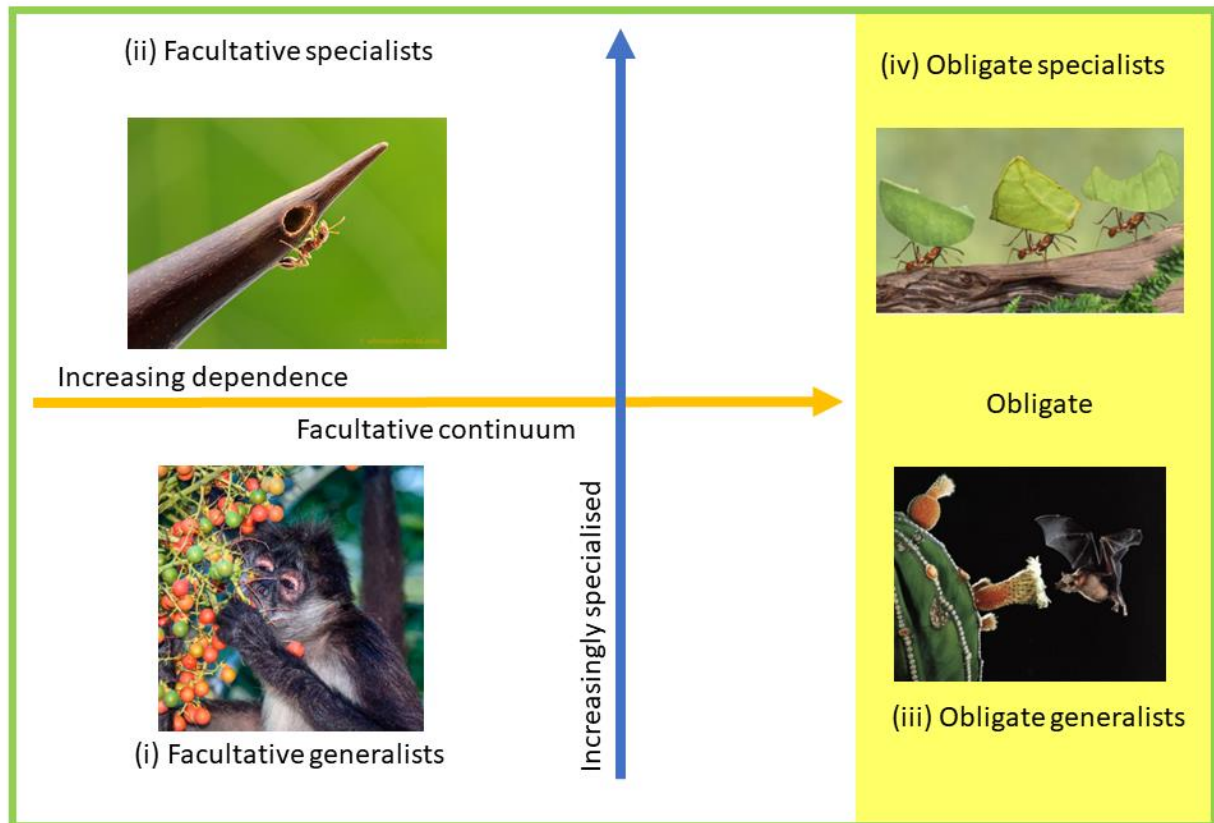


Figure 1.2 The two axes of mutualistic dependence. Dependence refers to the degree of fitness lost following the removal of the mutualist partner, ranging from obligate wherein the removal of the mutualist partner leads to either death or loss of the ability to reproduce, to facultative which itself varies in the level of fitness lost following partner removal. Obligacy is represented in a separate yellow box as it is a binary state, that does not exist on a continuum unlike facultative mutualisms and levels of specialisation. The second axis, specialisation, refer to the number of species a mutualist engages with, ranging from specialists which engage with one or a few species to generalists which engage with many.

2. Genome evolution

Most of our knowledge of genome evolution in response to mutualism comes from the study of bacterial endosymbionts. Bacterial endosymbionts, compared to their free-living relatives have been found to have smaller genomes, often smaller than 200 kilobases (kb), whereas most free-living bacteria have genome sizes of at least 1 megabase (Mb) (McCutcheon et al. 2024). This small genome size is accompanied by many gene losses (Toft and Andersson 2010, McCutcheon and Moran 2012, Moran and Bennett 2014, Latorre and Manzano-Marín 2017, McCutcheon et al. 2024), higher rates of genome evolution (Moran 1996, McCutcheon et al. 2024) and often an AT bias in genome sequence composition (Moran et al. 2008, Waneka et al. 2021).

Work examining the effects of mutualism on genome evolution between macro-mutualists (non-microorganisms) is less extensive. One study on the ant genus *Pseudomyrmex* compared three mutualist lineages with three closely related non-mutualists lineages and

found that the mutualist lineages had higher rates of molecular evolution, mirroring endosymbiont evolution (Rubin and Moreau 2016). The obligate mutualist *Ceratosolen solmsi* (a fig wasp) was found to have higher ratio of non-synonymous to synonymous substitutions (dN/dS) and have an AT-biased genome compared to other insect genomes published at the time, although this study did not compare *C. solmsi* to close non-mutualist relatives (Xiao et al. 2013). In contrast, a study comparing mutualist lichenised fungi with non-lichenised relatives found reduced rates of molecular evolution (Ametrano et al. 2022). So far, no evidence suggests that evolution of mutualism in macro-organisms leads to a reduction in genome size.

3. Ant-plant mutualisms

During the 140 million years that ants and plants have coexisted (Moreau et al. 2006), they have evolved a myriad of mutualisms. There are four main types of mutualism between ants and plants (i) seed dispersal, (ii) plant defence mediated by food rewards, (iii) ant-plants, (iv) plant farming by ants. These four categories are not distinct, for example there are ant-plants that are farmed by ants.

3.3.1 Seed dispersal

Seed dispersal is essential for the success of most plant species and is often mediated by animals, ants are the most significant invertebrate seed dispersers. Seed dispersal by ants (myrmecochory) occurs in ~ 11,000 angiosperm species in 80 families with a minimum of 101 origins (Lengyel et al. 2010) and is generally an multispecies, unspecialised interaction wherein ants benefit from the presence of elaiosomes (food rewards high in lipids and protein) and discard the seeds, increasing the reproductive success of plants (Youngsteadt et al. 2008).

3.3.2 Plant defence mediated by food rewards

Extra floral nectaries (EFNs) are widespread within angiosperms, with at least 332 genera from 93 angiosperm families and 11 genera of ferns having some interaction with ants wherein the plant offers the nutrient rich EFNs in return for protection from herbivores and pathogens (Bronstein et al. 2006). Fossil evidence combined with molecular clock data suggests that these interactions evolved in the early Eocene (56 to 33.9 million years ago) (Nelsen et al. 2018).

3.3.3 Ant-plants

Ant-plants (myrmecophytes) represent a large group with nearly 700 known species (50 families, 159 genera) of vascular plants with domatia and 113 species of ants involved in

ant-plant mutualisms from five subfamilies: Myrmicinae, Formicinae, Dolichoderinae, Pseudomyrmecinae and Ponerinae. Myrmecophytes provide their ant mutualists with domatia which they utilise as nesting space. Domatia can be derived from a variety of modified plant structures including stems, leaves, rachis, thorns, root tubers and hypocotyls (Chomicki and Renner 2015) and sometimes provide their ant mutualists with nutrition (Chomicki and Renner 2019a). The service provided to the ant-plant varies depending on ecological context and the subsequent requirements of the plant partner. Terrestrial plants primarily rely upon their ant partners as defence against herbivores whereas epiphytic ant-plants rely on ant mutualists primarily for nutrition as epiphytes are often nutrient stressed (Chomicki and Renner 2016a). Some myrmecophytes are cultivated in ant-gardens.

1.3.4 Plant farming by ants

Up until recently, it was believed that the only example of “true” agriculture was farming of fungi by ants, termites and Ambrosia beetles (Mueller et al. 2005), however, in 2016 a new highly specialised farming mutualism was discovered on two Fijian islands (Vanua Levu and Taveuni) (Chomicki and Renner 2016a). *Philidris nagasau* collects seeds of six *Squamellaria* species (*S. grayi* (occurs on Vanua Levu and Taveuni), *S. huxleyana*, *S. wilsonii*, *S. imberbis* (Vanua Levu) *S. major* and *S. thekii* (Taveuni)), and plants them in cracks in tree bark maximising for sunlight (Chomicki et al. 2020b), when *Squamellaria* seedlings emerge *P. nagasau* patrol them, protecting them from herbivory as well as actively fertilising them as soon as entrance holes emerge (Chomicki and Renner 2016a). In addition to providing nesting space in the form of domatia, five out of the six farmed *Squamellaria* species provide food rewards in the form of post-anthetic nectaries which are concealed from species other than *P. nagasau* through a thick epidermis, which *P. nagasau* bites through (Chomicki et al. 2016).

With regards to mutualistic dependence *P. nagasau* is an obligate specialist. *Philidris nagasau* obligately depends on farming *Squamellaria* for nesting space, which is required for ants to reproduce, differing from other “true” farmers which rely on their crops for food, this idea is explored more in Chapter 2 (Campbell et al. 2023). *Philidris nagasau* is also considered to be a specialist, engaging in mutualism with six species within one genus. The six farmed *Squamellaria* species are in one clade, and the crown age of this clade is approximately three million years suggesting that engaging with mutualism with *P. nagasau* led to a small adaptive radiation for *Squamellaria* mirroring the much more dramatic radiation seen within Attine ants (Chomicki and Renner 2016a). This obligate dependence is reciprocal, experimentally removing *P. nagasau* from farmed *Squamellaria huxleyana* led to high mortality rates, specifically leaves become chlorotic indicating nitrogen deficiency and sustained herbivory (Chomicki and Renner 2019a), additionally there were signs of fungal

disease (pers. Comms. G. Chomicki 2024). Non-farmed *Squamellaria* do not become diseased in the absence of ants, indicating that farmed *P. nagasau* may be preventing disease and the farmed species of *Squamellaria* have lost the disease resistance genes. The farmed species of *Squamellaria* are also specialists, exclusively engaging in mutualism with *P. nagasau*.

While the Fijian farming mutualism is by far the most specialised example of plant-cultivation by ants, it is not the first. Described by Ule in 1901 ant-garden ants first build a carton nest, a nutrient rich structure made from masticated plant material and ant secretions, then they collect and plant the seeds of epiphytes into the carton (Ule 1901). As the roots grow this provides a scaffolding, allowing for larger carton nests to be built. The epiphytes benefit from being in a nutrient rich environment. Ant-gardens are highly diverse mutualisms and are explored in greater detail in Chapter 2.

4. *Philidris*

Shattuck erected the *Philidris* genus in 1992, transferring several species from *Iridomyrmex* (*P. cordatus*, *P. cordatus fuscus*, *P. cordatus protensus*, *P. cordatus stewartii*, *P. crudus*, *laevigatus*, *myrmecodiae*, *myrmecodiae andamanensis*, *myrmecodiae mandibularis*, *myrmecodiae nigriventris*, *nagasau*, *nagasau agnatus*, *nagasau alticola*, *pubescens*). Additionally, the species *Pseudolasius brunneus* was transferred to *Philidris* (Shattuck 1992a). There is currently no key to *Philidris*, resulting in many researchers having to rely on morphospecies when reporting on *Philidris* observations. There are currently 9 accepted species of *Philidris* and a further 7 subspecies. The genus has a geographic distribution throughout much of Southeast Asia, South Asia and Australasia, see Table 1.1.

Table 1.1 Accepted *Philidris* species (AntCat, 2024). Species are differentiated from subspecies by bold text.

Species	Distribution
<i>Philidris brunnea</i>	New Guinea
<i>Philidris cordata</i>	Indonesia (type locality), Australia (north), New Guinea and Singapore.
<i>Philidris cordata fusca</i>	New Guinea
<i>Philidris cordata protensa</i>	Borneo (type locality), Indonesia and Malaysia.
<i>Philidris cordata stewartii</i>	Australia (type locality)
<i>Philidris cordata waldoi</i>	Thailand (type locality)
<i>Philidris cruda</i>	Indonesia (type locality)
<i>Philidris jiugongshanensis</i>	China (type locality)
<i>Philidris laevigata</i>	Myanmar (type locality), Singapore, India and China
<i>Philidris myrmecodiae</i>	Malaysia (type locality), Borneo, Brunei Darussalam, Indonesia, Krakatau Islands, Philippines, Singapore, Solomon Islands and India.
<i>Philidris myrmecodiae andamanensis</i>	India (type locality)
<i>Philidris myrmecodiae mandibularis</i>	Indonesia (type locality)
<i>Philidris myrmecodiae nigriventris</i>	New Guinea (type locality)
<i>Philidris nagasau</i>	Fiji (type locality)
<i>Philidris notiala</i>	China (type locality)
<i>Philidris pubescens</i>	New Guinea (type locality)

The genus *Philidris* is most well-known for its highly diverse mutualistic interactions with plants and displays a great range of mutualistic dependence. The most well-known *Philidris* species, *P. nagasau*, is an obligate specialist (as described in section 1.3.4). Several *Philidris* species are ant-garden ants (Kaufmann et al. 2001, Kaufmann 2002, Kaufmann and Maschwitz 2006, Peeters and Wiwatwitaya 2014), engaging in facultative generalist mutualism with an assembly of plants from several plant families. Additionally, there are populations of *Philidris* that do not engage in any plant mutualism¹. Our sampling covers a large proportion of *Philidris*' range, due to a lack of a clear key for *Philidris* it is not clear which species we have, thankfully we can still investigate mutualism strategy while referring to operational taxonomic units.

5. Aims

In this thesis, the broad aim is to better understand the evolution of mutualistic dependence and farming by studying genomes of *Philidris* ants.

¹ It is not currently clear whether the non-mutualist populations of *Philidris* represent truly non-mutualist species. Further taxonomic work and observations are needed to answer this question more definitively. However, discussions with a collaborator and *Philidris* collector indicates that the morphospecies represented as sample 66 (PNGspp66) has yet to be collected from plants, indicating that this may represent a non-mutualist species (pers comms Milan Janda, 2025).

First, I aim to understand the extent of plant-cultivation by ants and ask (i) how many types of plant cultivation mutualisms exist, and where are they located geographically? (ii) how taxonomically diverse are they? (iii) how do these plant cultivation systems compare in relation to taxonomic diversity, presence of domatia and food rewards, specificity, and dependence? (iv) How do they compare to the conceptualisation of insect agriculture (Mueller et al. 2005)? In Chapter 2, I perform a systematic literature search to understand the extent of plant cultivation by ants creating a database of ant-cultivated plants and plant-cultivating ants. I trace the number of evolutionary origins of plant cultivating behaviour by ants as well as introduce a new inclusive eco-evolutionary framework to better understand how farming evolves.

Next, I aim to understand the genomic consequences of a shift to obligate specialist mutualistic dependence. As highlighted in section 2, most of our current understanding of mutualist genomes comes from the study of bacterial endosymbionts. The main signatures of obligate dependence in microbial endosymbionts are (i) reduced genome size, (ii) AT bias, (iii) increases rates of molecular evolution – is this true of the evolution of macro-mutualists? Answering these questions requires a robust comparative genomic and phylogenomic framework. In Chapter 3, I assemble and annotate two reference quality genomes of *Philidris nagasau* and *P. laevigata* as well as a further 38 genomes from Illumina resequencing data. Using these genomes, I inferred a genome-wide phylogeny from 2100 orthologous genes.

The shift to obligate farming in *P. nagasau* has been enabled by dramatic shifts in behaviour. *Philidris nagasau* is highly specific in relation to its seed collecting behaviour, appears to prevent disease in *Squamellaria*, can access highly specialised food rewards (post-anthetic nectaries) that other ants cannot access and prioritises sunlight when planting seeds. These shifts indicate some level of specialisation in the chemosensory system. Simultaneously, *P. nagasau* has lost the ability to build its own carton nests. While detecting genetic shifts in behaviour is difficult compared to processes with clearly defined genetic pathways (for example, digestion and nutrition), I still expect genomic shifts as a result of these behavioural changes. Specifically, changes in changes relating to the nervous system as highlighted by Rubin and Moreau (2016) studying a classic ant-plant mutualism within the genus *Pseudomyrmex*. Mutualistic *Pseudomyrmex* provide plants with protection from herbivory in return for living space in the form of domatia and are thus highly aggressive, especially in contrast to their non-mutualist relatives which have been observed to flee from aggressors, even when this leaves their own nests at risk (Rubin and Moreau, 2016). Rubin and Moreau found evidence for eight genes related to the nervous system to be under positive selection in mutualist lineages including genes related to neurogenesis and neurotransmission and hypothesis that the extreme behavioural differences seen between

mutualist and non-mutualist *Pseudomyrmex* are as a result of modifications to the nervous system (Rubin and Moreau 2016). In addition to changes to the nervous system in general, I expect to find changes in the chemosensory system. In Chapter 4, I analyse gene family expansions and contractions throughout *Philidris* and investigate signatures of selection on the olfactory receptor (OR) repertoire of *Philidris*.

Finally, in Chapter 5, I compare rates of genome evolution to understand how the shift to obligate specialised mutualism in *P. nagasau* compared to the facultative generalist relatives. I ask whether the shift to obligate mutualism evolves similarly to antagonistic interactions between predator and prey and thus display Red Queen dynamics? Models predict that mutualists would benefit from evolving more slowly (Bergstrom and Lachmann 2003a, b), known as the Red King effect. Additionally, I investigate signatures of selection including positive selection and purifying selection and test for evidence shifts in selection intensity. I hypothesise that genes relate to the nervous system and sensory perception will experience shifts in selection, specifically under positive selection. With regards to selection intensity, entering a new ecological niche is associated with genome wide relaxed selection. Alternatively, it may be that some genes are experiencing intensified selection in response to a new ecological niche.

All previous work on genome evolution in mutualists studies the effect of shifting from non-mutualism to mutualism, this study system is unique in that it allows us to assess the effect of a shift in mutualism strategy (from facultative generalist to obligate specialist). This work highlights key genomic differences associated with the shift to obligate farming in *Philidris nagasau* compared to its facultative generalist and non-mutualist relatives and lays a foundation for future experimental work to investigate the basis of this highly specialised mutualism.

Chapter 2: The evolution of plant cultivation by ants

An adapted version of this chapter is published as Campbell, L. C. E., Kiers, E.T. & Chomicki, G. (2023) 'The evolution of plant cultivation by ants'. *Trends in Plant Science*. 28: 271–282.



Abstract

Outside humans, true agriculture was previously thought to be restricted to social insects farming fungus. However, obligate farming of plants by ants was recently discovered in Fiji, prompting a re-examination of plant cultivation by ants. Here, I generate a database of plant cultivation by ants, identify three main types, and show that these interactions evolved primarily for shelter rather than food. I find that plant cultivation evolved at least 68 times independently for crops (~200 plant species), and 16 times in farmer lineages (~37 ant taxa) in the Neotropics and Asia/Australasia. Because of their high evolutionary replication, and variation in partner dependence, these systems are powerful models to unveil the steps in the evolution and ecology of insect agriculture.

Glossary

Ancestral state reconstruction (or estimation): Phylogenetic comparative method which infers back in time the evolution of a trait throughout a phylogenetic tree, based on the distribution of the trait in extant species.

By-product mutualism: Model of stable mutualism implying that the mutualistic service or reward is a by-product and hence cost-free. In theory, by-product mutualisms should be free of conflicts, but they can be the evolutionary starting point for more complex mutualisms with high costs and benefits.

Carton nest: Nests actively built by the majority of arboreal ants. Carton nests are made of plant materials, other debris, and ant secretions.

Cheating: When an organism that is evolutionarily derived from a true mutualist obtains rewards from a partner without reciprocating in a way that increases their own fitness and decreases the inclusive fitness.

Domatium (plural domatia): Evolutionary derived plant structures that are used by symbiotic ants for nesting. Domatia can be derived from a variety of modified plant structures including stems, leaves, rachis, thorns, root tubers and hypocotyls.

Elaiosomes: Nutrient rich, fleshy structures attached to seeds. Involved in seed dispersal mutualisms wherein ants are attracted by the food reward in return for dispersal.

Extrafloral nectaries (EFNs): Plant structure that produces nectar outside of its flowers. Some plants produce EFNs as a nutrient reward to ants that defend them against herbivory.

Partner Choice: Mutualism stabilization mechanism wherein a mutualist partner exclusively rewards particular beneficial partner(s) and denies resources to others.

Partner Fidelity Feedback: A stabilisation mechanism wherein the fitness of mutualist partners are aligned, implying that “cheating” a mutualist partner would harm their own fitness, therefore there is no incentive to cheat.

Pearl bodies: Small lustrous food rewards rich in lipids, protein, and carbohydrates. Similar to EFNs, some plants offer pearl bodies to elicit a defensive response from ants.

Phorophyte: A plant on which an epiphyte grows. It should be noted that epiphytes do not gain nutrients from their phorophytes, and instead just anchor themselves onto them, often to grow higher up in the forest canopy.

Polydomous: Nesting strategy in which an ant colony inhabits several spatially separated nests.

Post-anthetic nectar rewards: Specialized food reward of epiphytic Rubiaceae (Hydnophytinae), consisting of large nectary discs, which become ant rewards once the corolla has fallen off. These nectaries maintain a high sucrose content by delaying fruit development. Symbiotic ants access these rewards by biting through the thick epidermis, which reduces attraction to opportunists.

1. Introduction

1.1 Insect agriculture – beyond fungiculture

Agriculture has been pivotal to the rise of a human-dominated world (Larson et al. 2014). Outside of humans, ‘farming-like’ systems –where organisms cultivate other species for food– have evolved across the tree of life (Silliman and Newell 2003, Hata and Kato 2006, Brock et al. 2011, Thurber et al. 2011, Pion et al. 2013, Pauli et al. 2014). However, true agriculture – which is defined by Mueller et al. as having four key characteristics: (i) habitual planting, (ii) cultivation, (iii) harvest and (iv) obligate dependency on the crop for food – has only arisen in humans and three groups of social or subsocial insects, namely ants, termites and beetles, all of which farm fungi (Mueller et al. 2005).

Recently, an additional true, obligate agricultural system has been discovered in the Fiji Islands. The system is unique in that it is the only known example of insects farming plants. The Dolichoderine ant *Philidris nagasau* obligately cultivates six species of the epiphytic plant genus *Squamellaria* (Chomicki and Renner 2016a). This means that insect agriculture is not restricted to fungal crops as previously thought. Outside this obligate plant farming by ants, there is also the cultivation of epiphytic plants by ants. These so-called “ant-gardens” were discovered over one hundred years ago (Ule 1901). However, it is unclear how they fit within the framework of farming mutualisms, and whether they represent, or are ecologically close to, true agricultural systems.

In this chapter, I performed a systematic review of ant-gardens literature and assembled a database of plant-cultivating ants and ant-cultivated plants to ask the following questions: i) how many types of plant cultivation mutualisms exist, and where are they located geographically? (ii) how taxonomically diverse are they? (iii) how do these plant cultivation systems compare in relation to taxonomic diversity, presence of domatia and food rewards, specificity and dependence? (iv) How do they compare to the conceptualisation of insect agriculture (Mueller et al. 2005)? I argue that (i) plant cultivation by ants involves a range of dependences, with partnerships ranging from loose facultative cultivation systems to tight obligate agricultures; (ii) several systems likely represent true agriculture systems, but quantifications are not possible yet as most of these symbioses are understudied; (iii) because plant-cultivation partnerships range in their level of specialization and dependence and involve many independent lineages of plants and ants, they are powerful model systems to study the ecological and evolutionary consequences of agriculture; (iv) a more inclusive eco-evolutionary framework focused on evolutionary stages is useful for understanding the evolution of farming behaviour across the tree of life.

2. Methods

2.1 Systematic Review

To assess the diversity of plant cultivation mutualisms I performed a systematic review of available literature. Using the search terms “Ant Garden” and “Ant-garden”, publications were retrieved from Web of Science (24), 21 from Google Scholar (initially 553, then specified to title only, 21), 72 from Scopus and 79 from ScienceDirect. Additionally, 23 records from other sources were used. Once duplicates were removed the remaining 168 records were screened for suitability using the title and abstract, irrelevant articles such as those that focused on ant-fungiculture, or other irrelevant topics removed. Finally, the remaining 108 records were screened via the full text to identify articles suitable for our databases. Any article with a list of species initiating or occurring in ant-gardens was included, except in the case where no new species were listed (e.g., there were several studies focusing on the behaviour and ecology between the parabiotic ants *Camponotus* and *Crematogaster*, so not every study featuring these species was included in the database). The final databases were created using information from 38 records. See Figure 2.1 for PRISMA flow diagram and supplementary Table S2.1 and S1.2 for information on the inclusion/exclusion of each record.

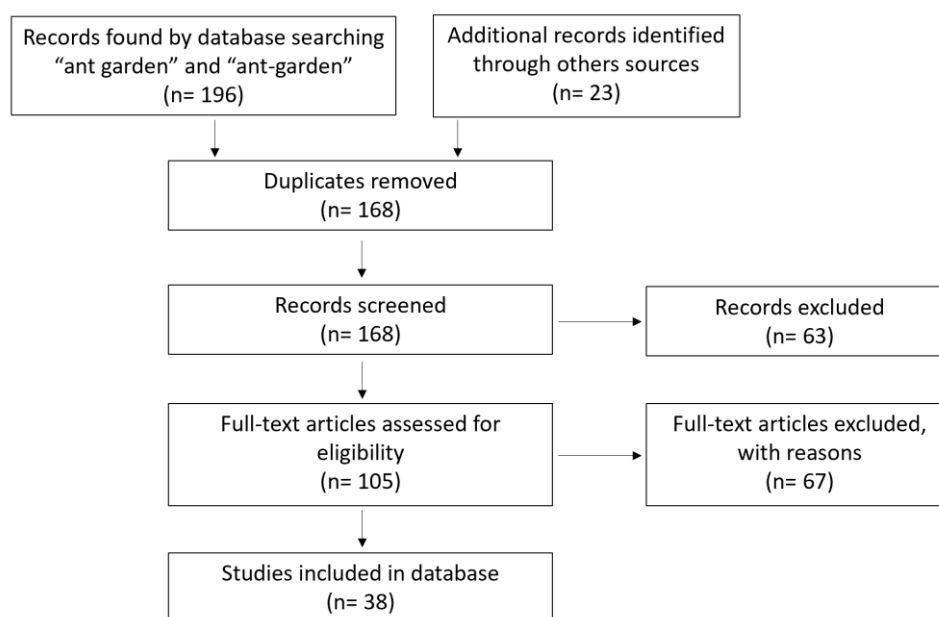


Figure 2.1 PRISMA diagram detailing our search and selection process during our systematic review.

2.2 Phylogeny

To understand the phylogenetic spread of ant-garden (AG) plants I mapped the plant species from our database onto the APGIII megatree phylogeny originally published by the Angiosperm Phylogeny Group (2009). We plotted bars to represent the number of known

ant-garden plants in each plant family, these values were normalised to make the values more comparable following the equation below:

$$\text{Normalised number of AG plants} = \frac{\text{No. of ant-cultivated of species per family}}{(\text{Total no. of species in the family} * \text{Crown Age of family})}$$

Bars were plotted using the `plotTree.wBars` function from the `Phytools` package in R (Revell, 2012). Graphical edits were made using Inkscape.

2.3 Species richness heatmap of ant cultivated plants

Geographical occurrence data for each plant species in the ant-garden plant database were collected from GBIF (GBIF.org 24 March 2021, see supplementary Table S2.7) as well as data from Chomicki and Renner (2019b) for Hydnophytinae occurrences. These data were plotted onto a map using ArcGIS Pro 2.7 (<https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview>). Data was screened for accuracy, for example occurrences of Cactaceae species outside of America were removed as well as removing occurrences where ant-gardens do not occur, for example outside of the tropics and in Africa. Then, utilising the fishnet function, a species richness heatmap was produced by calculating the number of different species in each square of the fishnet. Duplicate species were automatically removed (for example, one square containing only three occurrences of one species would have a species richness of one).

3. Results

3.1 Three Types of Plant Cultivation by Ants

Ants have coexisted with plants for at least 140 million years (Moreau et al. 2006). While they engage in a large diversity of mutualistic interactions with plants –including defence mutualisms based on **extrafloral nectaries** or **domatia**, and seed dispersal mediated by **elaiosomes** (Lengyel et al. 2010, Weber and Keeler 2013, Chomicki and Renner 2015, Nelsen et al. 2018)– actual plant cultivation only represents a small subset of these. Elaiosomes are almost always absent, consistent with the fact that the rewards come from the cultivation and not from these seed appendages as in terrestrial myrmecochorous systems.

We identified three types of plant cultivation mutualisms, which occur in three distinct geographical areas: (i) Neotropical ant-gardens, (ii) Southeast Asian and Australasian ant-gardens and (iii) Fijian agriculture (Figure 2.2-8, Figure 2.9 A-J). All three plant cultivation systems have the same core function: nutrient stressed epiphytes receive nutritional resources in return for providing ants with nesting space, however both nesting space and

nutrients are provided in very different ways across the three types of cultivation mutualisms. Nutrients are provided to plants by being dispersed into a nutrient-rich **carton nest**, constructed by ant-garden ants (Neotropical and South-East Asian ant-gardens) or by being actively fertilized with ant faeces (Fijian agriculture). The roots of planted epiphytes provide a scaffolding which increases the stabilisation of the carton nests which allows for the construction of larger nests (Neotropical and Southeast Asian ant-gardens). In addition to the structural support from the roots, epiphytes also remove excess moisture via transpiration (Yu 1994). Additionally, nest space is provided with domatia (Southeast Asian ant-gardens and Fijian agriculture).

3.1.1 Neotropical Ant-gardens

Ant-gardens were first described in Brazil by Ule in 1901 who reported ants as being precise and active constructors of ant-gardens (Ule 1901). Ule posited that foundress queens first prepare their **carton nests**, and plant epiphyte seeds. However, this was challenged by Wheeler (Wheeler 1921) and Weber (Weber 1943b) who both argued that young queens colonized the roots of already established epiphytes. Ule's hypothesis has subsequently been supported both by the presence of volatile compounds in ant-garden epiphyte seeds that exclusively attract ant-gardens ants in cafeteria experiments (Davidson 1988, Davidson et al. 1990, Seidel et al. 1990, Youngsteadt et al. 2008) and direct observations of ants collecting and planting seeds into their nests (Davidson et al. 1990, Corbara et al. 1999a, Morales-Linares et al. 2018).

The differential attractiveness of epiphyte seeds to ant-garden ants has been found to be directly correlated with the relative abundance of plants in ant-gardens (Morales-Linares et al. 2018). Elaiosomes are almost always absent apart from five Neotropical plant species (<6% of Neotropical species) (Madison 1979) and completely absent in other types of plant cultivation systems, strongly suggesting that ant-garden dispersal behaviour is not driven by food rewards. While some ant species do colonize established epiphytes (Belin-Depoux 1991) or abandoned ant-gardens (Dejean et al. 1997, Marini-Filho 1999), this behaviour is unusual and separate to the ontogeny of ant-gardens (Corbara et al. 1999a). Additionally, many Neotropical ant-gardens contain an additional mutualism wherein two ant species co-occur in the same nest referred to as parabiosis (Vantaux et al. 2007), whether it affects the farming mutualism is unknown (see *Outstanding questions*).

In terms of taxonomic diversity, we identified 75 records (including 69 identified at species level) of ant-garden epiphytes from 11 families (Araceae, Bromeliaceae, Cactaceae, Clusiaceae, Cyclanthaceae, Gesneriaceae, Moraceae, Orchidaceae, Piperaceae, Polypodiaceae and Solanaceae), scattered across the plant tree of life (Figure 2.6). The

evidence for individual species to be true ant-garden epiphytes varies widely (Table S2.3), mostly because of lack of data (see *Outstanding questions*). There are fewer ant species than plant species involved in these mutualisms: 17 records (15 reported at the species level) in 4 subfamilies Dolichoderinae, Formicinae, Myrmicinae and Ponerinae (Table S2.4).

3.1.2 Southeast Asian and Australasian Ant-gardens

While seed planting by ants was suspected in Central Java by Leeuwen in 1913 (Leeuwen and Leeuwen-Reijnvaan 1913), it was previously believed that ant-gardens do not occur in Southeast Asia or Australasia – their presence was only confirmed more recently (Kaufmann et al. 2001). Given the longer history of research on Neotropical ant-gardens, it was surprising that our systematic review found more ant-garden species in SE Asia and Australasia; with 112 potential species from 12 plant families (Apocynaceae, Araceae, Araliaceae, Gesneriaceae, Melastomataceae, Moraceae, Orchidaceae, Piperaceae, Polypodiaceae, Rubiaceae, Urticaceae and Zingiberaceae) (Table S2.3). The taxonomic diversity of Southeast Asian ant-gardens differs drastically from their Neotropical counterparts, with only 5 out of 12 families in common (Araceae, Moreaceae, Orchidaceae, Piperaceae and Polypodiaceae), but are similarly dispersed across the plant phylogeny (Figure 2.6). Like Neotropical ant-gardens, there are far fewer ant species involved: 4 subfamilies and 5 genera in the Dolichoderinae, Formicinae, Myrmicinae and Ponerinae with a total of 19 taxa, most of which only described to the genus level (Table S2.4). Southeast Asian ant-gardens also have the additional parabiotic mutualism. Interestingly, the same two genera (*Camponotus* and *Crematogaster*) that co-occur in the Neotropics also co-occur in SE Asia (Kaufmann 2002, Menzel and Schmitt 2012). Ant-gardens solely occupied by *Crematogaster* seem to be much less successful than when occurring in parabiotic association with *Camponotus* (Davidson 1988, Kaufmann 2002), both in the Neotropics and SE Asia. *Camponotus* is larger and more aggressive than *Crematogaster*, but benefits from *Crematogaster*'s superior foraging abilities (Vantaux et al. 2007, Menzel and Blüthgen 2010), again this has been noted in the Neotropics and SE Asia. Such differences in size and behaviour suggests that each species might play different roles in the ant garden, for instance in defense, seed planting, and cultivation. More work is needed to understand how parabiosis contributes to plant cultivation mutualisms (see *Outstanding Questions*).

Southeast Asian ant-gardens differ from Neotropical ones in two key ways. First, South-East Asian ant-gardens frequently involve stem or leaf **domatia** while these are absent in Neotropical ant-gardens, occurring in 46.7% of the SE Asian ant-garden plants, including four families and 10 genera (Figure 2.7). This correlates with the diversity of epiphytic ant-plants, which is concentrated in SE Asia and Australasia (Kaufmann 2002, Chomicki and

Renner 2015)). Despite the high prevalence of domatia in SE Asian ant-gardens, queens are not usually found to nest in domatia and instead nest within the carton (Kaufmann 2002)

Second, SE Asian ant-garden epiphytes (Kaufmann 2002) contribute less to symbiotic ant nutrition than in Neotropical ant-gardens: only 29% of SE Asian ant-garden plant species have food rewards as compared to 41% in the Neotropics (Figure 2.7, Table S2.3). Perhaps more strikingly, extrafloral nectaries (EFNs) are absent in SE Asian and Australasian ant-garden epiphytes (Figure 2.7, Table S2.3). Food reward in SE Asian and Australasia ant-gardens epiphytes are almost exclusively restricted to **post-anthetic nectar rewards**, found only in epiphytic Rubiaceae (34 species) (Chomicki et al. 2016), and **pearl bodies** occurring in two species (Clausing 1998).

3.1.3 Fijian agriculture

The first known system that can be defined as true plant agriculture by a non-human animal was discovered in Fiji in 2016 (Chomicki and Renner 2016a). In this system, *Philidris nagasau* (Dolichoderinae) obligately farms epiphytic *Squamellaria* plants (Rubiaceae). *Philidris nagasau* fulfils all four of the criteria for true agriculture. First, *P. nagasau* habitually collects and plants *Squamellaria* seeds under tree bark. Second, it actively fertilizes *Squamellaria* plants throughout their entire lifetime (Chomicki and Renner 2016a, 2019a) as well as protects them from herbivory and selects sites highly sun-exposed to optimize crop productivity (Chomicki et al. 2020b). Third, it harvests food rewards in the form of **post-anthetic nectar rewards** (Chomicki et al. 2016), but it does not obligately depend on crop-produced food, as workers can hunt insects when needed (Chomicki et al. 2016, Chomicki and Renner 2016a). Fourth, the ant farmer, however, obligately depends on *Squamellaria* for nesting space due to an evolutionary loss of the ability to construct its own carton nests (Chomicki and Renner 2019a).

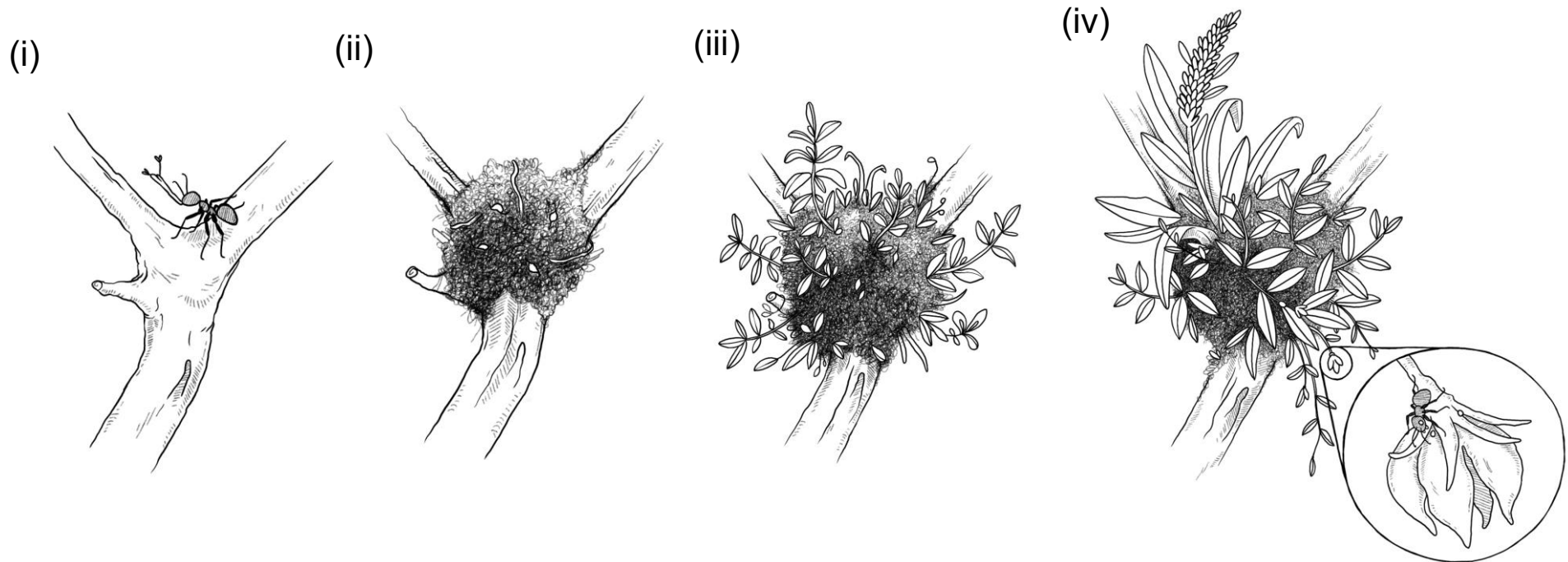


Figure 2.2 Neotropical ant-gardens. (i) Ant-garden ants build a carton nest, often aided by the phorophyte structure, such as here a branch fork, (ii) workers subsequently plant seeds inside the carton, (iii) the epiphyte roots stabilise the nest, and (iv) when epiphytes are mature, some provide rewards such as extrafloral nectar (inset). Drawings by Emma Hindhaugh.

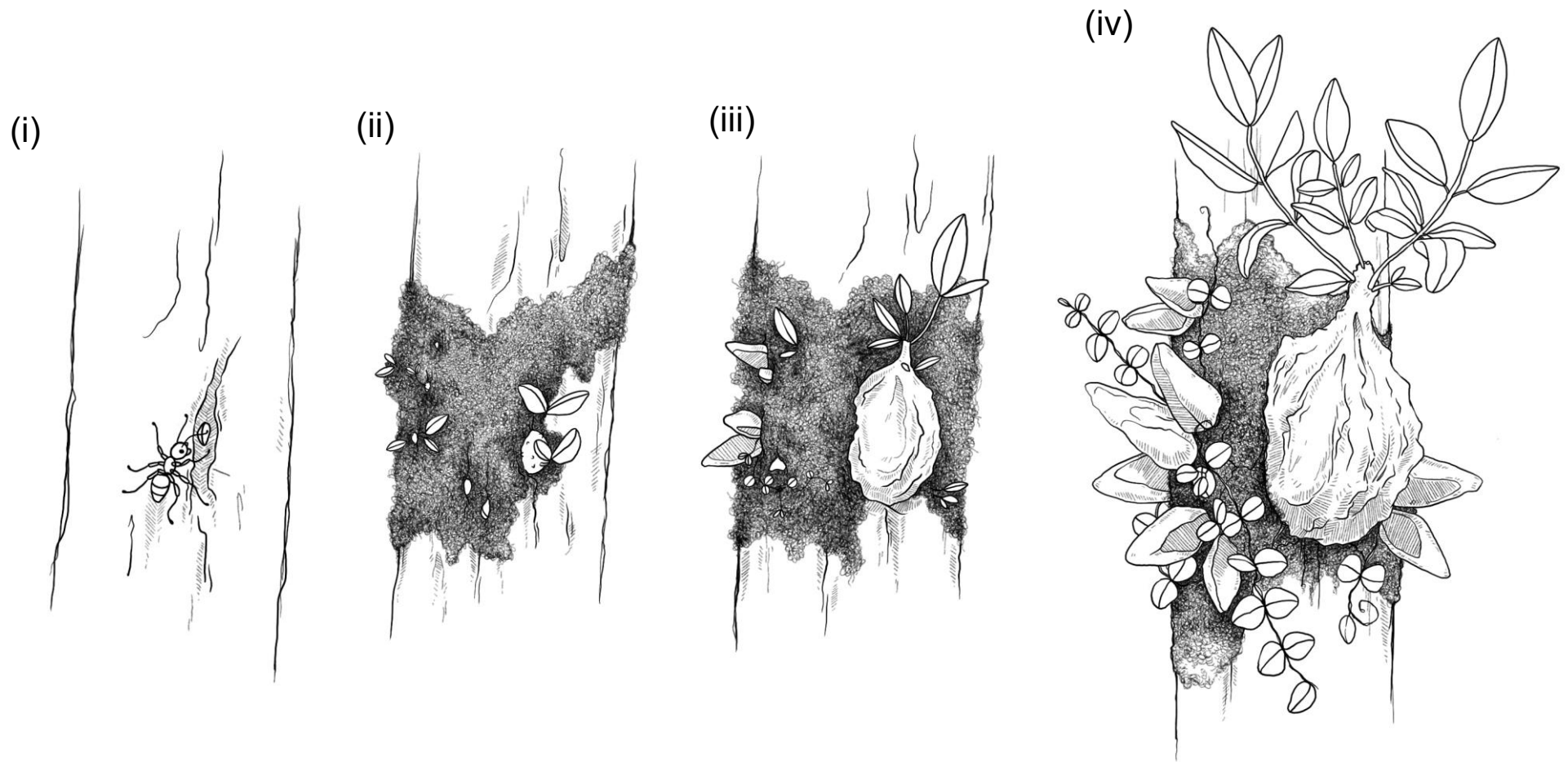


Figure 2.3 Southeast Asian/Australasian ant-gardens. (i) Ant-garden ants build a carton nest, often directly on the bark, and (ii) plant seeds of ant-garden epiphytes, some with and others without domatia. The epiphytes grow and stabilise the nest, with some starting to develop domatia where part of the (polydomous) ant colony will live, expanding the nesting site area of the colony (iii). In the mature ant-garden, domatium-bearing epiphytes provide substantial nesting site area (iv), and a large part of the colony nest there, with the majority of epiphytic Rubiaceae providing food rewards too. Drawings by Emma Hindhaugh.

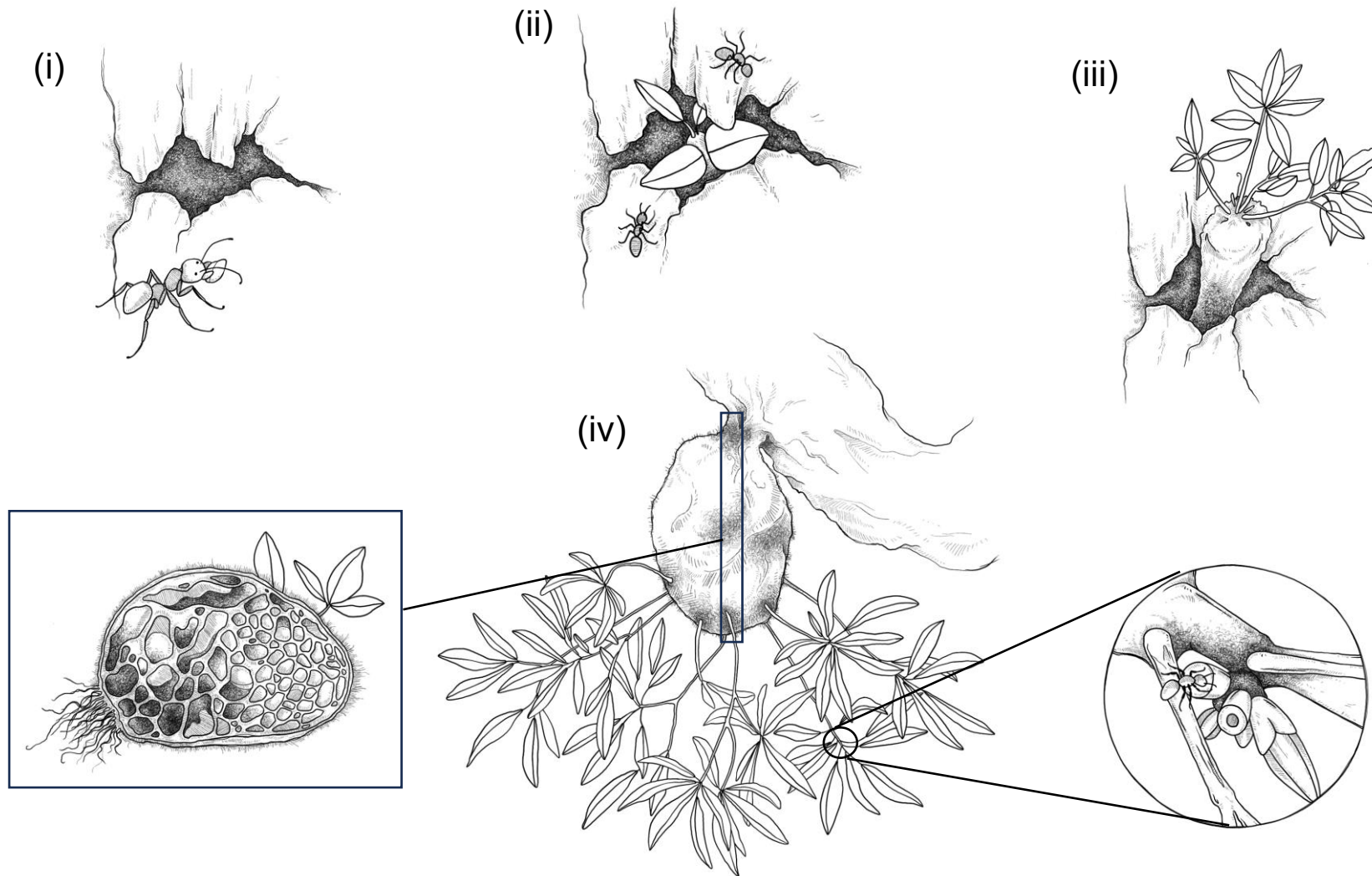


Figure 2.4 Fijian agriculture. (i) *Philidris nagasau* workers plant *Squamellaria* seeds directly in bark cracks, without a carton nest, and (ii) the seedlings emerge from the sunken cracks thanks to an adaptation of the hypocotyl ('hypocotyl foot'). Both seeds and seedlings are constantly monitored, and as soon as the domatium emerges, *P. nagasau* workers enter it to fertilise the plant by defaecation. As the *Squamellaria* plant grows (iii), it offers more nesting space to the colony, which typically consists of several dozen plants. Mature plants (iv) offer very large nesting sites in the domatia, which contain two types of wall: warty walls (in darker shade) where the ants defaecate, and smooth walls where the ants nest. They also offer post-anthetic nectar rewards, on which workers constantly feed. Drawings by Emma Hindhaugh.

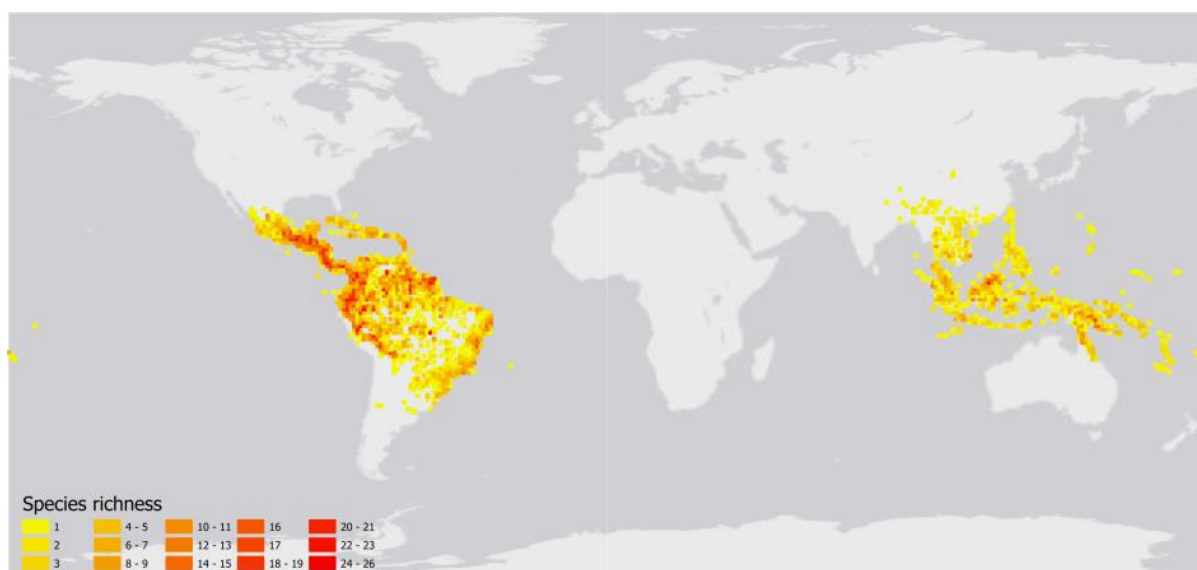


Figure 2.5 Species richness heatmap of ant-garden epiphytes based on Table S2.3 in the supplemental information online (see Table S2.7 in the supplemental information for GBIF references).

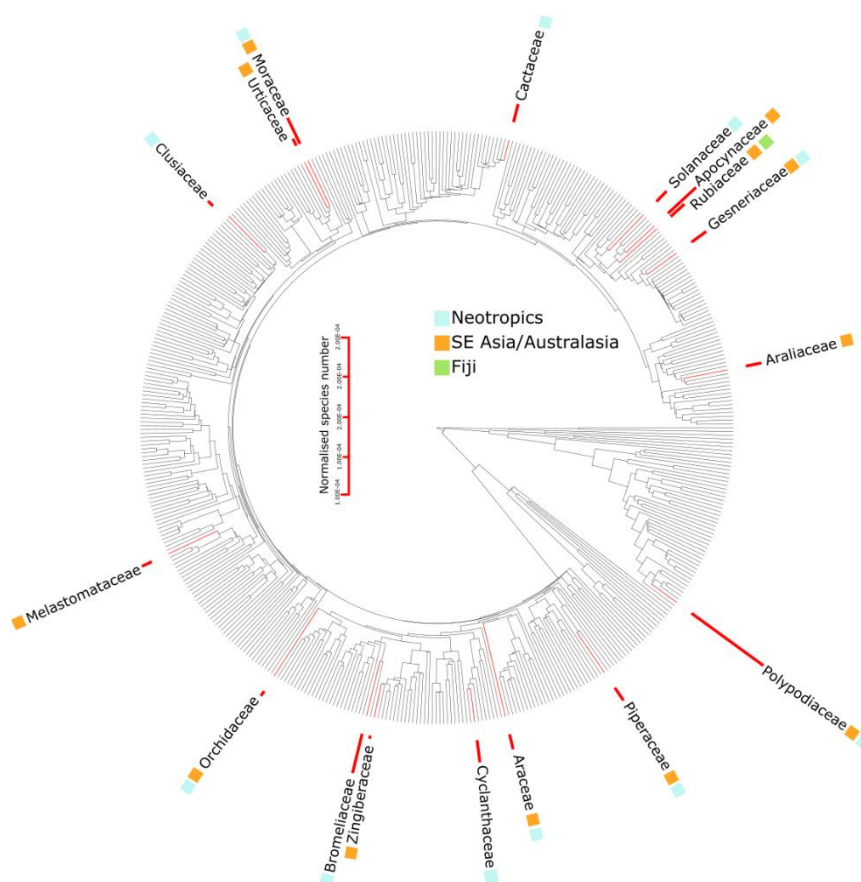


Figure 2.6 Phylogeny of vascular plant families with families containing ant-garden plants coloured in red. Bars indicate the normalised number of ant-garden species in each family see *Methods* for details of normalisation).

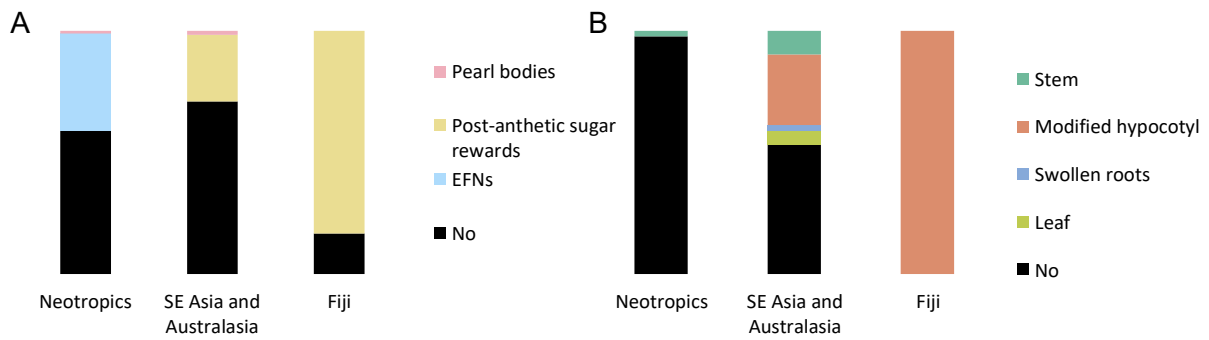


Figure 2.7 (A) Percentages of ant-garden plants with food rewards and frequency of food reward type. (B) Percentages of ant-garden plants with domatia and frequency of domatia types.

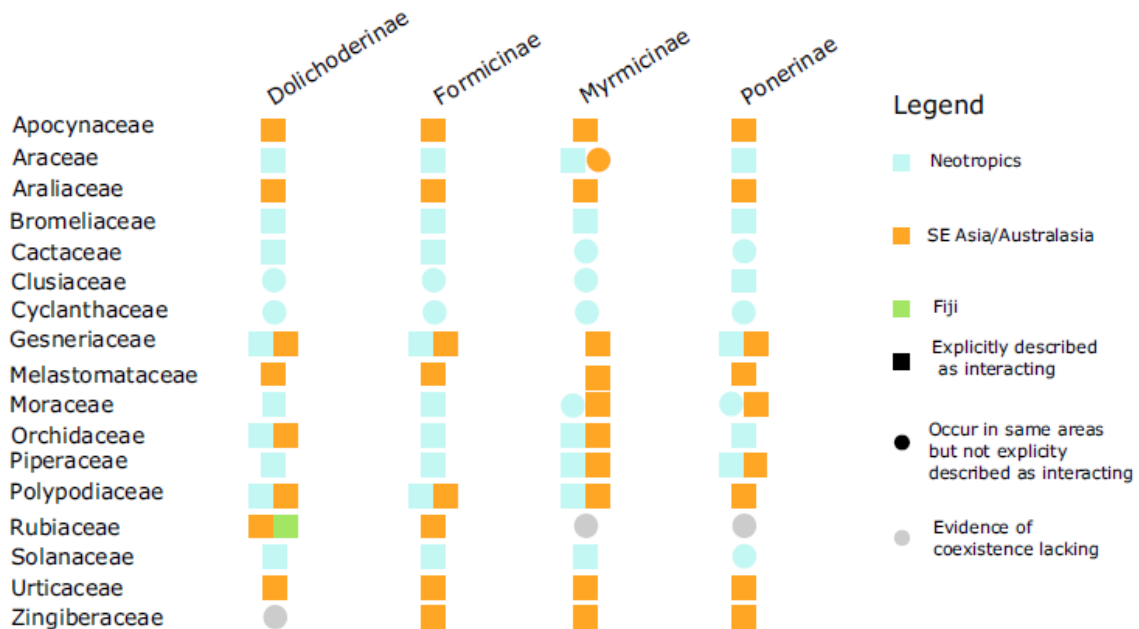


Figure 2.8 Interaction data of ant-garden ant subfamilies and ant-garden plant families. Squares indicate that there is strong evidence for these taxa interacting in ant-gardens, for example, explicit description of a plant family occurring in the ant-garden of an ant in a given subfamily. Circles indicate that these ants and plants may interact; for example, they are mentioned in the same ecological surveys. However, there is no explicit evidence of a given plant family occurring in the ant-gardens of a given ant subfamily. Grey circles indicate a lack of evidence of interaction, which, however, cannot be completely ruled out due to their co-occurrence in these geographic regions.

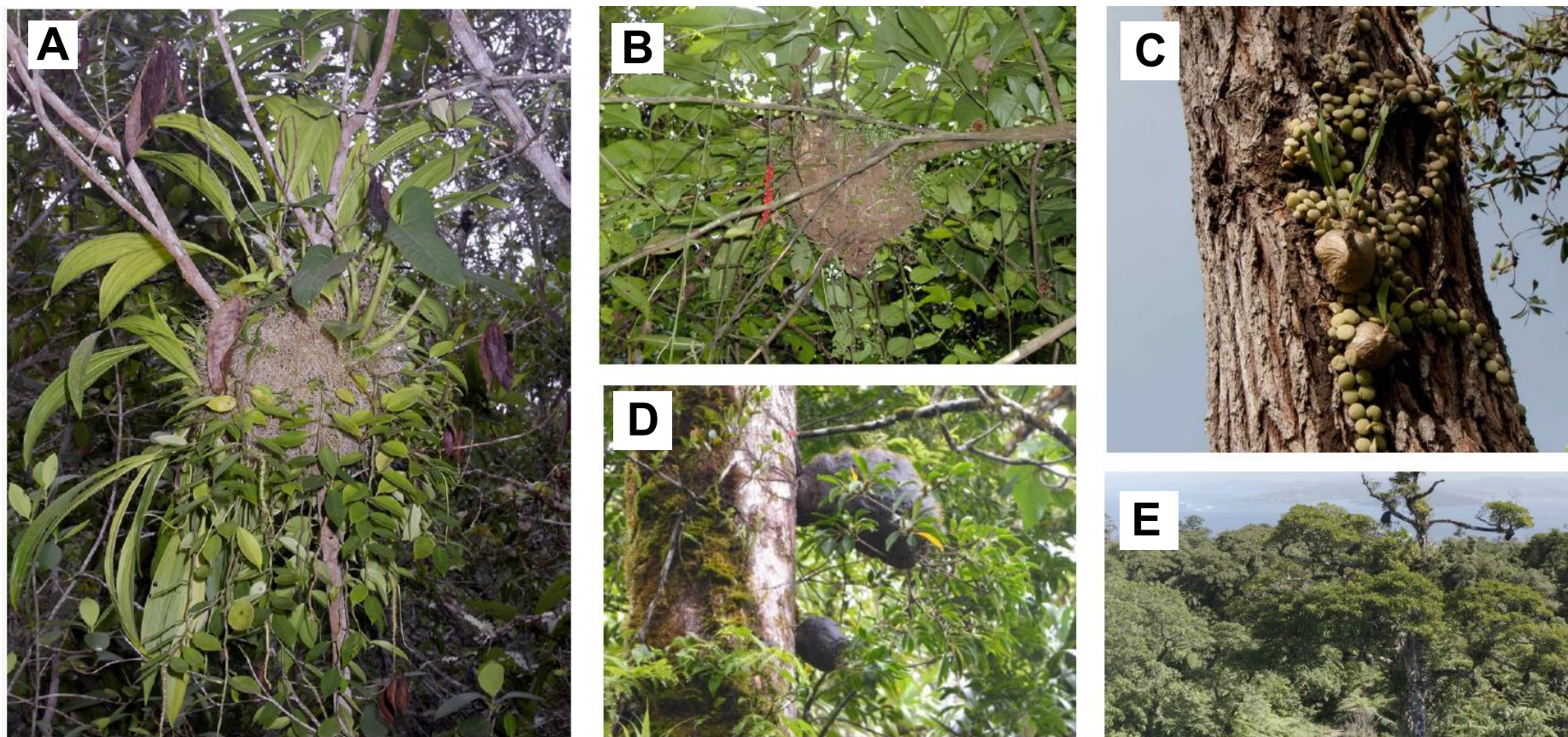


Figure 2.9 Photographs of plants cultivated by ants. (A) Neotropical ant-garden with *Coryanthes leucocorys* in Peru. (B) Neotropical ant-garden of parabiotic ants *Camponotus* and *Crematogaster*, (C) SE Asian ant-garden with *Myrmecodia tuberosa* and *Dischidia nummularia*, (D, E) *Squamellaria wilsonii* farmed by *Philidris nagasau*. Photographic credits: (A) Günter Gerlach; (B) Jérôme Orivel; (C) Milan Janda; (C) Guillaume Chomicki; (E) Benoît Chomicki.

4. Discussion

It is already accepted that *Philidris nagasau*'s farming behaviour constitutes "true" agriculture sensu Mueller (Mueller et al. 2005, Chomicki and Renner 2016a) which prompts the question are the other forms of plant cultivation by ants, Neotropical and SE Asian ant-gardens, "true" agriculture too?

Both Neotropical and SE Asian ant-gardens fulfil criterion one "habitual planting of the crop" with observations of ants collecting seeds widely reported as well as studies indicating seed preferences (Youngsteadt 2008, Youngsteadt et al. 2010). The second criterion, cultivation, is also met by both types of ant-gardens by the provisioning of a nutrient-rich environment in the form of carton nests, made up of organic debris (e.g. plant material, sand, soil, vertebrate faeces) cemented with ant secretions (Davidson and Epstein 1989, Kaufmann et al. 2001, Kaufmann 2002, Morales-Linares et al. 2017). Such carton nests are rich in nitrogen, phosphorus and potassium, thus providing a substrate that enhances plant growth. Not only do plants benefit from the nutrient-rich environment, but in at least some species, aggressive ant-garden ants also defend the plants against herbivory (Davidson and Epstein 1989, Dejean et al. 2000).

The third criterion –harvesting of the crop for food– is not ubiquitous (Figure 2.7, Table S2.3). It however occurs in 41% of Neotropical ant-garden species, mostly through extrafloral nectaries (Figure 2.7) and only 29% of SE Asian ant-garden species. Finally, the fourth requirement –obligate dependence on the crop for food– does not occur. However, there is experimental evidence suggesting that ant-garden ants are obligately dependent on epiphytes to maintain their nests structure wherein ant-garden epiphytes were experimentally defoliated, which led the nests to deteriorate by losing 75% of the carton in a week, meaning the ants could no longer nest and reproduce (Yu 1994). Similarly, SE Asian ant-garden ants are not obligate dependent on cultivated plants for food and it is unclear whether they obligately rely on plants for nesting, while SE Asian ant-garden ants have been described as obligate there is inconsistency in how the term "obligate" has been used and further work should focus on better quantifying dependence in these systems (see: Box 1).

The Fijian farming system differs from both Neotropical and Southeast Asian/Australasian ant-gardens in being more specialised and reciprocally obligately dependent. *Philidris nagasau* cultivates six species of *Squamellaria*. Typically *Squamellaria* 'farms' are monocultures of a single species, and more rarely farmers can cultivate up to three closely related *Squamellaria* species (Chomicki and Renner 2016a, 2019a) while in both Neotropical and South-East Asian ant-gardens these are typically diffuse assemblages spanning a large taxonomic breadth (Figure 2.8, 1.9 A-E, Table S2.3). In addition to the taxonomic

specialisation, farmed *Squamellaria* species show high levels of morphological specialisation including differentiated areas within the domatia smooth and highly absorptive areas, which defines different function in the symbiosis: nesting and rearing brood for the former, and defecating to fertilize the host in the latter (Chomicki and Renner 2019a) as well the evolution of food rewards which can only be accessed by *P. nagasau* (Chomicki et al. 2016).

Fijian agriculture also differs in relation to dependence, both the ant *P. nagasau* and the *Squamellaria* plants are obligately dependent on the mutualism. The ant farmer dependence on the crop is mediated by the loss of carton nest building behaviour (Chomicki and Renner 2016a), while the dependence of the crop on the farmer is not fully clear, but ant-exclusion experiments suggest that it might be mediated by a role of the ant farmer in disease protection (Chomicki and Renner 2019a).

4.1 Farming for shelter, not food

A major finding from our database is that less than half of the epiphyte species cultivated by ants provide food rewards (Figure 2.7A, Table S2.3), which strongly suggests that plant cultivation by ants is driven by an incentive for shelter rather than food. In contrast, structural support is ubiquitous. This is true across the gradient of specialization and dependence of these partnerships. A key illustration of this occurs in one of the six obligately farmed Fijian *Squamellaria* species (*S. grayi*), which has secondarily lost food rewards, yet is still cultivated in the same way by *P. nagasau* farming ants (Chomicki et al. 2016).

The idea of obligate nutritional dependence (Mueller et al. 2005) as one of the four steps of true agriculture put forward by Mueller et al. (Mueller et al. 2005) stems from the fact that high intensity agriculture for food generates food surpluses on which population size is dependent. In this regard, the fungiculture of attine ants and termites is remarkable with attines having large colonies containing millions of workers and nests as large as 600 square metres (Hölldobler and Wilson 1990). This shows a striking convergence with human agriculture.

Our findings in plant cultivation mutualisms argues for the inclusion of services other than nutrition –such as shelter– in Mueller et al. (Mueller et al. 2005)’s definition of true agriculture. The consequences for ants not having adequate access to nesting space is similar to not having adequate food: reduction in fitness/reproductive success. Not only are ants just as dependent on nesting space as they are on food, but nesting space is often the most limiting resource in tropical rainforest environments (Philpott and Foster 2005), largely because this is where ant abundance is maximal (Davidson et al. 2003). In rainforests, a large proportion of ants are cryptic herbivores, relying on the virtually unlimited plant carbon bank either directly by feeding on plant secretions such as extrafloral nectar or indirectly via

cultivating honeydew-producing Hemipteran insects (Blüthgen et al. 2003, Davidson et al. 2003). Given that food is not as limited, it makes sense to include farming for shelter within our understanding of agriculture. Besides the ecological relevance of farming for shelter, we humans also engage in agriculture for many non-nutritive purposes such as farming timber that is used for shelter, or for plants providing textiles such as cotton or plants farmed for pleasure such as coffee, cannabis or kava, or even ornament (Schultz 2022).

4.2 An Evolutionary Framework for Agriculture

While what should or should not be considered ‘true’ agriculture may be considered semantics, we argue that including a broader diversity of farming mutualisms, including those that are not obligate, within the framework of agriculture is essential for three reasons. First, it offers evolutionary replication to address a number of comparative questions on insect agriculture. Second, it provides younger systems in which to dissect the early evolutionary stages of the evolution of agriculture. Third, it provides farming systems with a wide range of dependence and specialization (see Box 1), which allow to ask how these two key variables shape the evolution of insect agriculture. We expand on the promise of plant cultivation mutualisms as models in insect agriculture (see Box 2).

Rather than a focus on particular characteristics as the defining feature of true agriculture, we propose an eco-evolutionary framework for agriculture wherein we recognize stages in the evolution of agriculture. This approach allows us to encompass all types of farming mutualisms, including those considered to be ‘farming-like’ systems, while at the same time retain the distinctiveness of true agriculture that Mueller et al. (Mueller et al. 2005) defined, and that we can further extend beyond other than food.

We recognize three stages in the evolution of agriculture, namely ‘resource expansion’, ‘adaptive cultivation’ and ‘obligate agriculture’. The first stage – ‘resource expansion’ – involves one species expanding its resource (such as food), through the cultivation of another species. This farming behaviour results from preadaptation of the farmer and the crops and does not involve any coevolutionary adaptation yet. This is seen in the damselfish farming mutualism, where the fish uses weeding to increase its algal crop resource (Hata and Kato 2006), the cultivation of bacteria by social amoeba (Brock et al. 2011) or fungal farming in marine snail (Silliman and Newell 2003). This is also seen in three-toed sloths which engage in a three-partner mutualism involving moths and algae. Sloths descend their trees once a week to defecate simultaneously delivering their mutualistic moths to their oviposition site. Adult moths actively seek out sloths and their presence in sloth fur increases nutrient availability to algae via decomposition and mineralisation, this in turn increases the growth of nutritious algae which the sloths benefit from eating – thus sloth farm algae as by-

product this behaviour (Pauli et al. 2014). Another example involving ant/plant systems are the so-called ‘devils gardens’, which involves *Myrmelachista* ants generating single-species strands of their plant hosts by killing other plant species using an ant-made herbicide (formic acid), yet lacking any form of seed dispersal (Renner and Ricklefs 1998, Frederickson et al. 2005).

The second stage – ‘adaptive cultivation’ – involves systems which are often generalist or facultative and show some adaptations yet lack reciprocal obligate dependence. Many Neotropical and South-East Asian ant-gardens fit in this category. Another example is the fungus, *Morchella crassipes*, which farms the bacteria *Pseudomonas putida*. *P. putida* is dispersed throughout the *M. crassipes* fungal network, *M. crassipes* transfers carbon to *P. putida* which is then harvested and consumed (Pion et al. 2013). Similarly, the polychaete worm *Platynereis dumerilii* attaches pieces of *Ulva* and *Cystoseria* algae to its own self-grown mucus tube which acts as a substrate for its algal crop (Woodin 1977, Gambi et al. 2000).

Finally, we argue that obligate agriculture should involve the four farming steps that Mueller et al. (Mueller et al. 2005) described, but with obligate dependency including services beyond nutrition, such as shelter – as discussed above. This includes the classic fungus farming in attine ants, termites, and beetle, and the Fijian *Squamellaria* plant farming by *Philidris nagasau* ants.

We recognize that these stages may not always be clear-cut cases, nor stable states.

However, we think that they are useful because they place the emphasis on processes in farming evolution. It is important to note that linear evolutionary transitions from resource expansion to adaptive cultivation, to obligate agriculture may happen only rarely. Rather, most farming mutualisms may end in the first, or more rarely the second category.

Documenting transitions between these three stages may also prove difficult as transitional stages may prove evolutionary unstable. In the farming ant-genus *Philidris*, a clear transition from adaptive cultivation to obligate agriculture occurred when ant-garden type ants from Southeast Asia colonized Fiji (Chomicki et al. 2017). Here, adaptive cultivation relied on a key pre-adaptation to farming (seed-dispersal in carton nest) in SE Asian *Philidris*, but subsequent coevolution drove the origin of obligate agriculture in Fiji (Chomicki and Renner 2016a).

In light of our analysis, we propose defining agriculture as the full spectrum of behaviours through which a species (the farmer) cultivates another one (the crop) for a service, such as

a food, shelter, chemicals, ornament, pleasure. It applies to both humans and non-human farmers alike, and includes minimally two factors, namely a form of cultivation, and a service harvested by the farmer. As explained above, this allows us to encompass the whole eco-evolutionary process of agricultural evolution, rather than only the few highly specialized obligate coevolved systems. This includes the cultivation of non-domesticated species, and early stages where cultivation occurs because of ecological pre-adaptations rather than adaptation resulting from cultivation. Our framework retains the four steps, whilst expanding to include functions other than nutrition, that Mueller et al. (2005) envisioned for obligate agriculture. We argue that such framework will help shed light on the evolutionary processes and forces at play in agriculture. Because plant cultivation mutualisms show a range of stages, they provide important model systems to study the evolution of agriculture (see Box 2).

Concluding remarks

Plant cultivation mutualisms include three main types –Neotropical and Southeast Asian ant-gardens, and Fijian obligate agriculture. In these systems, farming is for shelter, and is likely being driven by the limitation of nesting space in tropical forest canopies, rather than food. This forces us to reconsider our definition of agriculture to include the dependence on shelter rather than exclusively on food and may in turn help us to decipher the evolutionary steps in the origin of agriculture. These farming systems have an unrivalled level of evolutionary replication, and they also vary greatly in the level of dependence and specialization (see Box 1) of both crop and farmers. This offers unique opportunities to study the ecology and evolutionary of agriculture, using large cross-species comparative phylogenetic framework and field studies (see Box 2). Despite the great promise of plant cultivation mutualisms as tools to study agriculture, many aspects of their biology remain unknown. Below we raise a few key outstanding issues, from fundamental research in the ecology and evolution of these interactions to the improvement of our own agriculture (see *Outstanding questions*).

Outstanding questions

Are ant gardens truly absent from Africa?

Our systematic review suggest that ant-gardens are absent in Africa. Does it reflect a lack of research, or a biological reality? While much fewer as compared to the Neotropics and South-East Asia, there are still a wide range of ant-plant interactions in Africa, as well as a large number of vascular epiphytes. There are reports of arboreal ants nesting in the roots of epiphyte communities, which suggest that ant gardens are biologically plausible in Africa, especially in the West African rainforests. Further ecological surveys are needed to test this.

What is the effect of agriculture on farmer demography?

Demographic increase driven by the constant and large food production enabled by agriculture is often cited as the major reason for success of humans and leafcutter ants. Yet the extent to which agriculture truly contributes to demography has not been tested empirically. The evolutionary replication of plant cultivation mutualisms offers the opportunity to test this hypothesis. One approach would involve comparative phylogenetic analyses comparisons of colony size of farming ants and their non-farming closest relative. The ant genus *Philidris* would be a particularly useful as some species are facultative farmers, which would allow demographic comparisons at the population level. Experimental manipulations involving ant-garden epiphytes with and without food rewards could further dissect how shelter-only versus shelter plus food affect ant farmer demography.

What drives the obligate dependence of plant on ants?

While nutrient fertilization of *Squamellaria* by *Philidris nagasau* is highly efficient, nutrient starvation is unlikely the driver of obligate dependence on the farmers. Farmed *Squamellaria* species suffer more severe cases of disease than non-farmed but ant-occupied generalist *Squamellaria* during ant removal experiments, suggesting that *P. nagasau* provides protection from disease. The mechanistic basis is entirely unknown. Could bacteria associated with ant farmers be driving this function, as in the attine ant fungiculture?

What cues drive epiphyte selection by ant-garden ants?

An important study showed that seed odour consisting of five chemicals drives the seed-collecting behaviour in a Neotropical ant-garden. No such studies have been performed for Southeast Asian, Australasian, and Fijian systems. Are there convergent chemical signatures of seed odour across plant cultivation systems? Are all plant cultivation systems relying on odour or does morphology (for example seeds mimicking ant pupae) play a role? Do chemical cues drive the large epiphyte range of farmer specificity?

Ant garden occupants or exploiters

Some common ant garden occupants –such as some fern species (Table S2.3)– have no evidence of being ant-dispersed. Are they still inadvertently ant-dispersed through spores sticking on the ant cuticle or are they wind-dispersed? Or could they represent a separate category: ant-garden occupants. Further work should confirm whether ant-garden occupants exist, specifically ferns. If they are not ant-dispersed, what are their ecological role: Are they mutualists, commensalists or even parasites of ant-gardens?

Does parabiosis play a role in the ecology of ant gardens?

Parabiosis is a mutualisms involving two ant species nesting together and sharing tasks. Some ant-gardens are cultivated by parabiotic ants (e.g., *Camponotus femoratus* and *Crematogaster cf. limata parabiatica*). Because in a parabiosis there is a dimorphism with one of the ant species being larger-bodied than the other, does it play a role in ant-garden ecology?

Are there other obligate and specialized plant agricultural systems?

Obligate *Squamellaria* plant farming by ants was discovered in Fiji in 2016. Yet, these plants belong to a clade where highly specialized interaction with the same ant subfamily evolved four times. The majority of these interactions occurs in remote areas of New Guinea and have not been studied. Moreover, our database revealed that the existence of over 200 ant-garden plant species, some of which may form obligate relationships. More research is needed to study the level of dependence of these partnerships.

Could plant farming by ants inform human agriculture?

The use of bacteria by fungus-farming ants to combat disease has inspired some crop breeding programs using root microbiomes. Understanding more about plant farming by ants could similarly inform human agriculture. For instance, the extreme efficiency of nitrogen uptake inside the domatium of ant-farmed *Squamellaria* could hold clues on increasing nitrogen-use efficiency on our crops. More research is needed to understand the genetic basis of nitrogen absorption in this system.

Box 1: The challenge and opportunities of defining dependence in plant cultivation mutualisms.

Comparing and quantifying levels of dependence of ant-garden species has proven difficult for two reasons.

Firstly, specialisation (generalist vs. specialized) and dependence (facultative vs. obligate) are often conflated. This issue is not unique to the study of ant-gardens. A second problem is there is little consistency about how authors have referred to systems as being “obligate”. For example, Kaufmann (2002) refers to ant species which plant epiphyte seeds as obligate mutualists whereas Yu (1994) refers to ant-garden ants as being obligate partners of epiphytes due to their nests falling apart following defoliation experiments. Youngsteadt regularly refers to ant-garden ants as obligate but does not define what it means to be obligate (Youngsteadt, 2008). Determining whether a partnership is obligate typically requires experimentation. Experimental manipulations such as the removal of epiphytes (Yu 1994) or ants (Chomicki and Renner 2019a), permit to ascertain the level of dependence of a plant cultivation mutualism. In Southeast Asia, some ant-garden ant species are observed to nest outside of ant-gardens (E. Kaufmann, 2002; M. Janda pers. comm to G.C.) suggesting that there is a gradient of dependence amongst ant-garden ants.

Despite the difficulty to determine the level of dependence, plant cultivation mutualisms offer an unprecedented opportunity to probe how the level of dependence and specialization affect farming mutualisms (see: *Plant cultivation mutualisms as model systems in insect agriculture*).

Chomicki et al. (Chomicki et al. 2020a) proposed a framework wherein dependence and specialisation are clearly differentiated, splitting mutualism into four categories with each of the four combination of dependence and specialization (facultative generalist, facultative specialist, obligate generalist, obligate specialist). Because both dependence and specialization are frequently asymmetric, they have to be defined at the organism level rather than at the interaction level (Chomicki et al. 2020a).

Specialization is easier to assess compared to dependence, as it concerns the number of mutualistic partners. Occurrence data can inform of the partner breadth of a species. However, this can vary locally and be unevenly distributed, implying that sampling at different locations could yield distinct partner species and species number. The evolutionary conceptualization of specialization (Chomicki et al. 2020a) focuses on whether species associate with few specific (specialized) versus many unspecific partners (generalist). This place the emphasis on traits –which can in turn be traced along phylogenies– rather than on the absolute number of partners, which in generalist interactions, can be a function of sampling effort. In South-East Asian ant-gardens, the generalization of plant cultivation via the recruitment of (unrelated) new epiphyte species led to a specialization of the mutualism in plant side, sometimes resulting in new traits (Chomicki et al. 2017). Similarly to attines which can differentiate between different fungal cultivars (Mueller et al. 2004), it is clear that ant-garden ants can differentiate between seeds from ant-garden epiphytes and non-ant-garden epiphytes via chemical cues (Youngsteadt, 2008), likely also mediating the level of specialization of these mutualisms. Understanding how the level of specialization affects the stability and efficiency of plant cultivation mutualisms is an open question, and this in turn has the potential to inform insect agriculture in general.

Box 2: Plant cultivation mutualisms as model systems in insect agriculture

The study of insect fungiculture has provided many insights into mutualism, agriculture and social evolution (Currie et al. 1999, Poulsen and Boomsma 2005, Schultz and Brady 2008, Aanen et al. 2009, Nygaard et al. 2016). Here we argue that there are features of plant cultivation systems that have the potential to provide additional insights in insect agriculture for three main reasons.

First, it offers evolutionary replication to address various comparative questions on insect agriculture, such as the effect of agriculture on farmer demography (**Outstanding questions**). Such level of replication is unique within farming systems: there are at least 65 independent origins in the plant lineages (31 in Neotropical ant-gardens, 33 in Southeast Asian ant-gardens, and one in Fijian agriculture), and minimally 15 origins in the ant lineages (10 in Neotropical ant-gardens and 5 in Southeast Asian ant-gardens) (Supplementary Tables S3 and S4). This compare to 9 origins of fungiculture in insects (1 in ants, 1 in termites and 7 in beetles) and 6 in fungal lineages (3 farmed by ants, 1 farmed by termites, and 2 farmed by beetles (Mueller et al. 2005)). Such evolutionary replication provides statistical support to ask a wide range of evolutionary and ecological questions on insect agriculture.

Second, it provides younger systems (and evolutionary replication) in which to dissect the early evolutionary stages of the evolution of agriculture. For example, which traits were pre-adaptations to agriculture, and which ones have evolved subsequently? What traits are pre-requisite for agriculture to evolve? Phylogenetic comparative analyses can be used to compare agricultural systems with their close, non-agricultural relatives. Specifically, **ancestral state reconstructions** can be used to unveil the association and evolutionary directionality in the evolution of farming traits (Leeuwen and Leeuwen-Reijnvaan 1913, Kaufmann et al. 2001). For instance, seed and fruit traits have likely constrained the evolution of plant cultivation, since there are maximum weight and minimal size that an ant can carry (and this vary across species in function of traits such as body size). A tabulation of fruit types across plant-cultivated genera and their sister groups revealed that fleshy fruits are prevalent. Small dust-like seeds may not be suitable to ant dispersal (Table S2.3). Food rewards, such as EFNs, do not appear to have facilitated the evolution of plant cultivation, consistent with the key role of nest support rather than food (Table S2.3). The expression of plant compounds on seed cuticles that ants can identify such as 6-MMS or oleic acid might also play a role in the initial recruitment of epiphytes that become ant-cultivated.

Third, it provides farming systems with a wide range of dependence and specialisation, which together with the evolutionary replication, provides the opportunity to ask a number of evolutionary and ecological questions about how these two key variables shape insect agriculture. For example: how are farming mutualisms with different level of dependence stabilized? Recently, it has been shown that mutualisms with varying levels of dependence have been found to rely on different stabilising mechanisms, for example mutualisms with low levels of dependence often rely on **by-product** mutualisms whereas **partner choice** requires a closer association (Chomicki et al. 2020a). In the Fijian plant agriculture, two distinct mechanisms stabilize the partnership: (i) a crop-mediated mechanism involving the production of food rewards targeted to the farmer (**partner choice**) (Chomicki et al. 2016), and (ii) tight positive correlations between the ant colony size and the size of the domatium (**partner fidelity feedback**) (Chomicki and Renner 2019a). How the more diffuse ant-gardens are stabilised is unclear.

Along these lines, one particularly interesting area would be to investigate how stabilising mechanisms relate to the diversity of the “crops”, especially since both fungus farming insects and fungal crops appear to maintain high relatedness in fungiculture systems (Aanen et al. 2009), whereas ant-cultivated plant monocultures are rare except in the Fijian system. *In vivo* experiments varying the number of epiphyte species in ant-gardens and probing the potential plant-plant conflict or cooperation could be used to address this question. Crop-crop negative interaction – wherein fungal crops are hostile to the presence of unrelated fungi– are an important component of higher attine-fungal farming (Poulsen and Boomsma 2005), but whether it occurs at all in plant cultivation is unknown.

Supplemental material (Chapter 2)

Table S2.1 Systematic review of studies collated from our literature search (see Methods for details on search criteria). Studies were included in our database if they (a) describe a plant cultivation mutualism by ants – this does not include all seed dispersal and (b) the species discussed in the study were not already featured in our database.

Reference	Included?	Notes
(Bain et al. 2014)	Exclude	Title: How to be an ant on figs Review on interactions between ants and figs. No new recordings but does refer to work featured in our database (Wheeler 1921, Davidson 1988, Davidson and Epstein 1989).
(Beattie 1989)	Exclude	Title: Myrmecotrophy: Plants fed by ants Focus is ant-plants, not plant cultivation.
(Belin-Depoux 1991)	Exclude	Title: Ecology and evolution of ant gardens in French Guiana [Ecologie et evolution des jardins de fourmis en Guyane Francaise] Describes the epiphytes as establishing themselves into the gardens, not being cultivated by ants. "Whereas the plant partner settles first on a tree in a plantation, the ants secondarily entering the root-ball to build their nest, the ant partner is the first settler on rainforest trees, epiphytes later establishing themselves on their nests."
(Birer et al. 2020)	Exclude	Exclude – no new species here and described as plants establishing themselves. Title: Disentangling the assembly mechanisms of ant cuticular bacterial communities of two Amazonian ant species sharing a common arboreal nest Study focuses on bacterial communities in the cuticle of <i>Crematogaster</i> and <i>Camponotu</i> not on plant cultivation.
(Blüthgen et al. 2001)	Exclude	Title: Ants as epiphyte gardeners: Comparing the nutrient quality of ant and termite canopy substrates in a Venezuelan lowland rain forest Study about comparing the nutrient quality of ant gardens to other substrates not typically inhabited by epiphytes (specifically, ant and termite nests and galleries)
(Booth et al. 2009)	Exclude	Title: Characterization of 8 polymorphic microsatellite loci in the neotropical ant-garden ant, <i>Camponotus femoratus</i> (Fabricius)
(Bredeweg and Salles 2005)	Exclude	- Useful for population genetics only. Title: The Ants' Garden: Complex interactions between populations and the scalability of qualitative models
(Carrias et al. 2012)	Exclude	Development of modelling methods rather than description of new species / survey. Title: An ant-plant mutualism induces shifts in the protist community structure of a tank-bromeliad Study of protist communities, not about the ants / plants.

(Céréghino et al. 2010)	Exclude	Title: "Ants mediate the structure of phytotelm communities in an ant-garden bromeliad" "We studied the influence of its associated ant species (<i>Pachycondyla goeldii</i> and <i>Camponotus femoratus</i>) on the physical characteristics of the plants, and, subsequently, on the diversity of the invertebrate communities that inhabit their tanks." Not a survey of species / discovery of new species
(Céréghino et al. 2011)	Exclude	Title: "Ant-plant mutualisms promote functional diversity in phytotelm communities" No new species – focus of study is on invertebrate communities living within the AG plant <i>Aechmea mertensii</i> (which is also a phytotelm).
(Chomicki et al. 2017)	Include	Title "The assembly of ant-farmed gardens: Mutualism specialization following host broadening". Provides list and phylogenies of AG ants and plants.
(Clausing 1998)	Include	Title: Observations on ant-plant interactions in Pachycentria and other genera of the Dissochaeteae (Melastomataceae) in Sabah and Sarawak <i>Pachycentria constricta</i> and <i>P. glauca</i>
(Clausing and Renner 2001)	Exclude	Title: Evolution of growth form in epiphytic Dissochaeteae (Melastomataceae) Phylogenetic paper on evolution of growth forms
(Corbara and Dejean 1996)	Include	Title: Arboreal Nest Building and Ant-Garden Initiation by a Ponerine Ant <i>Pachycondyla goeldi</i> described from French Guiana
(Corbara and Dejan 1998)	Exclude	Title: A stingless bee nesting inside ant-gardens in French Guiana (Hymenoptera: Apidae) Discovery of presence of a stingless bee in ant-gardens, no new description/survey of ant or plant species.
(Corbara et al. 1999b)	Exclude	Title: Ant gardens, a unique epiphyte-ant association [Les « jardins de fourmis », une association plantes-fourmis originale] All species already in database.
(Corbara et al. 2018)	Exclude	Title: A mimetic nesting association between a timid social wasp and an aggressive arboreal ant Ecological study of commensalism between social wasps and ants.
(Cota-Sánchez 2004)	Exclude	Title: Vivipary in the Cactaceae: Its taxonomic occurrence and biological significance This is not about ants and should have been removed during the first screening.

(da Silva-Viana et al. 2020)	Exclude	Title: Beyond the gardens: The extended mutualism from ant-garden ants to nectary-bearing plants growing in Amazon tree-fall gaps No new species.
(Dacquin et al. 2021)	Exclude	Title: Relative colony size of parabiotic species demonstrates inversion with growth No new species
(Davidson et al. 1990)	Exclude	Title: Neotropical ant gardens II. Bioassays of seed compounds Bioassay experiment about chemical compounds associated with seeds – no new species.
(Davidson 1988)	Include	Title: Ecological Studies of Neotropical Ant Gardens Census of AGs taken in Peru's Manu National Park
(de Vega et al. 2014)	Exclude	Title: Floral volatiles play a key role in specialized ant pollination Only brief reference to ant-gardens, this paper is about ant pollination mediated by floral volatiles.
(Dejean et al. 2019)	Exclude	Title: Biotic and abiotic determinants of the formation of ant mosaics in primary Neotropical rainforests Investigates distribution of ants throughout forest canopies (ant mosaics) – no new AG species.
(Dejean et al. 2000)	Include	Title: The importance of ant gardens in the pioneer vegetal formations of French Guiana (Hymenoptera: Formicidae)
(Dejean et al. 1997)	Include	Survey of AGs in French Guiana. Title: Les jardins de fourmis de Guyane française: Relations entre arbres-suppôts, épiphytes et fourmis "four ant species, <i>Pachycondyla goeldii</i> (Ponerinae), <i>Crematogaster limata parabiatica</i> (Myrmicinae), <i>Dolichoderus bispinosus</i> (Dolichoderinae) and <i>Camponotus femoratus</i> (Formicinae), built carton nests on which we found epiphytes at different stages of development. For other ant species sheltering in ant gardens, we did not find young, developing epiphytes."
(Dejean et al. 2018a)	Exclude	Title: Ants impact the composition of the aquatic macroinvertebrate communities of a myrmecophytic tank bromeliad Focus is on aquatic invertebrate communities living within <i>Aechmea bracteata</i> which is a myrmecophyte but not an AG plant.
(Dejean et al. 2018c)	Exclude	Title: Ant-plant relationships in the canopy of an Amazonian rainforest: the presence of an ant mosaic No new species of AG ants.
(Dejean et al. 2018b)	Exclude	Title: Aquatic life in Neotropical rainforest canopies: Techniques using artificial phytotelmata to study the invertebrate communities inhabiting therein Focus is on aquatic invertebrates in "phytotelmata" not focused on ants.

(Denis et al. 2006)	Exclude	Title: First Record of Polydomy in a Monogynous Ponerine Ant: A Means to Allow Emigration Between <i>Pachycondyla goeldii</i> Nests Study on the nesting behaviour of <i>Pachycondyla goeldii</i> - already in our database.
(Denis et al. 2008)	Exclude	Title: Workers agonistic interactions in queenright and queenless nests of a polydomous ant society No new AG species.
(Edwards et al. 2007)	Exclude	Title: Protection in an ant–plant mutualism: an adaptation or a sensory trap? Focus is on ant protection mutualisms and only briefly refers to ant gardens.
(Frahm 1985)	Exclude	Title: A bryophyte in an ant garden Describes observation of a bryophyte species (<i>Brachymenium columbicum</i>) growing in ant-gardens.
(Hole 1981)	Exclude	No clear evidence that ants disperse these plants. Title: Effects of animals on soil
(Jouquet et al. 2006)	Exclude	Only briefly refers to ant-gardens. Title: Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops Focus is on effect of ants and ant-gardens on soils (as well as other spp).
(Kaufmann et al. 2001)	Include	Title: Ant-Gardens on the giant bamboo <i>Gigantochloa scortechinii</i> (Poaceae) in West-Malaysia Survey of AG ants and plants.
(Kaufmann and Maschwitz 2006)	Include	Title: Ant-gardens of tropical Asian rainforests Inventory of ant and plant species IDed in SE Asia. "The main study site was around the Ulu Gombak Field Studies Centre, Peninsular Malaysia, 3°19'N 101°45'E. Additional collections were made in other areas on Peninsular Malaysia and in Sabah and supplemented by observations in Sarawak, southern Thailand, and Java."
(Kenne et al. 1999)	Exclude	Title: Impact des fourmis sur les plantes cultivées en milieu tropical Study focuses on impact of ants on (human) cultivated plants, not on ecology of ant-gardens (brief mention).
(Kleijn and Van Donkelaar 2001)	Include	Title: Notes on the taxonomy and ecology of the genus <i>Hoya</i> (Asclepiadaceae) in Central Sulawesi Taxonomy and ecology of <i>Hoya</i> discussed, 3 additional ant-garden <i>Hoya</i> species described and one species of <i>Dendrobium</i>

(Leal et al. 2017)	Exclude	Title: The role of parabiotic ants and environment on epiphyte composition and protection in ant gardens
		No new AG species.
(Leroy et al. 2009a)	Exclude	Title: Potential sources of nitrogen in an ant-garden tank-bromeliad
		No new AG species.
(Leroy et al. 2009b)	Exclude	Title: Ants mediate foliar structure and nitrogen acquisition in a tank-bromeliad
		No new AG species.
(Leroy et al. 2017)	Exclude	Title: The influence of light, substrate and seed origin on the germination and establishment of an ant-garden bromeliad
		No new AG species.
(Leroy et al. 2012)	Exclude	Title: Ant species identity mediates reproductive traits and allocation in an ant- garden bromeliad
		No new AG species.
(Leroy et al. 2019)	Exclude	Title: How significant are endophytic fungi in bromeliad seeds and seedlings? Effects on germination, survival and performance of two epiphytic plant species
		Focus is on fungi – no new AG species.
(Maeyama and Matsumoto 2000)	Include	Title: Colonial system of <i>Philidris</i> ants (Formicidae; Dolichoderinae) occupying epiphytic myrmecophytes in a tropical mangrove forest
		- <i>Hydnophytum moseleyanum</i>
		- <i>Philidris</i> colony structure
(Marini-Filho 1999)	Include	Title: Distribution, composition, and dispersal of ant gardens and tending ants in three kinds of central Amazonian habitats
		Survey of ant-gardens in Brazil
(Martínez-Soto and Ruiz-Herrera 2017)	Exclude	Title: Functional analysis of the MAPK pathways in fungi
		Only brief reference to ant gardens, focus is fungi.
(McKey et al. 1999)	Exclude	Title: Les symbioses entre plantes et fourmis arboricoles
		Focus is on ant-plants rather than ant gardens/ ant-cultivated plants.
(Menzel and Blüthgen 2010)	Include	Title: Parabiotic associations between tropical ants: Equal partnership or parasitic exploitation?
		Description of parabiotic mutualism between <i>Campotonus</i> and <i>Crematogaster</i> in SE Asia (Borneo)
(Menzel et al. 2008)	Exclude	Title: Selective interspecific tolerance in tropical <i>Crematogaster</i> – <i>Camponotus</i> associations

Same species as described in Menzel and Bluthgen (2010).

(Morales-Linares et al. 2017)	Include	Title: Spatial structure of ant-gardens: vertical distribution on host trees and succession/segregation of associated vascular epiphytes
(Morales-Linares et al. 2016)	Exclude	Surveyed plants in AGs cultivated by <i>Azteca gnava</i> ants. Title: Vascular epiphytes and host trees of ant-gardens in an anthropic landscape in southeastern Mexico
(Morales-Linares et al. 2018)	Include	Same species as recorded from (Morales-Linares et al. 2017) Title: Orchid seed removal by ants in Neotropical ant-gardens Orchids grow in the AGs built by ant <i>Azteca gnava</i> .
(Ng and Hew 2000)	Exclude	Title: Orchid pseudobulbs – 'false' bulbs with a genuine importance in orchid growth and survival!
(Nieder et al. 2000)	Include	Only brief mention of AGs – no new species Title: Spatial distribution of vascular epiphytes (including hemiepiphytes) in a lowland amazonian rain forest (Surumoni crane plot) of southern Venezuela Survey of vascular epiphytes along Surumoni river in Venezuela. 50% of individuals recorded were obligate AG plants.
(Noble et al. 2009)	Exclude	Title: A qualitative model of limiting factors for a salmon life cycle in the context of river rehabilitation Brief comparison to ecology of ant gardens in text but the focus is salmon.
(Orivel and Dejean 2000)	Exclude	Title: Myrmecophily in HesperIIDae. The case of <i>Vettius tertianus</i> in ant gardens Focus is on the butterfly spp existing within AGs built by <i>Pachycondyla goeldii</i> (Ponerinae). It appears that the butterflies oviposit not just preferentially within the plants but also for the presence of ants.
(Orivel and Dejean 1999a)	Include	Title: Selection of epiphyte seeds by ant-garden ants An inventory of 501 ant gardens inhabited by <i>Camponotus femoratus</i> Fabr., <i>Crematogaster limata parabiotica</i> Forel in parabiosis or alone, and <i>Pachycondyla goeldii</i> Forel enabled us to record 11 epiphyte species
(Orivel et al. 1997)	Exclude	Title: Ant gardens: Interspecific recognition in parabioc ant species No new species
(Orivel and Dejean 1999b)	Exclude	Title: L'adaptation à la vie arboricole chez les fourmis Not about AGs, just arboreal ants in general.

(Paolucci et al. 2016)	Include	Title: Proximity shapes similarity in epiphytic composition of Neotropical ant gardens <i>Psychotria</i> reported (only to genus level)
(Raath et al. 2017)	Exclude	Title: Empty <i>Gonometa postica</i> cocoons function as nest sites and shelters for arboreal ants Focus is on ant-nesting, not AGs (brief comparisons to AGs).
(Richard et al. 2004)	Exclude	Title: Sugary food robbing in ants: a case of temporal cleptobiosis No new AG species
(Salles et al. 2006)	Exclude	Title: The ants' garden: Qualitative models of complex interactions between populations Paper is modelling fungus farming ants, not AGs
(Schmit-Neuerburg and Blüthgen 2007)	Exclude	Title: Ant-garden epiphytes are protected against drought in a Venezuelan lowland rain forest No new AG species
(Seidel et al. 1990)	Exclude	Title: Neotropical ant gardens - I. Chemical constituents No new species – instead focus is on chemical compounds that attract ants to seeds.
(Talaga et al. 2015)	Exclude	Title: Tank bromeliads as natural microcosms: A facultative association with ants influences the aquatic invertebrate community structure Focus is not on AGs, just a brief comparison.
(Vander Wall and Longland 2004)	Exclude	Title: Diplochory: are two seed dispersers better than one? Review of benefits to plants of diplochory (seed dispersal by two or more agents) wherein ant gardens are used as an example.
(Vantaux et al. 2007)	Exclude	Title: Parasitism versus mutualism in the ant-garden parabiosis between <i>Camponotus femoratus</i> and <i>Crematogaster levior</i> Study investigates whether this parabiosis is truly mutualistic or parasitic – no new AG species.
(Vicente et al. 2014)	Exclude	Title: Differential Recruitment of <i>Camponotus femoratus</i> (Fabricius) Ants in Response to Ant Garden Herbivory Focus is on response to chemical compounds associated with herbivory, no new AG species.
(Vicente and Izzo 2021)	Exclude	Title: Effect of dominant parabiotic Ant-Garden ants on the understory and ground-dwelling ant assemblage in the Amazon rainforest Focus is on effect on invertebrate community in relation to the parabiotic ants.

(Vicente and Izzo 2017)	Exclude	Title: Defining habitat use by the parabiotic ants <i>Camponotus femoratus</i> (Fabricius, 1804) and <i>Crematogaster levior</i> Longino, 2003
(Voglmayr et al. 2011)	Exclude	No new AG species. Title: The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions
(Wang and Wu 2015)	Exclude	Focus is on fungus and differences between fungi found in carton nests vs in domatia. Title: Stability of plant–pollinator–ant co-mutualism Focus is not an AG ants, brief comparisons are made to AGs.
(Wanntorp et al. 2006)	Exclude	Title: Wax plants disentangled: A phylogeny of <i>Hoya</i> (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences Only brief mention of ant-gardens and does not specify which species are AG plants.
(Watkins Jr. et al. 2008)	Exclude	Title: Ants mediate nitrogen relations of an epiphytic fern Focus is a myrmecophytic fern but is not ant-cultivated/AG plant
(Weber 1943a)	Include	Title: The queen of a British Guiana Eciton and a new ant garden <i>Solenopsis</i> (Hymenoptera: Formicidae). Description of <i>Solenopsis parabiatica</i> which lives in a parabiotic association with <i>Camponotus femoratus</i> .
(Weissflog et al. 2017)	Exclude	Title: Ant gardens of <i>Camponotus</i> (Myrmotarsus) <i>irritabilis</i> (Hymenoptera: Formicidae: Formicinae) and <i>Hoya elliptica</i> (Apocynaceae) in Southeast Asia No new AG species
(Youngsteadt et al. 2010)	Exclude	Title: Divergent chemical cues elicit seed collecting by ants in an obligate multi-species mutualism in lowland Amazonia
(Youngsteadt et al. 2008)	Exclude	No new AG species Title: Seed odor mediates an obligate ant-plant mutualism in Amazonian rainforests No new AG species

Table S2.2 Additional sources used not found in systematic literature search.

Reference	Notes
(E. Kaufmann, PhD thesis, Goethe University Frankfurt, 2002)	Title: Southeast Asian ant-gardens. Diversity, ecology, ecosystematic significance, and evolution of mutualistic ant-epiphyte associations Extensive surveys of SE Asian AGs.
(Gerlach 2011)	Table 4-2: Ant-garden inhabiting ants (morphospecies). Table 4-3: Ant-garden inhabiting epiphytes Title: The genus <i>Coryanthes</i> : a paradygm in ecology All <i>Coryanthes</i> spp are obligate AG plants.
(Huxley and Jebb 1993)	The tuberous epiphytes of the Rubiaceae 5: A revision of <i>Myrmecodia</i> All <i>Myrmecodia</i> spp are cultivated by ants.
(Haufler et al. 2003)	Title: Systematics of the ant-fern genus <i>Lecanopteris</i> (Polypodiaceae): Testing phylogenetic hypotheses with DNA sequences Additional info on <i>Lecanopteris</i> .
(Huxley and Jebb 1991a)	Title: The tuberous epiphytes of the Rubiaceae 2: The new genus <i>Anthorrhiza</i>
(Huxley and Jebb 1991b)	Title: The tuberous epiphytes of the Rubiaceae 3: A revision of <i>Myrmephytum</i> to include <i>Myrmedoma</i>
(Huxley and Jebb 1993)	Title: The tuberous epiphytes of the Rubiaceae 5: A revision of <i>Myrmecodia</i>
(Jebb 1993)	Title: <i>Anthorrhiza camilla</i> : a new species of rubiaceous ant-plant
(Jebb and Huxley 2019)	Title: The tuberous epiphytes of the Rubiaceae 7: A revision of the genus <i>Hydnophytum</i>
(Jebb, M.H.P, PhD thesis, Oxford University, 1985)	Title: Taxonomy and tuber morphology of the rubiaceous ant-plants Thesis by Jebb, additional info on Hydnophytinae
(Chomicki and Renner 2016b)	Title: Evolutionary relationships and biogeography of the ant-epiphytic genus <i>Squamellaria</i> (Rubiaceae: Psychotrieae) and their taxonomic implications
(Chomicki et al. 2016)	Title: Partner choice through concealed floral sugar rewards evolved with the specialization of ant-plant mutualisms Info on post-anthetic floral rewards in <i>Squamellaria</i>
(Kiew and Anthonysamy 1995)	Title: Ant-garden and ant-tree associations involving <i>Dischidia</i> species (Asclepiadaceae) in Peninsular Malaysia.
(Kiew and Anthonysamy 1987)	Title: A comparative study of vascular epiphytes in three epiphyte-rich habitats at Ulu Endau, Johore, Malaysia.
(Leeuwen and Leeuwen-Reijnvaan 1913)	Title: Beiträge zur Kenntnis der Lebensweise einiger <i>Dischidia</i> -Arten.
(Weir and Kiew 1986)	Title: A reassessment of the relations in Malaysia between ants (<i>Crematogaster</i>) on trees (<i>Leptospermum</i> and <i>Dacrydium</i>) and epiphytes of the genus <i>Dischidia</i> (Asclepiadaceae) including ant-plants.
(Kerr 1912)	Title: Notes on <i>Dischidia rafflesiana</i> Wall., and <i>Dischidia nummularia</i> , Blume.

- (Leeuwen 1929a) Title: Kurze Mitteilungen über Ameisenepiphyten aus Java.
- (Leeuwen 1929b) Title: Mierenepiphyten I.
- (Leeuwen 1929c) Title: Mierenepiphyten II.
- (Benzing and
Clements 1991) Title: Dispersal of the orchid *Dendrobium insigne* by the ant *Iridomyrmex cordatus* in Papua New
Guinea.
- (Madison 1979) Title: Additional observations on ant-gardens in Amazonas.
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Plant cultivation mutualism databases

Table S2.3 Database of ant-cultivated plants. “True AG plant?” refers to the strength of evidence for the given species being collected and planted into ant-gardens by ants. It is often not clear whether all plants in an ant-garden are truly cultivated by ants, or whether they were dispersed in another way.

Family	Genus	Species	Domatia		Food rewards		True AG plant?	Type of evidence	Region	Region details	Elaiosomes	Ant partners	Reference
			Presence	Type	Presence	Type							
Apocynaceae	<i>Dischidia</i>	<i>acutifolia</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>albida</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>albiflora</i>	Yes	External domatia with leaves appressed again tree bark forming nesting space for ants	No	N/A	Maybe	Only evidence is frequently growing in runways/ant nests, but many in this genus are true AG plants.	SE Asia and Australasia	Java	No	Not specified	(Leeuwen and Leeuwen-Reijnvaan 1913)
Apocynaceae	<i>Dischidia</i>	<i>astephana</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>bengalensis</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>fruticulosa</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>hirsuta</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>imbricata</i>	Yes	External domatia with leaves appressed again tree bark forming nesting space for ants	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Moog et al. 2003; Kaufmann and Maschwitz 2006)

Apocynaceae	<i>Dischidia</i>	<i>longepedunculata</i>	Yes	Leaf domatia - Whole leaf folded into a pouch	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Ridley 1912, Weir and Kiew 1986, Kaufmann 2002, Moog et al. 2003, Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>major</i>	Yes	Leaf domatia - Whole leaf folded into a pouch	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Griffith and Solly 1851, Groom 1893, Scott and Sargent 1893, Treseder et al. 1995, Kaufmann 2002, Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>nummularia</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>ovata</i>	No	N/A	No	N/A	Maybe	Referred to as an AG epiphyte but not reasoning provided	SE Asia and Australasia	Malaya	No	Not specified	(Kiew and Anthonysamy 1995)
Apocynaceae	<i>Dischidia</i>	<i>parvifolia</i>	No	N/A	No	N/A	Yes	Ants observed retrieving diaspores	SE Asia and Australasia	Malaya	No	Not specified	(Weir and Kiew 1986)
Apocynaceae	<i>Dischidia</i>	<i>punctata</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>singularis</i>	No	N/A	No	N/A	Maybe	Frequently growing in runways/ant nests and oil containing diaspores	SE Asia and Australasia	Thailand	No	Not specified	(Kerr 1912)
Apocynaceae	<i>Dischidia</i>	<i>spKfmE115</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Dischidia</i>	<i>subulata</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)

Apocynaceae	<i>Hoya</i>	<i>brevialata</i>	No	N/A	No	N/A	Maybe	Regularly occurs in AGs, but no observations of seed collecting behaviour specified	SE Asia and Australasia	Sulawesi	No	Not specified	(Kleijn and Donkelaar 2001)
Apocynaceae	<i>Hoya</i>	<i>elliptica</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>endauensis</i>	No	N/A	No	N/A	Maybe	Frequently growing in runways/ant nests but no other reasons provided	SE Asia and Australasia	Malaya	No	Not specified	(Kiew and Anthonyamy 1995)
Apocynaceae	<i>Hoya</i>	<i>imbricata</i>	Yes	External domatia formed by the leaves pressed against the host tree	No	N/A	Maybe	Frequently occurs in AGs but no observations of seed collecting behaviour specified	SE Asia and Australasia	Sulawesi	No	Not specified	(Jolivet 1996, Kleijn and Van Donkelaar 2001)
Apocynaceae	<i>Hoya</i>	<i>lacunosa</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>micrantha</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>mitrata</i>	Yes	External domatia formed by the leaves pressed against the host tree	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Moog et al. (2003); Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>multiflora</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>obtusifolia</i>	No	N/A	No	N/A	Maybe	Frequently occurs in AGs no further reasoning provided	SE Asia and Australasia	Malaya	No	Not specified	(Kiew and Anthonyamy 1995)
Apocynaceae	<i>Hoya</i>	<i>pallilimba</i>	No	N/A	No	N/A	Maybe	"Probably grow in association with ants"	SE Asia and Australasia	Sulawesi	No	Not specified	(Kleijn and Donkelaar 2001)
Apocynaceae	<i>Hoya</i>	<i>parasitica</i>	No	N/A	No	N/A	Yes	probably true AG-species but final proof missing	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>pubera</i>	No	N/A	No	N/A	Maybe	Always found in ant nests	SE Asia and Australasia	Java	No	Not specified	(Leeuwen 1929b)

Apocynaceae	<i>Hoya</i>	<i>pusilla</i>	No	N/A	No	N/A	Maybe	Frequently occurs in AGs no further reasoning provided	SE Asia and Australasia	Malaya	No	Not specified	(Kiew and Anthony'samy 1995)
Apocynaceae	<i>Hoya</i>	<i>spKfmE133</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>spKfmE51</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Apocynaceae	<i>Hoya</i>	<i>spKfmE94</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Araceae	<i>Anadendrum</i>	<i>latifolium</i>	No	N/A	No	N/A	Yes	probably true AG-species but final proof missing	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Araceae	<i>Anthurium</i>	<i>clavigerum</i>	No	N/A	No	N/A	Maybe	Occurs outside of AGs, seed collecting experiments found that 2/6 <i>A. clavigerum</i> seeds were collected by AG ants, but 4 were consistently rejected? V. common in AGs	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Araceae	<i>Anthurium</i>	<i>gracile</i>	No	N/A	No	N/A	Yes	Plants observed in AGs; Observed ants collecting seeds	Neotropics	Brazil, Peru, Venezuela, French Guiana	Yes	<i>Pachycondyla goeldii</i>	(Davidson 1988, Dejean et al. 1997, 2000, Orivel and Dejean 1999a, Nieder et al. 2000)
Araceae	<i>Anthurium</i>	<i>obtusum</i> (listed as <i>A. trinerve</i> in article)	No	N/A	No	N/A	Maybe	Nieder et al. describes as facultative in ant nests...	Neotropics	Surumoni River, Venezuela	No	Not specified	(Nieder et al. 2000)
Araceae	<i>Anthurium</i>	<i>scandens</i>	No	N/A	No	N/A	Maybe	Plant observed in AGs, no details on seed collection/planting in study, also doesn't specify whether plant occurs outside of AGs	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Araceae	<i>Anthurium</i>	<i>schlechtendalii</i>	No	N/A	No	N/A	Maybe	Plant observed in AGs, no details on seed collection/planting in study, also doesn't specify whether plant occurs outside of AGs	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)

Araceae	<i>Anthurium</i>	<i>solitarium</i>	No	N/A	No	N/A	Maybe	Regularly occurs in AGs, but no observations of seed collecting behaviour specified	Neotropics	Brazil	No	Not specified	(Madison, 1979)
Araceae	<i>Anthurium</i>	<i>sp.</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Brazil, Mexico	No	<i>Azteca gnava</i>	(Marini-Filho 1999; Morales-Linares et al. 2017)
Araceae	<i>Anthurium</i>	<i>sp. nov</i>	No	N/A	No	N/A	Maybe	AG restricted, not found outside AGs but seed collecting not observed	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Araceae	<i>Cercestis</i> (Listed as " <i>Rektophyllum</i> (<i>Cercestis</i>)" in paper)	<i>sp.</i>	No	N/A	No	N/A	Maybe	Paper refers to seeds being planted, but does not specify this for all species	Neotropics	French Guiana	Yes	Not specified	(Dejean et al. 1997)
Araceae	<i>Monstera</i>	<i>adansonii</i>	No	N/A	No	N/A	Maybe	"Not AG-restricted" occurs outside of AGs	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Araceae	<i>Philodendron</i>	<i>deflexum</i>	No	N/A	Yes	EFNs on leaf blade, leaf petiole, leaf bud, stem	Maybe	Describes as "obligatory in ant nests" but no mention of seed collecting or planting	Neotropics	Surumoni River, Venezuela	No	Not specified	(Blüthgen et al. 2000, Nieder et al. 2000)
Araceae	<i>Philodendron</i>	<i>insigne</i> (Lists as <i>P. calophyllum</i> in paper)	No	N/A	Yes	EFNs on leaf blade, spathe	Maybe	Paper refers to seeds being planted, but does not specify this for all species	Neotropics	French Guiana	No	Not specified	(Dejean et al. 1997, Gonçalves-Souza et al. 2016)
Araceae	<i>Philodendron</i>	<i>megalophyllum</i>	No	N/A	Yes	EFNs	Maybe	Non-AG restricted, grows on abandoned AGs as well as ant-tended AGs	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Araceae	<i>Philodendron</i>	<i>myrmecophilum</i>	No*	Tangled roots providing a scaffold for ant nesting: specialized ant garden epiphyte	No	N/A	Maybe	States that AG ants collect and incorporates seeds but no specific observations of seed collecting/carrying/planting in study.	Neotropics	French Guiana	No	Not specified	(Engler 1913; Dejean et al. 2000; Dejean et al. 1997; C.R. Huxley-Lambrick pers. Comm)
Araceae	<i>Philodendron</i>	<i>spp.</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds	Neotropics	Peru, Brazil, French Guiana	No	<i>Ca. femoratus</i> and <i>C. limata</i>	(Davidson 1988; Marini-Filho 1999; Orivel and Dejean 1999)
Araceae	<i>Philodendron</i>	<i>traunii</i>	No	N/A	Yes	EFNs	Maybe	Occurs in AGs, but no description of seeds being collected/planted	Neotropics	Brazil	No	Not specified	(Madison, 1979)
Araceae	<i>Rhektophyllum</i>	<i>sp.</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds	Neotropics	Sinnamary, French Guiana	No	<i>Ca. femoratus</i> and <i>C. limata</i>	(Orivel and Dejean 1999a)
Araceae	<i>Syngonium</i>	<i>spp</i>	No	N/A	No	N/A	Maybe	"Non-AG restricted"	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)

Araliaceae	<i>Schefflera</i>	<i>sp.</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002)
Araliaceae	<i>Schefflera</i>	<i>spKfmE132</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Araliaceae	<i>Schefflera</i>	<i>spKfmE155</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Araliaceae	<i>Schefflera</i>	<i>spKfmE75</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Araliaceae	<i>Schefflera</i>	<i>spKfmE91</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Bromeliaceae	<i>Aechmea</i>	<i>longifolia</i> ²	No	N/A	No	N/A	Maybe	States that AG ants collect and incorporates seeds. Orivel and Dejean 1999 paper doesn't test seed collection of this species	Neotropics	Peru; French Guiana	No	<i>Cr. limata</i>	(Davidson 1988; Dejean et al. 1997; Orivel and Dejean 1999; Dejean et al. 2000)
Bromeliaceae	<i>Aechmea</i>	<i>mertensii</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds	Neotropics	Sinnamary, French Guiana	Yes	<i>Pachycondyla goeldii</i>	(Dejean et al. 1997; Orivel and Dejean 1999; Dejean et al. 2000)
Bromeliaceae	<i>Aechmea</i>	<i>tillandsioides</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds	Neotropics	Mexico, Venezuela	No	<i>Azteca gnava</i>	(Nieder et al. 2000; Morales-Linares et al. 2017; Morales-Linares et al. 2018)

² Listed as *Streptocalyx angustifolium* in Dejean et al. 2000, as *S. angustifolius* in Orivel and Dejean 1999 and Dejean et al. 1997 and listed as *S. longifolius* in Davidson (1988) these are all both synonyms of *S. longifolia*.

Bromeliaceae	<i>Neoregelia</i>	<i>eleutheroptala</i> (note - in maintext listed as sp. but ID added in proof)	No	N/A	No	N/A	Maybe	AG restricted, not found outside AGs but seed collecting not observed	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Bromeliaceae	<i>sp. 1</i>		No	N/A	No	N/A	Maybe	"It may grow in complexity, as it is a good site for the deposition and establishment of wind-dispersed epiphytes, like Orchidaceae, Bromeliaceae, Asclepiadaceae, Nepenthalaceae, Ericaceae, and Melas- tomataceae" ... suggests, no? but does not rule out completely...	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Bromeliaceae	<i>Tillandsia</i>	<i>polystachia</i>	No	N/A	No	N/A	Maybe	Present in AGs, no specification about seed collection/planting	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Bromeliaceae	<i>Tillandsia</i>	<i>schiedeana</i>	No	N/A	No	N/A	Maybe	Present in AGs, no specification about seed collection/planting	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Bromeliaceae	<i>Tillandsia</i>	<i>sp. 1</i>	No	N/A	No	N/A	Maybe	"It may grow in complexity, as it is a good site for the deposition and establishment of wind-dispersed epiphytes, like Orchidaceae, Bromeliaceae, Asclepiadaceae, Nepenthalaceae, Ericaceae, and Melas- tomataceae" ... suggests, no? but does not rule out completely...	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Bromeliaceae	<i>Tillandsia</i>	<i>sp. 2</i>	No	N/A	No	N/A	Maybe	"It may grow in complexity, as it is a good site for the deposition and establishment of wind-dispersed epiphytes, like Orchidaceae, Bromeliaceae, Asclepiadaceae, Nepenthalaceae, Ericaceae, and Melas- tomataceae" ... suggests, no? but does not rule out completely...	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Bromeliaceae	<i>Tillandsia</i>	<i>variabilis</i>	No	N/A	No	N/A	Maybe	Present in AGs, no specification about seed collection/planting	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Cactaceae	<i>Epiphyllum</i>	<i>oxypetalum</i>	No	N/A	Yes	EFNs (formless, distributed along the floral buds and sepals in front of the anthers)	No	"Species found in the lower AG zone, such as the cacti <i>Epip. oxypetalum</i> and <i>Selenicereus boeckman- nii</i> (Otto ex Salm-Dyck) Britton & Rose, may be more shade-tolerant or exploit the spaces where obligate AG are less frequent" Suggests that authors don't believe this to be true AG... but again do not specify otherwise	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Aguirre et al. 2013; Morales-Linares et al. 2017)

Cactaceae	<i>Epiphyllum</i>	<i>phyllanthus</i>	No	N/A	Yes	EFNs (outer surface of flower on aereoles)	Yes	Observed ants collecting seeds, however, also frequently occur outside AGs	Neotropics	Peru, Mexico, Venezuela	No	<i>Azteca gnava</i>	(Davidson 1988, Nieder et al. 2000, Almeida et al. 2013, Morales- Linares et al. 2017, 2018)
Cactaceae	<i>Hylocereus</i>	<i>undatus</i>	No	N/A	Yes	EFNs	Maybe	Present in AGs, no other details provided	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Mauseth et al. 2016, Morales- Linares et al. 2017)
Cactaceae	<i>Rhipsalis</i>	<i>baccifera</i>	No	N/A	No	N/A	No	Described as an "opportunistic species" Suggests that authors don't believe this to be true AG... but again do not specify otherwise	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales- Linares et al. 2017)
Cactaceae	<i>Selenicereus</i>	<i>boeckmannii</i>	No	N/A	No	N/A	Maybe	Present in AGs, no other details provided	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales- Linares et al. 2017)
Cactaceae	<i>Selenicereus</i>	<i>testudo</i>	No	N/A	No	N/A	No	Described as an "opportunistic species" Suggests that authors don't believe this to be true AG... but again do not specify otherwise	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales- Linares et al. 2017)
Clusiaceae	<i>Clusia</i>	<i>spp</i>	No	N/A	No	N/A	Maybe	Differentiates between ants that initiate AGs vs those that occupy them... but no specific observations of Clusia seeds being collected/planted	Neotropics	Brazil; French Guiana	No	<i>Cr. limata</i>	(Dejean et al. 1997; Marini- Filho 1999; Orivel and Dejean 1999; Dejean et al. 2000)
Cyclanthaceae	<i>Asplundia</i>	<i>sp.</i>	No	N/A	No	N/A	Maybe	Described as occurring in AGs by one author and no further discussions of the genus in text, no other mention of this genus or family elsewhere in literature	Neotropics	Brazil	No	Not specified	(Madison, 1979)
Ericaceae	<i>Vaccinium</i>	<i>bancanum</i>	No	N/A	No	N/A	No	Growth form of this species does not make sense as an AG species (tree, facultative epiphyte).	SE Asia and Australasia	Malaya	No	Not specified	(Kiew and Anthonysamy 1987)
Gesneriaceae	<i>Aeschynanthus</i>	<i>albidus</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002; Kaufmann and Maschwitz 2006)
Gesneriaceae	<i>Aeschynanthus</i>	<i>angustifolius</i>	No	N/A	No	N/A	Maybe	Frequently grows in AGs, always found in ant nests, oil containing diaspores	SE Asia and Australasia	Java	No	Not specified	(Leeuwen 1929abc)

Gesneriaceae	<i>Aeschynanthus</i>	<i>fecundus</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002; Kaufmann and Maschwitz 2006)
Gesneriaceae	<i>Aeschynanthus</i>	<i>myrmecophilus</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Gesneriaceae	<i>Aeschynanthus</i>	<i>spKfmE122</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Gesneriaceae	<i>Aeschynanthus</i>	<i>spKfmE33</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Gesneriaceae	<i>Codonanthe/</i> <i>Codonanthopsis</i> 3	<i>calcarata</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds; Plants observed in AGs; differentiates between ants that intitiate AGs v those that occupy... assume observations of seed planting and/or collecting	Neotropics	French Guiana, Venezuela	Yes	<i>Ca. femoratus</i> and <i>C. limata</i>	(Orivel and Dejean 1999; Dejean et al. 2000; Nieder et al. 2000)
Gesneriaceae	<i>Codonanthe/</i> <i>Codonanthopsis</i> 2	<i>crassifolia</i>	No	N/A	Yes	EFNs (stem; petiole; calyx)	Yes	Observed collecting and planting seeds	Neotropics	French Guiana, Mexico	No	<i>Azteca gnava</i>	(Kleinfeldt 1978; Dejean et al. 1997; Blüthgen et al. 2000; Morales-Linares et al. 2017)
Gesneriaceae	<i>Codonanthe/</i> <i>Codonanthopsis</i> 2	<i>dissimulata</i>	No	N/A	Yes	EFNs	Yes	Observed ants collectig seeds	Neotropics	Brazil	No	Not specified	(Madison, 1979)

³ *Codonanthe* species that have an association with ants were reclassified as into the genus *Codonanthopsis* in 2013 (Chautems and Perret 2013), however, this classification does not seem to be have been adopted within ant-garden literature.

Gesneriaceae	<i>Codonanthe/Codonanthopsis</i> 2	sp	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Gesneriaceae	<i>Codonanthe/Codonanthopsis</i> 2	sp.2	No	N/A	No	N/A	Maybe	AG restricted, not found outside AGs but seed collecting not observed	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Gesneriaceae	<i>Codonanthe/Codonanthopsis</i> 2	<i>uleana</i>	No	N/A	Yes	EFNs	Yes	Observed ants collecting seeds	Neotropics	Mexico, Peru	No	<i>Azteca gnava</i>	(Davidson 1988; Morales- Linares et al. 2017; Morales- Linares et al. 2018)
Melastomataceae	<i>Medinilla</i>	<i>crassifolia</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Melastomataceae	<i>Medinilla</i>	spp.	No	N/A	No	N/A	N/A genus level	N/A (but <i>M. crassifolia</i> is a true AG epiphyte)	SE Asia and Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	No	Not specified	(Kaufmann et al. 2001)
Melastomataceae	<i>Pachycentria</i>	<i>constricta</i>	Yes	Swellings on adventitious roots 4	Yes	Pearl bodies	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Burma (now Myanmar), Thailand, Peninsular Malaysia, Sumatra, Borneo, Java, Philippines and Sulawesi	No	<i>Crematogaster</i>	(Clausen, 1998; Kaufmann et al. 2001; Kaufmann 2002; Kaufmann and Maschwitz 2006)
Melastomataceae	<i>Pachycentria</i>	<i>glauca</i>	Yes	Swellings on adventitious roots*	Yes	Pearl bodies	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Lowland forests of Borneo	No	<i>Pholidris</i>	(Clausen, 1998; Kaufmann et al. 2001)
Melastomataceae	<i>Pachycentria</i>	<i>glauca</i> subsp. <i>glauca</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)

⁴ Domatia can only be used when they have dried out and are not commonly observed to be occupied by ants, even when the plant is in an ant-gardens.

Melastomataceae	<i>Pachycentria</i>	<i>glauca</i> subsp. <i>maingayi</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Melastomataceae	<i>Pachycentria</i>	<i>spp.</i>	No	N/A	No	N/A	N/A genus level	N/A	SE Asia and Australasia	Malaysia	No	Not specified	(Kaufmann et al. 2001)
Melastomataceae	<i>Pachycentria</i>	<i>tuberculata</i>	Yes	Root domatia - swollen root structures with hollow cavities	No	N/A	Maybe	EK: ants retrieve dispores (refs Janzen) however, in Janzen paper they say, "This small shrub appears to be totally dependent on Hydnophytum formicarium" and makes reference to a "half hearted" suggestion that ants may hollow out <i>P. tuberosa</i> and live in it...	SE Asia and Australasia	Borneo	No	Not specified	(Janzen 1974, Kiew and Anthony 1995)
Moraceae	<i>Ficus</i>	<i>paraensis</i> ⁵	No	N/A	Yes	Pearl bodies	Maybe	Differentiates between ants that initiate AGs vs those that occupy them but no observation of seed collecting described. Davidson: AG restricted	Neotropics	French Guiana, Peru	No	Not specified	(Davidson 1988; Dejean et al. 1997; Dejean et al. 2000)
Moraceae	<i>Ficus</i>	<i>sp.</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Moraceae	<i>Ficus</i>	<i>sp. 1</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Moraceae	<i>Ficus</i>	<i>sp. 2</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Moraceae	<i>Ficus</i>	<i>sp. 3</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Moraceae	<i>Ficus</i>	<i>sp. 4</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Moraceae	<i>Ficus</i>	<i>spKfmE134</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Moraceae	<i>Ficus</i>	<i>spKfmE144</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Moraceae	<i>Ficus</i>	<i>spKfmE96</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and

⁵Dejean et al 1997 and 2000 list *F. myrmecophila*, this is a synonym of *F. paraensis*.

Orchidaceae	<i>Acriopsis</i>	<i>gracilis</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	Maschwitz 2006) (Kaufmann 2002; Kaufmann and Maschwitz 2006)
Orchidaceae	<i>Acriopsis</i>	<i>lilifolia</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002; Kaufmann and Maschwitz 2006)
Orchidaceae	<i>Coryanthes</i>	<i>albertinae</i>	No ⁶	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011, G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>alborosea</i>	No ⁶	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>boyi</i>	No ⁶	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>bruchmuelleri</i>	No ⁶	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>cataniapoense</i>	No ⁶	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)

⁶ Some authors consider the tangled roots of *Coryanthes* to be domatia, in that they appear to be a modified structure that evolved to house ants, and some do not, as ants do not nest inside the roots.

Orchidaceae	<i>Coryanthes</i>	<i>feildingii</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>gerlachiana</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>gernotii</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>hunteriana</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>lafontainei</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>leferenziorum</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>leucocorys</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)

Orchidaceae	<i>Coryanthes</i>	<i>macrantha</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>maculata</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>mastersiana</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>picturata</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Yes	Observed ants collecting seeds	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; Morales-Linares et al. 2017; Morales-Linares et al. 2018; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>seegeri</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>senghasiana</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>speciosa</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster, Azteca and Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)

Orchidaceae	<i>Coryanthes</i>	<i>speciosa</i> var. <i>espiritosantense</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>speciosa</i> var. <i>punctata</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>trifoliata</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>vasquezii</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>verrucolineata</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>vieirae</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Coryanthes</i>	<i>villegasiana</i>	No ⁵	Tangled roots providing a scaffold for ant nesting	Yes	EFNs	Maybe	<i>C. picturata</i> seeds have been observed to be collected by ants and <i>Coryanthes</i> have a close relationship with ants. However, there are some observations of mature <i>Coryanthes</i> growing outside of ant-nests.	Neotropics	Range: Mexico - Bolivia - Brazil	No	<i>Crematogaster</i> , <i>Azteca</i> and <i>Camponotus</i>	(Gerlach 2011; G. Gerlach pers. comm. to G.C.)
Orchidaceae	<i>Dendrobium</i>	<i>bicaudatum</i>	No	N/A	No	N/A	Maybe	Frequently occurs in AGs no further reasoning provided	SE Asia and Australasia	Sulawesi	No	Not specified	(Kleijn and Donkelaar, 2001)

Orchidaceae	<i>Dendrobium</i>	<i>insigne</i>	No	N/A	No	N/A	Yes	Observed collecting and planting seeds	SE Asia and Australasia	Papua New Guinea	No	<i>Pholidris cordata</i>	(Benzing and Clements 1991)
Orchidaceae	<i>Dendrobium</i>	<i>sp.</i>	No	N/A	No	N/A	Maybe	Kaufmann: no data available	SE Asia and Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	No	Not specified	(Kaufmann et al. 2001; Kaufmann et al. 2002)
Orchidaceae	<i>Dendrochilum</i>	<i>pallidiflavens</i>	No	N/A	No	N/A	Maybe	Always found in AGs, oil-containing disappores.	SE Asia and Australasia	Java	No	Not specified	(Leeuwen 1929a, b, c)
Orchidaceae	<i>Epidendrum</i> ⁷	<i>sp.</i>	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Peru, Brazil	No	Not specified	(Davidson 1988; Marini-Filho 1999)
Orchidaceae	<i>Epidendrum</i> ⁸	<i>flexuosum</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017; Morales-Linares et al. 2018)
Orchidaceae	<i>Epidendrum</i>	<i>macrocarpum</i> ⁹	No	N/A	No	N/A	Maybe	Differentiates between ants that initiate AGs and those that occupy them, however no observation of seed collecting/planting described	Neotropics	French Guiana	No	<i>Pachycondyla goeldii</i>	(Orivel and Dejean 1999; Dejean et al. 2000)
Orchidaceae	<i>Epidendrum</i>	<i>pachyrachis</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017; Morales-Linares et al. 2018)
Orchidaceae	<i>Liparis</i>	<i>lacerata</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002; Kaufmann and Maschwitz 2006)

⁷ Davidson (1988) and Marini-Filho (1999) both refer to *Epidendron sp.* however this is a common name for species within the genus *Epidendrum*.

⁸ Davidson (1988) and Marini-Filho (1999) both refer to *Epidendron sp.* however this is a common name for species within the genus *Epidendrum*.

⁹ Reported as *Epidendrum schomburgkii* Lindl in Dejean et al. 2000 and as *Epidendron schomburgkii* in Orivel Dejan 1999 these are synonyms of *E. macrocarpum*.

Orchidaceae	<i>Pseudolalelia</i> 10	sp.	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	French Guiana	No	Not specified	(Dejean et al. 1997, Oscar A. Pérez- Escobar, pers. comm. to G.C. January 2022)
Orchidaceae		sp.	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Orchidaceae	<i>Vanilla</i>	<i>planifolia</i>	No	N/A	Yes	N/A	Maybe	AG restricted, not found outside AGs but seed collecting not observed. Also "Vanilla planifolia grew luxuriantly from three AGs on a single host tree inhabited by no other epiphytes" if it's a true AG and only V. planifolia is there then it must have been planted by ants. However, Davidson notes that it was v. rare so difficult to decide whether it is exclusive to AGs	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
kOrchidaceae	<i>Vanilla</i>	sp.	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Piperaceae	<i>Peperomia</i>	<i>glabella</i>	No	N/A	No	N/A	Maybe	No description of seed collecting/ any further details on this species in text.	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales- Linares et al. 2017)
Piperaceae	<i>Peperomia</i>	<i>macrostachya</i>	No	N/A	No	N/A	Yes	Observed ants collecting seeds.	Neotropics	French Guiana, Peru	Yes	Not specified	(Davidson 1988; Dejean et al. 1997; Orivel and Dejean 1999; Dejean et al. 2000)
Piperaceae	<i>Peperomia</i>	<i>macrostachyos</i>	No	N/A	No	N/A	Maybe	No description of seed collecting/ any further details on this species in text.	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales- Linares et al. 2017)
Piperaceae	<i>Peperomia</i>	<i>rotundifolia</i>	No	N/A	No	N/A	Maybe	No description of seed collecting/ any further details on this species in text.	Neotropics	Mexico	No	<i>Azteca gnava</i>	(Morales- Linares et al. 2017)
Piperaceae	<i>Peperomia</i>	sp.	No	N/A	No	N/A	N/A genus level	N/A	Neotropics	Brazil	No	Not specified	(Marini-Filho 1999)
Piperaceae	<i>Peperomia</i>	sp. 2	No	N/A	No	N/A	Maybe	Not AG restricted, but may be planted	Neotropics	Peru, Manu National Park	No	Not specified	(Davidson 1988)
Piperaceae	<i>Peperomia</i>	spKfmE117	No	N/A	No	N/A	Probably	EK describes "probably true AG- species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002)

¹⁰ Reported as *Pseudolatia* by Dejean et al. 1997, this is not a valid genus name. However, *Pseudolalelia* sp. are known to occur in ant-gardens (Oscar A. Pérez-Escobar, pers. comm. to G.C. January 2022)

Piperaceae	<i>Peperomia</i>	<i>spKfmE135</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann and Maschwitz 2006)
Polypodiaceae	<i>Asplenium</i>	<i>nidus</i>	No	N/A	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Polypodiaceae	<i>Lecanopteris</i>	<i>balgooyi</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003, Chomicki et al. 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>carnosa</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>celebica</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>crustacea</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>curtisii</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Maybe	Always occurs alongside ants	SE Asia and Australasia	Java	No	Not specified	(Leeuwen 1929a and b; Hauffer et al. 2003)
Polypodiaceae	<i>Lecanopteris</i>	<i>depariodies</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>linguiforme</i>	No	N/A	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>lomarioides</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>luzonensis</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>mirabilis</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>pumila</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Hauffer et al. 2003; Chomicki et al 2017)

Polypodiaceae	<i>Lecanopteris</i>	<i>sarcopus</i>	No	N/A	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Haufler et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Lecanopteris</i>	<i>sinuosa</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Leeuwen 1929a and Haufler et al. 2003; Chomicki et al 2017, Kaufmann 2002; Kaufmann and Maschwitz 2006)
Polypodiaceae	<i>Lecanopteris</i>	<i>spinosa</i>	Yes	Stem domatia - hollow rhizome	No	N/A	Probably	Always occurs alongside ants	SE Asia and Australasia		No	Not specified	(Haufler et al. 2003; Chomicki et al 2017)
Polypodiaceae	<i>Microgramma</i>	<i>lycopodioides</i>	No	N/A	No	N/A	Maybe	Occurs in AGs, but no description of spores being collected/planted	Neotropics	Teapa, state of Tabasco and Amatan, state of Chiapas, Mexico	No	<i>Azteca gnava</i>	(Morales-Linares et al. 2017)
Polypodiaceae	<i>Platyserium</i>	<i>ridleyi</i>	Yes	External domatia - dimorphic leaves forming external domatia between the epiphytic fern and the host tree	No	N/A	Probably	EK describes "probably true AG-species but final proof missing"	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Moog et al. (2003); Kaufmann and Maschwitz 2006)
Polypodiaceae	<i>Polypodium</i>	<i>ciliari</i>	No	N/A	No	N/A	Maybe	No description of seed collecting/ any further details on this species in text.	Neotropics	French Guiana	No	Not specified	(Dejean et al. 1997)
Polypodiaceae	<i>Pyrossia</i>	<i>lanceolata</i>	No	N/A	No	N/A	Maybe	Kaufmann: no data available	SE Asia and Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002)
Polypodiaceae	<i>Pyrossia</i>	<i>sp.</i>	No	N/A	No	N/A	Maybe	Kaufmann: no data available	SE Asia and Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	No	Not specified	(Kaufmann et al. 2001; Kaufmann 2002)
Rubiaceae	<i>Anthorrhiza</i>	<i>caerulea</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea, Morobe Province	No	Not specified	(Jebb 1985, Huxley and Jebb 1991a)
Rubiaceae	<i>Anthorrhiza</i>	<i>camilla</i>	Yes	Modified hypocotyl	No	N/A	Yes		SE Asia and Australasia	Papua New Guinea, Morobe Province	No	Not specified	(Jebb 1993)
Rubiaceae	<i>Anthorrhiza</i>	<i>chrysacantha</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea, Central and Morobe provinces	No	Not specified	(Jebb 1985, Huxley and Jebb 1991a)

Rubiaceae	<i>Hydnophytum</i>	<i>formicarium</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia	No	Not specified	(Kaufmann 2002, Kaufmann and Maschwitz 2006, Jebb and Huxley 2019)
Rubiaceae	<i>Hydnophytum</i>	<i>moseleyanum</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	<i>Philidris</i> observed living in domatia and protecting from herbivores	SE Asia and Australasia	Papua New Guinea	No	<i>Philidris</i>	(Maeyama and Matsumoto 2000)
Rubiaceae	<i>Hydnophytum</i>	<i>spKfmE104</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Ulu Gombak Field Studies Centre, Peninsular Malaysia, 3°19'N 101°45'E. ¹¹	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Rubiaceae	<i>Myrmecodia</i>	<i>alata</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Indonesian New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>albertsii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia		No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>angustifolia</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Indonesian New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>antoinii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea, Australia (North Queensland)	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>archboldiana</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Indonesian New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>aureospina</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Indonesian New Guinea, Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>beccarii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	North Queensland, Australia	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>brassii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Indonesian New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)

¹¹ Additional collections were made in other areas on Peninsular Malaysia and in Sabah and supplemented by observations in Sarawak, southern Thailand, and Java.

Rubiaceae	<i>Myrmecodia</i>	<i>dahlil</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea, New Ireland	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>erinacea</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Indonesian New Guinea, Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>ferox</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>gracilispina</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>horrida</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>jobiensis</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Indonesian New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>kutubuensis</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea, southern highlands	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>lamii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Indonesian New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>longifolia</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Indonesian New Guinea Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>longissima</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Indonesian New Guinea Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>melanacantha</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>oblongata</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>oksapminensis</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)

Rubiaceae	<i>Myrmecodia</i>	<i>paradoxa</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>pendens</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>platytyrea</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Mollucas, Indonesian New Guinea, Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>pteroaspida</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>rumphii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Moluccas, Eastern Indonesia, Lesser Sunda islands	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>salomonensis</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Papua New Guinea, New Ireland, Bougainville; Solomon Islands	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>schlechteri</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia		No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>sterrophylla</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes		SE Asia and Australasia	Indonesian New Guinea Papua New Guinea	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993)
Rubiaceae	<i>Myrmecodia</i>	<i>tuberosa</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Indonesia, New Guinea, Borneo, Thailand, Malaysia	No	<i>Iridomyrmex</i>	(Huxley and Jebb 1993; Kaufmann 2002; Kaufmann and Maschwitz 2006)
Rubiaceae	<i>Myrmephytum</i>	<i>arfakianum</i>	Yes	Modified hypocotyl	No ¹²	N/A	Yes		SE Asia and Australasia	Philippines (Northern)	No	Not specified	(Jebb 1985, Huxley and Jebb 1991b)
Rubiaceae	<i>Psychotria</i>	<i>sp.</i>	No	N/A	No	N/A	No	Only 2/1907 species are epiphytic, and both are located in SE Asia not the Neotropics	Neotropics	Brazil	No	Not specified	(Paolucci et al. 2016)

¹² It is unknown whether this species has food rewards.

Rubiaceae	<i>Squamellaria</i>	<i>grayi</i>	Yes	Modified hypocotyl	No	N/A	Yes	Observations of seed collection, planting, fertilising and harvesting.by <i>Philidris nagasau</i>	Fiji	Fiji	No	<i>P. nagasau</i>	(Chomicki and Renner 2016a)
Rubiaceae	<i>Squamellaria</i>	<i>huxleyana</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Observations of seed collection, planting, fertilising and harvesting.by <i>Philidris nagasau</i>	Fiji	Fiji	No	<i>P. nagasau</i>	(Chomicki and Renner 2016a; Chomicki et al. 2016)
Rubiaceae	<i>Squamellaria</i>	<i>imberbis</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Observations of seed collection, planting, fertilising and harvesting.by <i>Philidris nagasau</i>	Fiji	Fiji	No	<i>P. nagasau</i>	(Chomicki and Renner 2016a; Chomicki et al. 2016)
Rubiaceae	<i>Squamellaria</i>	<i>major</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Observations of seed collection, planting, fertilising and harvesting. by <i>Philidris nagasau</i>	Fiji	Fiji	No	<i>P. nagasau</i>	(Chomicki and Renner 2016a; Chomicki et al. 2016)
Rubiaceae	<i>Squamellaria</i>	<i>thekii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Observations of seed collection, planting, fertilising and harvesting. by <i>Philidris nagasau</i>	Fiji	Fiji	No	<i>P. nagasau</i>	(Chomicki and Renner 2016a; Chomicki et al. 2016)
Rubiaceae	<i>Squamellaria</i>	<i>wilsonii</i>	Yes	Modified hypocotyl	Yes	Post-anthetic sugar rewards	Yes	Observations of seed collection, planting, fertilising and harvesting. by <i>Philidris nagasau</i>	Fiji	Fiji	No	<i>P. nagasau</i>	(Chomicki and Renner 2016a; Chomicki et al. 2016)
Solanaceae	<i>Markea</i>	<i>formicarum</i>	Yes	Stem domatia - swollen tuber-like stems	No	N/A	Maybe	Differentiates between ants that initiate AGs vs those that occupy them but no observation of seed collecting described.	Neotropics	French Guiana	No	Not specified	(Spruce 1908, Weber 1943b, Knapp et al. 1997, Orivel and Dejean 1999a, Dejean et al. 2000, Stehmann and Giacomini 2012)
Solanaceae	<i>Markea</i>	<i>ulei</i>	Yes	Stem domatia - swollen tuber-like stems	Yes	EFNs	Maybe	AG restricted (Davidson), no description of seed collection/planting	Neotropics	Peru, Manu National Park	No	Not specified	(Spruce 1908; Weber 1943; Knapp et al. 1997; Davidson 1988; Stehmann and Giacomini 2012)
Solanaceae	<i>Markea</i> (reported as <i>Marchea</i>)	<i>coccinea</i>	No	N/A	No	N/A	Maybe	No mention of <i>Markea</i> (<i>Marchea</i>) within wider text.	Neotropics	French Guiana	No	Not specified	(Dejean et al. 1997)
Urticaceae	<i>Poikilospermum</i>	<i>cordifolium</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)

Urticaceae	<i>Poikilospermum</i>	<i>microstachys</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Urticaceae	<i>Poikilospermum</i>	<i>sp.</i>	No	N/A	No	N/A	N/A genus level	N/A	SE Asia and Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	No	Not specified	(Kaufmann et al. 2001)
Zingiberaceae	<i>Hedygium</i>	<i>longicomutum</i>	No	N/A	No	N/A	Yes	Proved by observation of AG-ants carrying the seeds	SE Asia and Australasia	Malaysia, southern Thailand, Java	No	Not specified	(Kaufmann 2002; Kaufmann and Maschwitz 2006)

Table S2.4 Database of plant-cultivating ants.

Subfamily	Genus	Species	Taxonomic authority	Associated plants	Region	Region details	Reference
Dolichoderinae	<i>Azteca</i>	<i>cf. traili</i>	Emery, 1893	Not specified per ant species	Neotropics	Peru, Manu National Park	(Davidson 1988)
Dolichoderinae	<i>Azteca</i>	<i>gnava</i>	Forel, 1906	Not specified per ant species	Neotropics	Teapa, state of Tabasco and Amatan, state of Chiapas, Mexico	(Morales-Linares et al. 2017)
Dolichoderinae	<i>Azteca</i>	<i>sp.</i>	Forel, 1878	Not specified per ant species	Neotropics	Brazil	(Marini-Filho 1999)
Dolichoderinae	<i>Azteca</i>	<i>ulei</i>	Forel, 1904	Not specified per ant species	Neotropics	Brazil	(Madison 1979)
Dolichoderinae	<i>Dolichoderus</i>	<i>bispinosus</i>	(Olivier, 1792)	Not specified per ant species	Neotropics	French Guiana	(Dejean et al. 1997)
Dolichoderinae	<i>Dolichoderus</i>	<i>debilis</i>	Emery, 1890	Not specified per ant species	Neotropics	Brazil	(Madison 1979)
Dolichoderinae	<i>Dolichoderus</i> ¹³	<i>sp</i>	Lund, 1832	Not specified per ant species	Neotropics	Brazil, French Guiana	(Marini-Filho 1999, Dejean et al. 2000)
Dolichoderinae	<i>Philidris</i>	<i>cordata</i> ¹⁴	(Smith, F., 1859)	<i>Dendrobium insigne</i>	Southeast Asia/Australasia	Papua New Guinea, Central Sulawesi	(Benzing and Clements 1991, Kleijn and Van Donkelaar 2001)
Dolichoderinae	<i>Philidris</i>	<i>myrmecodiae</i>	(Emery, 1887)	<i>Myrmecodia</i>	Southeast Asia/Australasia	Papua New Guinea	(Chomicki et al. 2017; pers comms M. Janda)

¹³ Reported as *Monacis* by Marini-Filho, 1999 which is a junior synonym of *Dolichoderus*.

¹⁴ Reported as *Iridomyrmex cordatus*, which was assigned to *Philidris* by Shattuck (1992).

Dolichoderinae	<i>Philidris</i>	<i>nagasau</i>	Mann, 1921	<i>Squamellaria grayi</i> , <i>S. major</i> , <i>S. thekii</i> , <i>S. huxleyana</i> , <i>S. imberbis</i> and <i>S. wilsonii</i>	Oceania	Fiji	(Chomicki and Renner 2016a)
Dolichoderinae	<i>Philidris</i>	<i>sp.</i>	Shattuck, 1992	Not specified	Southeast Asia/Australasia	Malaysia	(Kaufmann et al. 2001)
Dolichoderinae	<i>Philidris</i>	spKfmA159	Kaufmann	<i>Hydnophytum formicarium</i> [32%] <i>Myrmecodia tuberosa</i> [22%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002, Kaufmann and Maschwitz 2006)
Dolichoderinae	<i>Philidris</i>	spKfmA160	Kaufmann	<i>Myrmecodia tuberosa</i> [34%] <i>Hydnophytum formicarium</i> [23%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002, Kaufmann and Maschwitz 2006)
Dolichoderinae	<i>Philidris</i>	spKfmA208	Kaufmann	<i>Dischidia major</i> [100%]	Southeast Asia/Australasia	Malaysia	Kaufmann and Maschwitz (2006)
Dolichoderinae	<i>Philidris</i>	spKfmA209	Kaufmann	<i>Myrmecodia tuberosa</i> [43%] <i>Pachycentria constricta</i> [29%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Dolichoderinae	<i>Philidris</i>	spKfmA37	Kaufmann	<i>Pyrossia lanceolata</i> [21%] <i>Dischidia nummularia</i> [20%] <i>Pachycentria constricta</i> [7%] <i>Pachycentria glauca</i> subs. <i>maingayi</i> [7%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Dolichoderinae	<i>Philidris</i>	spKfmA85	Kaufmann	<i>Dischidia major</i> [40%] 6 other species with 10% each	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)

Formicinae	<i>Camponotus</i>	<i>atriceps</i> ¹⁵	(Smith, 1858)	Not specified per ant species	Neotropics	Brazil	(Marini-Filho 1999)
Formicinae	<i>Camponotus</i>	<i>femoratus</i>	(Fabricius, 1804)	Not specified per ant species	Neotropics	Peru, Brazil, French Guiana	(Davidson 1988, Dejean et al. 1997, 2000, Marini-Filho 1999)
Formicinae	<i>Camponotus</i>	<i>irritabilis</i>	(Smith, F., 1857)	<i>Poikilospermum cordifolium</i> (Cecropiaceae)	Southeast Asia/Australasia	Sabah (Malaysian Borneo)	(Menzel and Blüthgen 2010)
Formicinae	<i>Camponotus</i>	<i>rufifemur</i>	Emery, 1900	<i>Poikilospermum cordifolium</i> (Cecropiaceae)	Southeast Asia/Australasia	Sabah (Malaysian Borneo)	(Menzel and Blüthgen 2010)
Formicinae	<i>Camponotus</i>	sp.	Mayr, 1861	Not specified per ant species	Southeast Asia/Australasia	Malaysia	(Kaufmann et al. 2001)
Formicinae	<i>Camponotus</i>	spKfmA168	Kaufmann	Not specified per ant species	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002)
Formicinae	<i>Camponotus</i>	spKfmA240	Kaufmann	<i>Hoya mitrata</i> [50%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Formicinae	<i>Camponotus</i>	spKfmA241	Kaufmann	4 species in equal frequencies	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Formicinae	<i>Camponotus</i>	spKfmA9	Kaufmann	<i>Hoya elliptica</i> [41%] <i>Lepisorus longifolius</i> [21%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Crematogaster</i>	<i>limata</i> ¹⁶	Smith, 1858	Not specified per ant species	Neotropics	Brazil, Malaysian Borneo, French Guiana	(Davidson 1988; Marini-Filho 1999;

¹⁵ listed as *C. abdominalis* by Marini-Filho (1999), this is an invalid name.

¹⁶ Davidson 1988 lists at *C. cf limata parabiatica*; DeJean et al. 1997 and 2000 lists at *C. limata parabiatica*.

Myrmicinae	<i>Crematogaster</i>	<i>modiglianii</i>	Emery, 1900	<i>Poikilospermum cordifolium</i> (Cecropiaceae)	Southeast Asia/Australasia	Sabah (Malaysian Borneo)	Dejean et al. 1997; Dejean et al. 2000) (Menzel and Blüthgen 2010)
Myrmicinae	<i>Crematogaster</i>	<i>sp. 1</i>		Not specified per ant species	Southeast Asia/Australasia	Malaysia	(Kaufmann et al. 2001)
Myrmicinae	<i>Crematogaster</i>	<i>sp.2</i>		Not specified per ant species	Southeast Asia/Australasia	Malaysia	(Kaufmann et al. 2001)
Myrmicinae	<i>Crematogaster</i>	<i>sp.4</i>		Not specified per ant species	Southeast Asia/Australasia	Malaysia	(Kaufmann et al. 2001)
Myrmicinae	<i>Crematogaster</i>	spKfmA113	Kaufmann	<i>Poikilospermum microstachys</i> [50%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Crematogaster</i>	spKfmA18	Kaufmann	<i>Dischidia nummularia</i> [45%] <i>Pyrossia lanceolata</i> [9,6%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Crematogaster</i>	spKfmA19	Kaufmann	<i>Dischidia nummularia</i> [47%] <i>Pyrossia lanceolata</i> [27%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Crematogaster</i>	spKfmA200	Kaufmann	<i>Aeschynanthus albidus</i> [71%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Crematogaster</i>	spKfmA21	Kaufmann	<i>Hoya mitrata</i> [23%] <i>Poikilospermum microstachys</i> [14%] <i>P. cordifolia</i> [10%] <i>Aeschynanthus albidus</i> [10%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)

Myrmicinae	<i>Crematogaster</i>	spKfmA99	Kaufmann	<i>Hoya multiflora</i> [25%] <i>Aeschynanthus albidus</i> [25%]	Southeast Asia/Australasia	Malaysia	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Pheidole</i>	sp.	Westwood, 1839	Not specified per ant species	Southeast Asia/Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	(Kaufmann et al. 2001)
Myrmicinae	<i>Pheidole</i>	sp. 1	Westwood, 1840	Not specified per ant species	Neotropics	Peru, Manu National Park	(Davidson 1988)
Myrmicinae	<i>Pheidole</i>	sp. 2	Westwood, 1841	Not specified per ant species	Neotropics	Peru, Manu National Park	(Davidson 1988)
Myrmicinae	<i>Pheidole</i>	spKfmA120	Kaufmann	<i>Aeschynanthus albidus</i> [46%] <i>Pachycentria constricta</i> [15%]	Southeast Asia/Australasia	Ulu Gombak Field Studies Centre, Peninsular Malaysia, 3°19'N 101°45'E. ¹⁷	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Pheidole</i>	spKfmA33	Kaufmann	Not specified per ant species	Southeast Asia/Australasia	Ulu Gombak Field Studies Centre, Peninsular Malaysia, 3°19'N 101°45'E. ⁴	(Kaufmann 2002; Kaufmann and Maschwitz 2006)
Myrmicinae	<i>Solenopsis</i>	n/a	Westwood, 1840	Not specified per ant species	Neotropics	Peru, Manu National Park	(Davidson 1988)
Myrmicinae	<i>Solenopsis</i>	<i>parabiotica</i>	Weber, 1943	<i>Codonanthe calcaratus</i> (Miq.)	Neotropics	Venezuela, British Guiana, Suriname	(Weber 1943a, b) ¹⁸
Ponerinae	<i>Anochetus</i>	emarginatus	(Fabricius, 1804)	Not specified per ant species	Neotropics	Brazil	(Madison, 1979)
Ponerinae	<i>Anochetus</i>	sp.	Mayr, 1861	Not specified per ant species	Neotropics	Brazil	(Marini-Filho 1999)
Ponerinae	<i>Diacamma</i>	sp.	Kaufmann	Not specified per ant species	Southeast Asia/Australasia	Gombak Field Studies Center, 3°19' N, 101°45' E	(Kaufmann et al. 2001)

¹⁷ Additional collections were made in other areas on Peninsular Malaysia and in Sabah and supplemented by observations in Sarawak, southern Thailand, and Java.

¹⁸ Author asserts that ants do not plant seeds, otherwise the description appears to be an ant-garden.

Ponerinae	<i>Diacamma</i>	spKfmA111	Kaufmann	<i>Schefflera</i> spKfmE75 [28%] <i>Poikilospermum microstachys</i> [20%]	Southeast Asia/Australasia	Ulu Gombak Field Studies Centre, Peninsular Malaysia, 3°19'N 101°45'E. ¹⁹	(Kaufmann 2001; Kaufmann and Maschwitz 2006)
Ponerinae	<i>Odontomachus</i>	<i>affinis</i>	Guérin-Ménéville, 1844	<i>Aeschynanthus albidus</i> [13%] Not specified	Neotropics	Brazil	(Madison 1979)
Ponerinae	<i>Odontomachus</i>	<i>haematodus</i>	(Linnaeus, 1758)	Not specified per ant species	Neotropics	Peru, Manu National Park	(Davidson 1988)
Ponerinae	<i>Odontomachus</i>	<i>mayi</i> ²⁰	Mann, 1911	Not specified per ant species	Neotropics	Brazil; French Guiana	(Marini-Filho 1999; Dejean et al. 2000)
Ponerinae	<i>Pachycondyla</i>	<i>goeldii</i>	(Forel, 1912)	<i>Aechmea mertensii</i> , <i>Anthurium gracile</i> and <i>Codonanthe crassifolia</i>	Neotropics	Brazil, French Guiana	(Corbara & Dejean, 1996; Dejean et al. 1997; Marini-Filho 1999; Dejean et al. 2000)
Ponerinae	<i>Pachycondyla</i>	<i>villosa</i>	(Fabricius, 1804)	Not specified per ant species	Neotropics	Brazil	(Marini-Filho 1999)

¹⁹ Additional collections were made in other areas on Peninsular Malaysia and in Sabah and supplemented by observations in Sarawak, southern Thailand, and Java.

²⁰ Marini-Filho reports as *O. mayri*, this is likely a typographical error.

Evolutionary origins of ant-garden epiphytes and ants

Evolutionary origins of ant-cultivated plants

Table S2.5 Estimated number of evolutionary origins of ant-garden epiphytes.

Family	Genus	Number of origins			Notes	Reference
		Neotropics	SE Asia	Fiji		
Apocynaceae	Dischidia	0	1	0	Singular origin seen in Phylogeny from Chomicki et al. (2017), however many species missing in this phylogeny.	(Chomicki et al. 2017)
	Hoya	0	7	0	Conservative estimate as <i>H. lacunosa</i> and <i>H. pubera</i> were not included in the phylogenetic analysis by Wantorp et al. (2014) due to strong conflict between the 2cp- and 2nr-based trees and <i>H. obtusiflora</i> and <i>H. parasitica</i> not included in the paper, likely due to these names being unresolved/ambiguous)	(Wanntorp et al. 2014) www.worldfloraonline.org
Araceae	Anadendrum	0	1	0	Only <i>A. latifolium</i> in database	N/A
	Anthurium	3	0	0	Potentially conservative due to <i>A. schlechtendalii</i> was not included in the Carlsen and Croat (2013) phylogeny.	(Carlsen and Croat 2013)
	Cercestis	1	0	0	Only one report of <i>Cercestis</i> and not identified to species level	N/A
	Monstera	1	0	0	Only <i>M. adansonii</i> in database	N/A
	Philodendron	2	0	0	Potentially conservative <i>P. deflexum</i> and <i>P. myrmecophilum</i> not included in Gauthier et al. (2008) phylogeny	(Gauthier et al. 2008)
	Rhektophyllum	1	0	0	Only one report of <i>Rhektophyllum</i> and not reported to species level	N/A
Araliaceae	Syngonium	1	0	0	Only one report of <i>Syngonium</i> and not reported to species level	N/A
	Schefflera	0	1	0	No reports of <i>Schefflera</i> to the species level apart from four morphospecies by Kaufmann (2002)	N/A
Bromeliaceae	Aechmea	2	0	0	Potentially conservative as <i>A. longifolia</i> not included in Sass and Specht (2010) analysis.	(Sass and Specht 2010)
	Neoregelia	1	0	0	Only <i>N. eleutheroptala</i> in database.	N/A
	Tillandsia	1	0	0	No phylogeny available that contains any of the three <i>Tillandsia</i> species that are in the database (Terry et al. 1997, Barfuss et al. 2005, Givnish et al. 2011)	N/A
Cactaceae	Epiphyllum	1	0	0	Only <i>E. Phyllanthus</i> is a true ant-garden species, whereas <i>E. oxypetalum</i> is an opportunistic occupant of ant-gardens.	N/A

	Hylocereus	1	0	0	Only <i>H. undatus</i> in database	N/A
	Rhipsalis	1	0	0	Only <i>R. baccifera</i> in database	N/A
	Selenicereus	2	0	0	Note: While Morales-Linares et al. (Morales-Linares et al. 2017) refers to <i>Selenicereus testudo</i> , Korotkova et al. (Korotkova et al. 2017) refers to <i>Strophocactus testudo</i> which is them reallocated to the genus <i>Dreamia</i> . <i>Selenicereus boeckmanni</i> is referred to as <i>Selenicereus pteranthus</i> . Regardless, these taxa are clearly two separate origins.	(Korotkova et al. 2017)
Clusiaceae	Clusia	1	0	0	While “Clusia spp” was reported in four articles it was never reported to species level	N/A
Ericaceae	Vaccinium	0	0*	0	While this species is in the database this is highly unlikely to be a true ant-garden species.	N/A
Gesneriaceae	Aeschynanthus	0	4	0	See phylogeny in Denduangboripant et al. (Denduangboripant et al. 2001). All four species listed in database appear to be separate origins although they are closely related.	(Denduangboripant et al. 2001)
Gesneriaceae	Codonanthe/ Codonanthis	1	0	0	All three <i>Codonanthe</i> (since re-allocated to <i>Codonanthis</i>) species are closeted together along with <i>C. erubescens</i> , ant-garden trait potentially subsequently lost from <i>C. erubescens</i> .	(Perret et al. 2013)
Melastomataceae	Medinilla	0	1	0	Only <i>M. crassifolia</i> described to species level in database	N/A
	Pachycentria	0	1	0	No phylogeny to my knowledge containing relevant <i>Pachycentria</i> spp.	N/A
Moraceae	Ficus	1	1	0	Only one Ficus species described to species level (<i>F. paraensis</i>), however, this species is restricted to Mexico (Serrato et al. 2004), so the Ficus sp. is almost definitely a separate origin. Even one origin in the Neotropics and SE Asia each this is likely to be an under estimation as in the Neotropics there is mention of <i>Ficus</i> sp. 1,2,3 and 4 (Morales-Linares et al. 2017) and in SE Asia there is description of 3 <i>Ficus</i> morphospecies (E. Kaufmann, PhD thesis, Goethe University Frankfurt, 2002).	N/A
Orchidaceae	Acropsis	0	1	0	No genus level phylogeny of Acropsis to my knowledge, even phylogeny of the subtribe Cymbidieae (Li et al. 2016) only features Acropsis at the genus level.	N/A
	Coryanthes	1	0	0	All species of <i>Coryanthes</i> share the character of being involved in ant-gardens, meaning that it is likely to have had a single origin.	(Gerlach, 2011; pers comms Gerlach to GC)
	Dendrobium	0	1	0	No phylogeny that includes the Dendrobium species included in our database to my knowledge, including in extensive phylogeny (Xiang et al. 2013).	N/A
	Dendrochilum	0	1	0	Only <i>D. pallidiflavens</i> in database	N/A
	Epidendron	1	0	0	No Epidendron species described to species level	N/A

	Epidendrum	1	0	0	No phylogeny with the three Epidendrum species from the database together to my knowledge.	N/A
	Liparis	0	1	0	Only <i>L. lacerata</i> in database	N/A
	Pseudolatia	1	0	0	No Pseudolatia species described to species level in database	N/A
	Vanilla	1	0	0	Only <i>V. planifolia</i> described to species level	N/A
Piperaceae	Peperomia	3	1*	0	See phylogeny by Wanke et al. (Wanke et al. 2006). <i>P. glabella</i> , <i>P. macrostachya</i> and <i>P. rotundifolia</i> clearly separate origins. Other instances of <i>Peperomia</i> species being reported suggests that there may be other <i>Peperomia</i> species and thus more origins. *SE Asian <i>Peperomia</i> not reported to species level in Asian studies, however, given the large size of the genus (1500-1700 species) and the clear separation between the Neotropical species it seems likely that there is at least one additional origin in SE Asia.	(Wanke et al. 2006)
Polypodiaceae	Asplenium	0	1	0	Only <i>A. nidus</i> in database	N/A
	Lecanopteris	0	1	0	All Lecanopteris species are involved in ant-gardens, highly likely that this is a single origin (Haufler et al. 2003, Chomicki et al. 2017).	N/A
	Microgramma	1	0	0	Only <i>M. lycopodioides</i> in database	N/A
	Myrmecophila	0	1	0	Only <i>M. sinuosa</i> in database	N/A
	Platyterium	0	1	0	Only <i>P. ridleyi</i> in database	N/A
	Polypodium	1	0	0	Only <i>P. ciliary</i> in database	N/A
	Pyrossia	0	1	0	Only <i>P. lanceolata</i> described to species level	N/A
Rubiaceae	Anthorrhiza	0	1	0	Possibly an underestimate as Chomicki et al. (2017) phylogeny does not include <i>A. camilla</i> . <i>A. caerulea</i> and <i>A. chrysacantha</i> on same node.	(Chomicki et al. 2017)
	Hydnophytum	0	2	0	<i>H. formicarium</i> and <i>H. moseleyanum</i> clearly two separate origins.	(Chomicki et al. 2017)
	Myrmecodia	0	1	0	All <i>Myrmecodia</i> species are ant-dispersed: one origin	(Chomicki et al. 2017)
	Squamellaria	0	0	1	All ant-dispersed species of <i>Squamellaria</i> are clustered together on one node, single origin.	(Chomicki et al. 2017)
Solanaceae	Markea	3	0	0	Clearly three origins. Note: <i>M. ulei</i> is referred to as <i>Hawkesiophyton ulei</i> by Orejuela et al. (Orejuela et al. 2017) but this is a synonym of <i>Markea ulei</i> .	(Orejuela et al. 2017)
Urticaceae	Poikilospermum	0	1	0	No phylogeny containing either <i>P. cordifolium</i> or <i>P. microstachys</i> to my knowledge	N/A
Zingiberaceae	Hedychium	0	1	0	Only <i>H. longicornutum</i> in database	N/A

Evolutionary origins of plant-cultivating ants

Table S2.6 Estimated number of evolutionary origins of ant-garden ants.

Subfamily	Genus	Estimated number of origins			Notes	Reference
		Neotropical	SE Asia	Fiji		
Dolichoderinae	<i>Azteca</i>	1	0	0	No phylogeny containing <i>A. cf. traili</i> and <i>A. gnava</i> to my knowledge.	N/A
	<i>Dolichoderus</i>	1	0	0	Only <i>D. bispinosus</i> described to species level.	N/A
	<i>Philidris</i>	0	1	0*	<i>Philidris</i> taxonomy is in great need of revision and therefore it is difficult to accurately estimate the number of origins of AG initiating behaviour amongst <i>Philidris</i> , however, given that there are three <i>Philidris</i> species that cultivate plants and an additional six morphospecies described by Kaufmann (2002) this is likely to be an underestimated. However, in the absence of a well resolved phylogeny we can only confidently claim one origin of plant cultivation behaviour in <i>Philidris</i> in SE Asia since this is the older clade.	N/A
Formicinae	<i>Camponotus</i>	1*	1*	0	No phylogeny to my knowledge containing the <i>Camponotus</i> spp. In our database, however, given the large size of the genus (over 1000 species (Feldhaar et al. 2007)) it is likely that there is at least one origin of plant cultivation behaviour in the Neotropics and SE Asia.	N/A
Myrmicinae	<i>Crematogaster</i>	1	1	0	No phylogeny to my knowledge containing the <i>Crematogaster</i> spp. In our database, however, Blaimer et al. (Blaimer 2012) refer to a SE Asian and Australasian clade of <i>Crematogaster</i> which contains <i>C. modiglianii</i> . We can infer that the Neotropical species of <i>Crematogaster</i> occur in a separate clade.	(Blaimer 2012)
	<i>Pheidole</i>	1*	1*	0	*Records of plant cultivating <i>Pheidole</i> in both Neotropics and SE Asia are only reported to genus level. However, given that <i>Pheidole</i> comprises over 1100 species (Moreau 2008) it seems likely that plant cultivation evolved separately in the Neotropics and Southeast Asia.	N/A
	<i>Solenopsis</i>	1	0	0	Only <i>Solenopsis parvirostris</i> described to species level	N/A
Ponerinae	<i>Anochetus</i>	1	0	0	No records of AG initiating <i>Anochetus</i> described to species level	N/A
	<i>Diacamma</i>	0	1	0	No records of AG initiating <i>Diacamma</i> described to species level	N/A
	<i>Odontomachus</i>	2	0	0	See phylogeny by Fernandes et al. (Fernandes et al. 2021) two clear origins for <i>O. mayi</i> and <i>O. haematodus</i> . Possible third origin as this paper refers to species <i>Anochetus mayri</i> (which is clearly a separate origin to <i>O. mayi</i> and <i>O. haematodus</i>), however, it is unclear which species was observed due to the reporting of " <i>Odontomachus mayri</i> " as this could be either <i>A. mayri</i> or <i>O. mayi</i> (Marini-Filho 1999).	(Fernandes et al. 2021)

<i>Pachycondyla</i>	1	0	0	No phylogeny to my knowledge containing both <i>Pachycondyla</i> spp. in our database.	N/A

GBIF citations

Table S2.7 GBIF citations for data used in ant-cultivated plant species richness heatmap (Figure 2.5).

Species	Citation
<i>Acriopsis gracilis</i> Minderh. & de Vogel	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.5m7n9z
<i>Acriopsis liliifolia</i> (J.Koenig) Seidenf.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.845rmt
<i>Aechmea longifolia</i> (Rudge) L.B.Sm. & M.A.Spencer	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.vj73n3
<i>Aechmea mertensii</i> (G.Mey.) Schult. & Schult.f.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.bq7ens
<i>Aechmea tillandsioides</i> (Mart. ex Schult. & Schult.f.) Baker	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ardcdh
<i>Aeschynanthus albidus</i> (Blume) Steud.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.terhj7
<i>Aeschynanthus angustifolius</i> (Blume) Steud.	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ye7ec3
<i>Aeschynanthus fecundus</i> P. Woods	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.h2kjit
<i>Anadendrum latifolium</i> Hook.f.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.h4ggw6
<i>Anthorrhiza caerulea</i> C.R. Huxley & Jebb	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.59qq2y
<i>Anthorrhiza chrysacantha</i> C.R. Huxley & Jebb	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.d68ehy
<i>Anthurium clavigerum</i> Poepp.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.g5hszm
<i>Anthurium gracile</i> (Rudge) Lindl.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.gfyye4
<i>Anthurium obtusum</i> (Engl.) Grayum	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.hyab4w
<i>Anthurium scandens</i> (Aubl.) Engl.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.aavzsk
<i>Anthurium schlechtendalii</i> Kunth	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.65qnpp
<i>Anthurium solitarium</i> Schott	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.9gxqmh
<i>Asplenium nidus</i> L.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.kk9kqu
<i>Codonanthe calcarata</i> (Miq.) Hanst.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.r8ycsh
<i>Codonanthe crassifolia</i> (Focke) C.V. Morton	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ybm3s

<i>Codonanthe uleana</i> Fritsch	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.hae8ay
<i>Codonanthopsis dissimulata</i> (H.E. Moore) Wiehler	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.4ph7se
<i>Coryanthes hunteriana</i> Schltr.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.23wb33
<i>Coryanthes macrantha</i> (Hook.) Hook.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.fkupj6
<i>Coryanthes maculata</i> Hook.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.7brdij
<i>Coryanthes mastersiana</i> F. Lehm.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.7b79nz
<i>Coryanthes picturata</i> Rchb.f.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.9jzc4y
<i>Coryanthes senghasiana</i> G. Gerlach	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.9sqmf6
<i>Coryanthes speciosa</i> Hook.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.rhmrq5
<i>Coryanthes vasquezii</i> Dodson	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.4c4cej
<i>Coryanthes verrucolineata</i> G. Gerlach	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.n7p5kk
<i>Dendrobium bicaudatum</i> Reinw. ex Lindl.	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.p4qy3m
<i>Dendrobium insigne</i> (Blume) Rchb.f. ex Miq.	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.6ty7uh
<i>Dendrochilum pallidiflavens</i> Blume	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.73775p
<i>Dischidia acutifolia</i> Maingay ex Hook.fil.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.fsd4cy
<i>Dischidia albida</i> Griff	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.rwj9cz
<i>Dischidia albiflora</i> Griff.	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.wy7du9
<i>Dischidia astephana</i> Scort. ex King & Gamble	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.vvr4if
<i>Dischidia bengalensis</i> Colebr.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.vxzcxt
<i>Dischidia fruticulosa</i> Ridl.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.6drb8k
<i>Dischidia hirsuta</i> (Blume) Decne.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.3qzt6d
<i>Dischidia imbricata</i> (Blume) Steud.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.4xc5xd
<i>Dischidia major</i> (Vahl) Merr.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.9cy6x3

<i>Dischidia nummularia</i> R.Br.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ap7qac
<i>Dischidia ovata</i> Benth.	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.7qx4r8
<i>Dischidia punctata</i> (Blume) Decne.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.4by2xy
<i>Dischidia singularis</i> Craib	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.94rbxp
<i>Epidendrum flexuosum</i> G. Mey.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.q7vc3b
<i>Epidendrum macrocarpum</i> Rich.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.2mqjer
<i>Epidendrum pachyrachis</i> Ames	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.wtb3sa
<i>Epiphyllum oxypetalum</i> (DC.) Haw.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.76ebbn
<i>Epiphyllum phyllanthus</i> (L.) Haw.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.6n9cwc
<i>Ficus paraensis</i> (Miq) Miq.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ybz53a
<i>Hedychium longicornutum</i> Griff. ex Baker	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.7a5rdb
<i>Hoya brevialata</i> Kleijn & Donkelaar	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.wsr6ji
<i>Hoya elliptica</i> Hook.fil.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.7t2683
<i>Hoya imbricata</i> Decne.	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.q4d32j
<i>Hoya lacunosa</i> Blume	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.beqcr3
<i>Hoya micrantha</i> Hook.fil.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.u5rj5k
<i>Hoya mitrata</i> Kerr	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.wgqn67
<i>Hoya multiflora</i> Blume	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.emb69g
<i>Hoya obtusifolia</i> Wight	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.45sb94
<i>Hoya pallilimba</i> Kleijn & Donkelaar	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.6pbumy
<i>Hoya parasitica</i> (Roxb.) Wall. ex Traill	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.5z74fq
<i>Hoya pubera</i> Blume	GBIF.org (01 December 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.z2r8rq
<i>Hydnophytum formicarum</i> Jack	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.punkiq
<i>Hydnophytum moseleyanum</i> Becc.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.3wb6x5

<i>Hylocereus undatus</i> (Haw.) Britton & Rose	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.34afc4
<i>Lecanopteris balgooyi</i> Hennisman	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.t8vmc
<i>Lecanopteris carnososa</i> (Reinw.) Blume	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.f6eajp
<i>Lecanopteris celebica</i> Hennisman	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.2b6e6p
<i>Lecanopteris crustacea</i> Copel.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.rw8yhn
<i>Lecanopteris deparioides</i> (Ces.) Baker	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.jegen7
<i>Lecanopteris lomarioides</i> (Kunze ex Mett.) Copel.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.7ga36u
<i>Lecanopteris luzonensis</i> Hennisman	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.m8n96g
<i>Lecanopteris mirabilis</i> Copel.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.j66uzx
<i>Lecanopteris pumila</i> Blume	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.n3dwy6
<i>Lecanopteris</i> Reinw.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.gttzqp
<i>Lecanopteris sarcopus</i> (Teijsm. & Binn.) Copel.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.aerjpf
<i>Lecanopteris sinuosa</i> (Wall. ex Hook.) Copel.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.v9a67j
<i>Lecanopteris spinosa</i> Jermy & T. Walker	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.yzwwns
<i>Liparis lacerata</i> Ridl.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.kuj78b
<i>Markea coccinea</i> Rich.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.er2nwx
<i>Markea formicarum</i> Dammer	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.j8mfjg
<i>Markea ulei</i> (Dammer) Cuatrec.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.uyencx
<i>Medinilla crassifolia</i> (Reinw. ex Blume) Blume	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.fxftzt
<i>Microgramma lycopodioides</i> (L.) Copel.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.gjie7t
<i>Monstera adansonii</i> Schott	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.hqdvu5
<i>Myrmecodia alata</i> Becc.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.q44h8n
<i>Myrmecodia albertisii</i> Becc.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.nafawd
<i>Myrmecodia angustifolia</i> Valetton	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.wuz2g5

<i>Myrmecodia antoinii</i> Becc.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.79qb2n
<i>Myrmecodia archboldiana</i> Merr. & L.M. Perry	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.hxcyt6
<i>Myrmecodia aureospina</i> Huxley & Jebb	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.mbysu2
<i>Myrmecodia beccarii</i> Hook.f.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.n5g3nr
<i>Myrmecodia brassii</i> Merr. & L.M. Perry	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.y8fpe8
<i>Myrmecodia dahlia</i> K. Schum.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.sx87vq
<i>Myrmecodia ferox</i> Huxley & Jebb	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.h89hqz
<i>Myrmecodia gracilispina</i> Huxley & Jebb	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.eq597n
<i>Myrmecodia horrida</i> Huxley & Jebb	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.g2mezh
<i>Neoregelia eleutheropetala</i> (Ule) L.B.Sm.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.rh3a6a
<i>Pachycentria constricta</i> (Blume) Blume	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.x73g9b
<i>Pachycentria glauca</i> subsp. maingayi (C.B. Clarke) Clausing	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.kypymv
<i>Pachycentria glauca</i> Triana	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.mwvpsk
<i>Peperomia glabella</i> (Sw.) A. Dietr.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.jsasee
<i>Peperomia macrostachya</i> (Vahl) A. Dietr.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.xwd6sn
<i>Peperomia macrostachyos</i> (Vahl) A. Dietr.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.53vude
<i>Peperomia rotundifolia</i> (L.) Kunth	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.sdyf3n
<i>Philodendron deflexum</i> Poepp. ex-Schott	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.bnfqxy
<i>Philodendron insigne</i> Schott	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.mamene
<i>Philodendron megalophyllum</i> Schott	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ffwgnc
<i>Philodendron myrmecophilum</i> Engl.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.8mduu5
<i>Platyserium ridleyi</i> Christ	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.cxhr4h
<i>Poikilospermum cordifolium</i> (Barg. -Petr.) Merr.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.zw635j
<i>Poikilospermum microstachys</i> (Barg. -Petr.) Merr.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.grf29f

<i>Pyrrosia lanceolata</i> (L.) Farw.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.w2u37s
<i>Rhipsalis baccifera</i> (J.S. Muell.) Stearn	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.p8kmtw
<i>Selenicereus boeckmannii</i> (Otto) Britton & Rose	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.y4k6cq
<i>Selenicereus testudo</i> (Karw. ex Zucc.) Buxb.	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.t4ccba
<i>Tillandsia polystachia</i> (L.) L.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.xpbth8
<i>Tillandsia schiedeana</i> Steud.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.d3k2ru
<i>Tillandsia variabilis</i> Schltdl.	GBIF.org (24 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.whs4ex
<i>Vanilla planifolia</i> Andrews	GBIF.org (25 March 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ke9n5e

Chapter 3: Building the comparative genomic and phylogenomic framework for the study of *Philidris* ants.



Abstract

Ants dominate life on Earth and consequently as soon as genome sequencing efforts were applicable to non-model organisms there was an explosion of interest in ant genomics. However, only a tiny fraction (~ 0.007) of described ant species has been sequenced. Here, I produce two high-quality genome assemblies and annotations of the genus *Philidris* (species *P. nagsau* and *P. laevigata*) as well as 38 genomes assembled and annotated from Illumina resequencing data to generate an evolutionary framework for *Philidris* that will be used in this thesis. I compare three different annotation approaches, and find that a new machine-learning approach, Helixer, produces the highest quality annotations. Next, I use these genome assemblies to produce a well-supported genome wide phylogeny relying on 2,100 single copy orthologous genes using concatenated and coalescence approaches. This chapter provides the genomic and phylogenetic framework for comparative genomic work on the ant genus *Philidris* for this thesis. Additionally, I compare the genome size and GC content of *Philidris* genomes, comparing three mutualist strategies (obligate specialist, facultative generalist and non-mutualist) and compare the amino acid biosynthesis capacities of one genome from each strategy.

1. Introduction

Ants are the most ecologically dominant insect group on Earth (Hölldobler and Wilson 1990, Schultheiss et al. 2022). Ants have long fascinated many scientists and general public alike; particularly because of their unusual eusocial lifestyle, characterised by reproductive division of labour wherein either one queen (monogynous) or a few queens (polygynous) are the sole reproductives in a colony, males are produced by laying unfertilised eggs and exist only to reproduce and promptly die, the remaining females in the colony, the workers, cannot reproduce (except in the case where queens have been replaced by gamergates, egg laying workers (Peeters 1991)), and instead care for the queen's brood.

In addition to their eusocial lifestyle, ants engage in a wide array of mutualisms (Chomicki and Renner 2017a), varying from seed dispersal (myrmecochory) in return for fatty seed appendages called elaiosomes (Gammans et al. 2005, Lengyel et al. 2010), protection from herbivores in return for food rewards such as extra floral nectaries (EFNs) (Bronstein et al. 2006, Nelsen et al. 2018) or for nesting space as in the form of plant-made structures called domatia (Chomicki et al. 2015, 2017). In the case of symbioses relying on domatium-bearing plants, ants can also provide nutritional benefits to their plant hosts in addition on instead of defence (Chomicki et al., 2015; Campbell et al., 2023). Ants farm fungi (Mueller et al. 2005), hemipterans (Hölldobler and Wilson 1990, Shingleton et al. 2005, Yao 2014) and plants (Davidson et al. 1990, Kaufmann and Maschwitz 2006, Youngsteadt et al. 2010, Chomicki and Renner 2016a, Campbell et al. 2023). In addition to these charismatic mutualisms, ants engage in a wide range of species interactions from mutualistic to parasitic, but also competition and commensalism with other ants and a wide range of other organisms from plants to arthropods, molluscs, fungi and bacteria (see Chomicki and Renner, 2017, for a review). Especially notable parasitism includes slave-making ants (D'Ettorre and Heinze 2001, Foitzik et al. 2001) and army ants which “raid” nest of invertebrates, often including other species of ants (Kronauer 2009) and inquiline social parasites, which live and exploit an ant' colony –often by reproducing or acquiring the host chemical hydrocarbon signature– but do not provide any benefits (Lenoir et al. 2001). Such huge diversity of unique lifestyles, behaviours, species interactions has driven a large interest in ant genomics when genomic resources became affordable and feasible for non-model organisms.

In 2011, Gadagkar declared that we were witnessing the birth of ant genomics (Gadagkar 2011) following the publication of the first six ant genomes between 2010 and 2011. The first two: the Florida carpenter ant *Camponotus floridanus* (Formicinae) and the Indian jumping ant *Harpegnathos saltator* (Ponerinae) (Bonasio et al. 2010) were chosen due to their contrasting levels of caste specialisation and social organisation with the aim to understand

the genomic basis of eusociality. Next, the invasive Argentine ant *Linepithema humile* (Dolichoderinae) (Smith et al. 2011a), the Red harvester ant *Pogonomyrmex barbatus* (Myrmicinae) (Smith et al. 2011b) and the fire ant *Solenopsis invicta* (Myrmicinae) (Wurm et al. 2011), followed by the leafcutter ant *Atta cephalotes* (Myrmicinae) (Suen et al. 2011). This provided many insights on ants genome as well as the specific adaptation of these distinct systems, such as specific nutritional adaption in *Atta* (Suen et al. 2011).

Research effort and interest in ant genomics has been sustained with 96 ant genomes of 72 species available to download on NCBI at the time of writing. While this number is impressive, there is still a great amount of work to do to understand the entire Formicidae family, which currently has over 14,000 described species (Bolton 2024). There are four Poneroid ant genomes, all within the subfamily Ponerinae, the most speciose Poneroid subfamily. Of the Formicoids, there are genomes published from Dolichoderine (1 genome, 1 species), Dorylinae (3 genomes, 2 species), Formicinae (23 genomes, 17 species), Myrmicinae (56 genomes, 42 species) and Pseudomyrmecinae (8 genomes, 8 species²¹) see Table 3.1 and Supplementary Table S3.1. Myrmicinae is slightly overrepresented relative to their number of species but overall, the sampling of ant genomes is roughly representative of the number of described species (Table 3.1).

²¹ One of these eight species is a morphospecies.

Table 3.1 Number of published ant genome assemblies available on NCBI GenBank on 16/05/2024 for each ant subfamily with the number of accepted species and subspecies (AntCat 2024).

Subfamily	Number of genomes	Number of species
Poneroids		
Agroecomyrmecinae	0	2
Amblyoponinae	0	145
Paraponerinae	0	1
Ponerinae	4	1,277
Proceratiinae	0	166
Formicoids		
Aneuretinae	0	1
Apomyrminae	0	1
Dolichoderinae	1	717
Dorylinae	5	759
Ectatomminae	0	305
Formicinae	23	3,269
Leptanillinae	0	81
Martialinae	0	1
Myrmeciinae	0	94
Myrmicinae	56	7,154
Pseudomyrmecinae	8 ¹	235

Eusociality is key to the ecological dominance of ants. Thus, a great amount of ant genomic work has focused on the basis of eusociality. The *Doublesex* gene has been implicated in the evolution of higher social complexity seen in Formicoids compared to Poneroids (Jia et al. 2018). The existence of a “social chromosome” that mediates levels of social organisation has been identified in *Solenopsis invicta* (Wang et al. 2013) and convergently evolved in *Formica selysi* (Purcell et al. 2014). These social chromosomes share architectural similarities in that they are non-recombining but do not share gene content (Purcell et al. 2014). More recently, a new form of sex determination was recently discovered in the yellow crazy ant (*Anoplolepis gracilipes*) wherein males are haploid chimeras derived from two divergent lineages, workers are interlineage hybrids and queens have a pure lineage genome (Darras et al. 2023). It has been suggested that this unusual mode of genetic inheritance may have contributed to *A. gracilipes* invasion success, allowing for a singularly mated queen to hold the genetic variation to sustain the system indefinitely. Hybrids develop into non-reproductive workers, which prevents admixture between the two lineages and therefore genetic bottlenecks and inbreeding depression (Kronauer 2023). There are other invasive ants that engage in unusual reproduction, *Paratrechina longicornis* and *Wasmannia auropunctata* males and queen reproduce clonally whereas the workers are produced sexually (Fournier et al. 2005, Tseng et al. 2023). More generally, expansions in gene

families relating to chemoreception, particularly olfactory receptors (ORs) are related to the evolution of social complexity in ants (Zhou et al. 2015, Ferguson et al. 2023, Mikhailova et al. 2024) and contractions in OR families have been noted in socially parasitic ants such as slave-makers (Jongepier et al. 2022) and inquilines (Schrader et al. 2021).

Of the 96 currently published ant genomes, 13 are of Attine ants. Whole genome sequencing of attine ants has helped us understand many aspects of the mutualism between attines and their cultivated fungi. One key finding is that both *Atta cephalotes* and *Acromyrmex echinator* have lost the arginine biosynthesis pathway leading these ants to rely on their fungal cultivars for arginine (Suen et al. 2011, Nygaard et al. 2011) this loss appears to have occurred in the earliest attine farmers and may explain why there has never been a reversal to hunter-gatherer life following the evolution of fungiculture in the attines (Nygaard et al. 2016). Study of socially parasitic, inquiline attines revealed convergent genome-wide relaxed selection is response to the shift to parasitism (Schrader et al. 2021).

A huge amount of omics work has been carried out on the Clonal Raider Ant (*Ooceraea biroi*). *Ooceraea biroi* is a queenless ants, workers are reproduced clonally. This unusual mode of reproduction makes *O. biroi* easy to maintain in a laboratory setting (Kronauer 2024). Insights gained from the study of *O. biroi* include understanding the regulation of brood care (Chandra et al. 2018, Libbrecht et al. 2018) and the development and expression of the chemosensory system (Trible et al. 2017, Ryba et al. 2020, McKenzie et al. 2021, Brahma et al. 2023), showing that the system is different to *Drosophila*, and more similar to mammals (Ryba et al. 2020).

One fascinating ant lifestyle that is yet to be studied at the genomic level is plant cultivation by ants. *Philidris nagasau* has lost the ability to build its own carton nests, and therefore obligately depends on farming *Squamellaria domatia* for nesting space. In addition to providing nesting space, five of the six farmed *Squamellaria* species provide food rewards in the form of post anthetic nectaries. Other *Philidris* species including the species *P. laevigata* engage in a facultative²² generalist ant-garden mutualism with an assemblage of plants from a variety of plant families. Ant-garden ants collect epiphyte seeds and planting them into their nutrient rich carton nests. As the plants' roots grow this provides a scaffolding, increasing the stability of nests and allowing them to build larger carton nests.

²² Sometimes Southeast Asian ant-garden ants are described as being obligate mutualists including in the most comprehensive work on SE Asian ant-gardens (Kaufmann, 2002), however, this seems to be as a result of the inconsistent use of the word "obligate". In this thesis the term obligate refers to interactions wherein the removal of the partner results in either death, or loss of the ability to reproduce. While removing plants from ant-gardens does appear to have a fitness cost, it does not seem to stop the colony from reproducing or cause death of the colony.

Shattuck erected *Philidris* as a genus in 1992 transferring the species *P. cordatus*, *P. cordatus fuscus*, *P. cordatus protensus*, *P. cordatus stewartii*, *P. crudus*, *laevigatus*, *myrmecodiae*, *myrmecodiae andamanensis*, *myrmecodiae mandibularis*, *myrmecodiae nigriventris*, *nagasau*, *nagasau agnatus*, *nagasau alticola*, *pubescens* from the genus *Iridomyrmex* to *Philidris*. Additionally, the species *Pseudolasius brunneus* was transferred to *Philidris* (Shattuck 1992a). There is currently no key for *Philidris*, making identification of specimens difficult, resulting in observations of *Philidris* species being reported as morphospecies or just at the genus level. A previous phylogeny of *Philidris* based on five nuclear genes had little statistical support (Chomicki et al. 2017), highlighting the need for more phylogenetic datapoints.

Here, I present two new reference genomes for the ants *Philidris nagasau* and *Philidris laevigata*. *Philidris nagasau*, the Fijian farming ant, engages in an obligate specialised mutualism with six species of the plant genus *Squamellaria*. In addition to the two reference quality genomes, I assemble and annotate a further 38 ant genomes from short-read sequencing data, including obligate specialist, true agriculturalists, facultative generalist ant-garden ants and non-mutualist ants. I use these genome datasets to produce a genome-wide phylogeny of *Philidris*, relying on 2,100 single-copy orthologous genes. Additionally, I perform some cursory genomic comparison between mutualist strategies (obligate-specialist, facultative generalist and non-mutualist), comparing genome size, GC content and amino acid biosynthesis capacities.

2. Methods

2.1 Reference genomes

2.1.1 Biological material

Philidris nagasau samples were collected in July 2016 by Guillaume Chomicki and Alivereti Naikatini in Vanua Levu, around Waisali forest park, along the cross-road between Sasusavu and Labasa (S 16 38'19.8", E 179 13'19.7"), and immediately immersed in RNAlater (ThermoFisher) for subsequent PacBio sequencing. The *Philidris nagasau* specimens were collected by dissecting a *Squamellaria imberbis* plant. Specimens of the ants and its associated plant are kept in the Fiji Regional Herbarium (SUVA), at the University of the South Pacific, Laucala campus, Suva, Fiji, under accession (Chomicki 146) and G. Chomicki, J. Aroles & A. Naikatini 46 (SUVA, M) [duplicate in Munich herbarium [M], Germany). Samples were then stored at -80°C until high molecular weight DNA extraction.

I sampled *Philidris laevigata* from a live colony grown at the University of Sheffield, UK, originally coming from an ant-garden forming population associated with *Dischidia* species in Northern Thailand, obtained from a commercial supplier.

2.1.2 Nucleic acid library preparation and DNA sequencing

High molecular weight (HMW) DNA extraction was carried out at the Natural Environment Research Council (NERC) environmental omics facility (NEOF) in Sheffield, United Kingdom. For *Philidris nagasau* we used one male specimen and for *P. laevigata* we used thirty workers. First 600 µl Lysis Buffer, 40 µl 10% SDS, and 100 µl Proteinase K solution were mixed in a 2 ml Eppendorf tube. Then, sterile forceps were used to fully immerse the specimens in the solution. A sterile pestle was used to homogenise the specimens using a rolling motion until there were no visible clumps. Then the samples were vortexed for 5 seconds and centrifuged briefly. Then, the samples were left to digest overnight (12 – 18 h) at 37°C in a ThermoMixer®. Then, the HMW DNA was cleaned with paramagnetic beads (PacBio AMPure® PB). Samples were quality controlled for concentration using a Qubit, purity on a Nanodrop (260/280 & 260/230 ratios between 1.8-2.2) and integrity on a Femto Pulse (minimal to no degradation).

For both species, we relied on an ultra low input procedure for PacBio sequencing given the amount of DNA. Some 20 ng of DNA was sheared with a Covaris g tube to approximately 10 kb by centrifugation at 4000 rpm. After shearing the DNA, a Femto Pulse system (Agilent) was used to verify the size of the DNA fragments to ensure that the shearing process produced fragments of the desired length (approximately 10 kb). The DNA was damaged and end repaired followed by ligation to the amplification adapters using SMRTbell template prep kit 2.0. Qiagen Pronex beads were used to remove excess adapters and ligation reaction components. The sample was split into two equal volumes and amplified for 13 cycles using 2 different master mixes and cycling conditions as described by the manufacturer. The reactions were cleaned separately with Pronex beads and checked for concentration and size. On meeting the suggested requirements, the 2 reactions were equally combined based on mass input. This pooled sample was then used to construct the SMRTbell library via damage repair, end repair and ligation to an overhang adapter using SMRTbell template prep kit 2.0. This was size selected using the Sage Blue pippin system and 0.75% gel cassette with s1 marker in the range 8000-17000bp. The fraction recovered from the cassette was purified with Pronex beads and checked for size and concentration. The library was sequenced using v5 primer and Sequel polymerase 2.2 on the Sequel 2e system PacBio platform (that generates HiFi reads) using an 8M SMRT cell and 30 hour movie time.

2.1.3 RNA library preparation and RNA sequencing

RNA was extracted from one male and one worker for *P. nagasau* and from one queen and ten workers for *P. laevigata* at the Natural Environmental Research Council (NERC) environmental omics facility (NEOF) in Sheffield, United Kingdom using the Quiagen (Hilden, Germany) RNAeasy extraction kit according to the manufacturer's instructions.

Ribosomal RNA depletion was achieved via poly A selection using NEBnext Poly(A)mRNA magnetic isolation module. Samples (100ng or less if not available) were combined with beads with a poly dT tail. These were washed to remove material not containing poly A tails. The enriched poly A samples were recovered and checked for the absence of rRNA. This material was then used for input in NEBnext Ultra 2 RNA kit.

Samples were fragmented at 94 degrees for 7 minutes and used for creation of cDNA. Samples were end repaired and adapter added. These were treated with USER enzyme to generate a single nucleotide gap at the site of a 'U' residue. Samples were cleaned with ampure beads and used for amplification (14 cycles) aiding the addition of barcodes and i5 and i7 sequences. Libraries were recovered after ampure purification, and these were checked for concentration (Qubit assay) and size and profile (Fragment Analyser). Samples were pooled based on molarity. The samples were sequenced 2x 150bp on Novaseq using SP chemistry. RNA sequencing data was quality checked by technicians at NEOF.

2.2 Genome assembly

All analysis in this thesis was performed on the Hamilton8 supercomputer at Durham University. Unless otherwise specified, programs were ran using default settings.

I used jellyfish version 2.3.0 to estimate the genome size and sequencing error rate (Marçais and Kingsford 2011). Jellyfish relies on k-mers, and we employed a k value of 21, see Table S3.2. The results were visualised using GenomeScope version 2 (Ranallo-Benavidez et al. 2020).

The *Philidris nagasau* genome was assembled using HiFiasm version 0.16.1 (Cheng et al. 2021) using the setting *-n-hap 1* as the *P. nagasau* reference genome was sequenced from a haploid male. The *Philidris laevigata* genome was assembled using wtdbg version 2.5 (Ruan and Li 2020) (*P. laevigata* was initially assembled using HiFiasm, however, the assembly was poor see Tables 3.3-5). Mitochondrial genomes were assembled and annotated using MitoHiFi (Uliano-Silva et al. 2023). Following the initial genome assemblies, BlobToolKit2 (Challis et al. 2020) and a custom R script were utilised to identify and remove potential contamination as well as mitochondrial sequences, using the mitochondrial assemblies constructed using MitoHiFi (Uliano-Silva et al. 2023) as described by Patterson

et al. (2023). Transposable elements (TEs) were annotated using EDTA version 2.0.1 using default settings (Ou et al. 2019).

2.2.1 Quality assessment

Genome assemblies were assessed using BUSCO version 5.3.2 (Simão et al. 2015, Manni et al. 2021), using the Arthropoda odb10 and Hymenoptera odb10 dataset (Manni et al. 2021), and Quast version 5.2.0 (Gurevich et al. 2013)

2.2 Resequencing data

2.2.1 Genome sequencing

All 38 genomes were processed by BGI genomics, Hong Kong, including DNA extraction and library preparation. The samples were sequenced using short-read Illumina sequencing by BGI genomics, Hong Kong. See supplemental Table S3.3 for statistics of the resequencing data.

2.2.2 Genome assembly

The resequencing data was assembled using SPAdes (Prjibelski et al. 2020) Then, the assemblies were assessed using BUSCO version 5.3.2 and Quast version 5.2.0 (Gurevich et al. 2013, Simão et al. 2015, Manni et al. 2021), Following the initial genome assemblies, BlobToolKit2 (Challis et al. 2020) and a custom R script was utilised to identify and remove potential contamination as well as mitochondrial sequences, using the reference mitochondrial assemblies constructed using MitoHiFi from the PacBio data (Uliano-Silva et al. 2023) as described by Patterson et al. (2023).

2.3 Annotation

I compared three annotation approaches, Funannotate version 1.8.16 (Palmer and Stajich 2019), Braker3 (Gabriel et al. 2024) and Helixer (Holst et al. 2023). Following an initial trial I found that Braker3 yielded poor results resequencing genomes (see section 3.2.2), therefore I compared only Funannotate version 1.8.16 (Palmer and Stajich 2019) and Helixer (Holst et al. 2023) for the resequencing genomes.

2.3.1 Funannotate

In preparation for annotation, I utilised the Funannotate Clean to filter out contigs below 1000 bp. Then contigs were rearranged into size order using Funannotate Sort and soft-masked using Funannotate Mask (Palmer and Stajich 2019). Regions with high levels of repeats are often problematic for annotation software, soft-masking is a bioinformatics technique wherein regions high in repeats such as tandem repeats (<60 base pairs), or interspersed repeats (short interspersed repeats (SINES) and long interspersed repeats (LINES) are identified

and converted from uppercase to lowercase in the FASTA file, allowing the annotation software to ignore them. Soft-masking differs from hard-masking wherein the repeated regions are converted to “N”s. It was not necessary to use Funannotate Clean on the reference *P. nagasau* genome as there were no contigs below 1000bp. RNAseq evidence was utilised for the two HiFi genomes but was not available for approximately half of the resequencing genomes, and therefore for consistency was not used for any of them. RNAseq data was aligned to the reference genome using STAR (Dobin et al. 2013) using `--outSAMstrandField intronMotif`. Additionally, RNAseq data was assembled using Trinity version 2.13.2 (Grabherr et al. 2013). Both formats were used as inputs to Funannotate Predict using the options `--rna_bam` and `--transcript_evidence` respectively. RNAseq data was not available for resequencing genomes, therefore Funannotate predict was ran using the option `--busco_seed_species bombus_terrestris2` (the closest available species). Then functional annotation was performed by InterProScan version 5.47-82.0 using options `-goterms` and application options (`-appl`) CDD, COILS, Gene3D, HAMAP, MobiDBLite, PANTHER, Pfam, PIRSF, PRINTS, PROSITEPATTERNS, PROSITEPROFILES, SFLD, SMART, SUPERFAMILY, TIGRFAM (Jones et al. 2014), these results were combined with the gene predictions using Funannotate Annotate using the options `--busco_db arthropoda` and `--iprscan`.

2.3.2 Braker3

In preparation for annotation, regions of the reference genomes with high levels of repeats were soft-masked using Red (Repeat Detector) using default settings (Girgis 2015) (Reference genomes only). Then, I utilised the Funannotate Clean to filter out contigs below 1000 bp (Resequencing genomes only). Then contigs were rearranged into size order using Funannotate Sort and further soft-masked using Funannotate Mask (Reference and resequencing genomes) (Palmer and Stajich 2019). RNAseq data was aligned to the reference genome using STAR (Dobin et al. 2013) using `--outSAMstrandField intronMotif`.

The reference genomes of *P. nagasau* and *P. laevigata* were annotated with Braker3 utilising RNAseq data (using STAR aligned reads) and protein evidence, using the *Arthropoda.fa* dataset (Gabriel et al. 2023). One resequencing genome was annotated with Braker3, without protein evidence, and the resulting annotation was of low quality (see *Results*).

2.3.3 Helixer

Helixer does not accept soft-masked genomes (Holst et al. 2023) therefore I utilised the Funannotate Clean to filter out contigs below 1000bp followed by Funannotate Sort, to sort

the contigs into size order (Palmer and Stajich 2019), prior to annotation with Helixer (both reference and resequencing genomes). The only input for Helixer is a genome assembly, therefore RNAseq was not utilised here. To verify whether the annotations of the reference genomes were missing data due to the lack of RNA input, we aligned the RNAseq data to both the Braker and Helixer annotations using bowtie2 (Langmead and Salzberg 2012) and then extracted the unmapped reads using Samtools version 1.9 (Danecek et al. 2021) and then mapped the unmapped reads from Helixer to Braker (and *vice versa*).

2.3.4 Quality assessment

All annotations were assessed with BUSCO version 5.3.2 (Simão et al. 2015, Manni et al. 2021) and genomeqc (<https://genomeqc.maizegdb.org/>).

2.4 Phylogenetic reconstruction

To generate a strongly supported phylogenetic framework for *Philidris*, we first identified 2,100 single copy orthologous genes that were present in all 40 genomes, relying on Busco genes, extracted using BUSCO version 5.3.2 with the mode “w -augustus” enabled to extract DNA sequenced instead of the default proteins. We subsequently used two approaches for phylogenetic reconstruction: (i) a concatenated supermatrix of the 2,100 BUSCO genes, analysed under maximum likelihood phylogeny in IQTree2 (Minh et al. 2020) and (ii) a coalescent phylogeny using ASTRAL using 2,100 individual gene trees. BUSCO genes (Hymenoptera odb10 dataset (Manni et al. 2021)) were aligned using MAFFT version 7.505 using automatic mode (Kato and Standley 2013). Alignments were trimmed using trimAL version 1.4 (Capella-Gutiérrez et al. 2009) using standard settings. Only gene alignments in which all 40 samples were present were included in the concatenated maximum likelihood analysis and coalescent analysis.

2.4.1 Concatenated maximum likelihood analysis

The 2100 gene alignments were concatenated using catfasta2phyml (Nylander 2022) and a maximum likelihood tree was calculated using IQTree2 with 1000 bootstrap iterations utilising -safe to determine the most suitable substitution model (Minh et al. 2020).

2.4.2 Coalescent analysis

We calculated a maximum likelihood tree for each of the 2100 genes using IQTree2 with 1000 bootstrap iterations and utilising -safe mode (Minh et al. 2020). The gene trees were concatenated using the ‘cat’ command and then branches with bootstrap support under 10% were collapsed using Newick Utilities (Junier and Zdobnov 2010) before being used to calculate a coalescent tree using ASTRAL version 5.7.8 using standard settings (Zhang et al. 2018).

2.5 Reconstruction of amino acid biosynthesis pathways

To investigate whether a shift to obligate mutualism results in shifts amino acid biosynthesis pathways, specifically a species' ability to produce amino acids, genome annotations of the obligate specialist (*P. nagasau*), facultative generalist (*P. laevigata*) and non-mutualist outgroup (*Anonychomyrma* sp., sample 62) were KEGG pathways investigated using KofamKOALA (Aramaki et al. 2020).

2.6 Statistical Analysis

To compare whether mutualist strategy (obligate specialist, facultative generalist, non-mutualist) had a significant effect on genome size and GC content an ANOVA test followed by a Tukey's HSD (honestly significant difference) test were calculated in R version 4.3.1 (R Core Team 2023).

3. Results

3.1 Sequence output and genome assemblies

3.1.1 Reference genomes

Philidris nagasau and *P. laevigata* were sequenced using PacBio HiFi reads and yielded reads with average lengths of 10,222.5 bp and 9,189.8 bp respectively, see Table 3.2.

Table 3.2 Sequencing output from PacBio HiFi sequencing of *Philidris nagasau* and *P. laevigata*. Coverage was calculated by dividing the sum length of the reads by the size of the genome assembly.

Species	No. of reads	Avg. length	Max length	Min length	Coverage
<i>P. nagasau</i>	2,964,806	10,222.5	29,238	86	129.31
<i>P. laevigata</i>	2,769,531	9,189.8	32,093	95	116.39

Philidris nagasau was sequenced from a haploid male, and consequently had a homozygosity of 100%. *Philidris laevigata* was sequenced from a pool of diploid workers and had a minimum homozygosity of 94.2% and maximum homozygosity of 95.6%.

The reference genome of *Philidris nagasau* was assembled using HiFiasm version 0.16.0 (Cheng et al. 2021) and produced an assembly with high BUSCO completeness and low BUSCO duplication (Arthropoda completeness (C): 99% single copy completeness (S): 97.9% duplicated (D): 1.1% fragmented (F): 0.4% missing (M): 0.6%, Hymenoptera C: 93.0 S: 92.7 D: 0.3 F: 1.8 M: 5.2), see Table 3.4 and 3.5) before filtering for potential contamination and mitochondrial sequences from the assembly. Initially, *P. laevigata* was also assembled using HiFiasm, however, this gave a poor result with a high BUSCO

duplication score (Arthropoda 33.9% and Hymenoptera 30.7%) which was not resolved by using *purge_dups* (Guan et al. 2020), and the size of assembly was 398Mb, which is much larger than the only other published Dolichoderinae ant, *Linepithema humile* (219.5 Mb) (Smith et al. 2011a) and the *P. nagasau* assembly (234 Mb pre-filtering, 228.2 Mb post-filtering). The assembly consisted of nearly four times as many contigs compared to *P. nagasau* (7715 compared to *P. nagasau* 2228). In light of this result, I re-assembled the genome using wtdbg version 2.5 (Ruan and Li 2020), which greatly improved the *P. laevigata* assembly with a low BUSCO duplication rate (Arthropoda: 3.8 and Hymenoptera: 0.8) and a more reasonable assembly size of 256Mb (see Tables 3.3-5.). While the assembly was still less contiguous than *P. nagasau*, the wtdbg assembly was improved compared the HiFiasm for *P. laevigata*, see Tables 2.3-5. I also reassembled *P. nagasau* using wtdbg version 2.5, however, the HiFiasm assembly was more complete and more contiguous, so we utilised the HiFiasm assembly for *P. nagasau* and the wtdbg assembly for *P. laevigata* for downstream analysis. After filtering with Blobtoolkit2 (Challis et al. 2020) and further filtering with Funannotate Clean (Palmer and Stajich 2019) (for *P. laevigata* only, due to having lower contiguity which was causing issues with annotation), measures of contiguity slightly improved and BUSCO completeness slightly decreased. see Tables 2.6-8 and Figures 3.1 and 3.2. GC content was typical of Hymenopteran genomes, 36.5% for *P. nagasau* and 36.09% for *P. laevigata*.

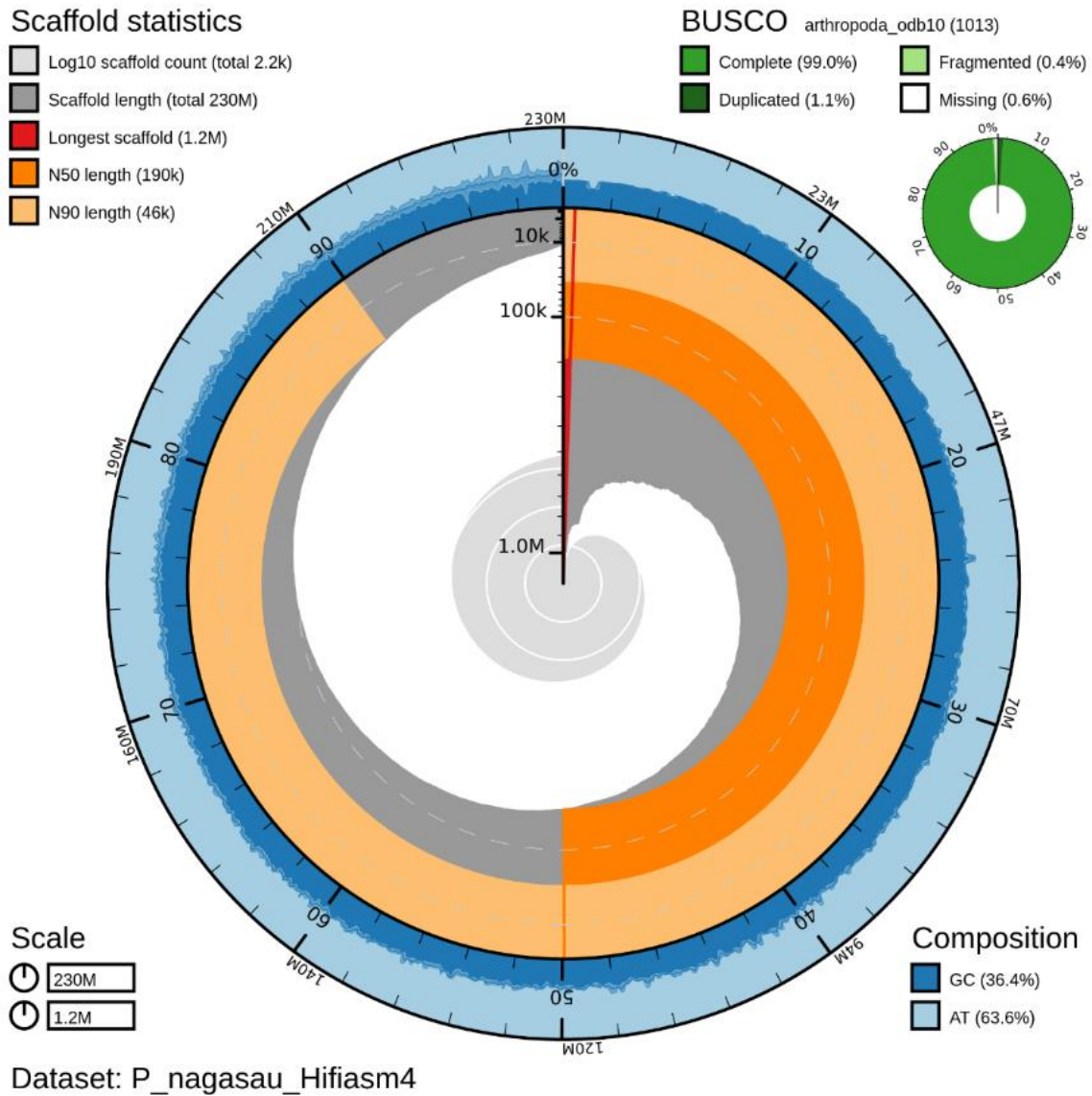


Figure 3.1 Graphical representation of filtered genome assembly of *Philidris nagasau* produced by Blobtoolkit2 (Challis et al. 2020). The main centre plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 234,525,867bp assembly. The distribution of scaffold lengths is shown as blocks moving clockwise with the plot radius scaled to the longest contig present in the assembly (120 Mb, denoted by the first radial line). The N50 and N90 record lengths (186,826 and 45,110bp) are denoted by the second and third radial lines, respectively. The spiral shows the cumulative record count on a log scale with dashed lines showing successive orders of magnitude. The outside of the plot shows the distribution of GC, AT, and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the arthropoda_odb10 set is shown in the top right (see Tables 3.6-8 for further details).

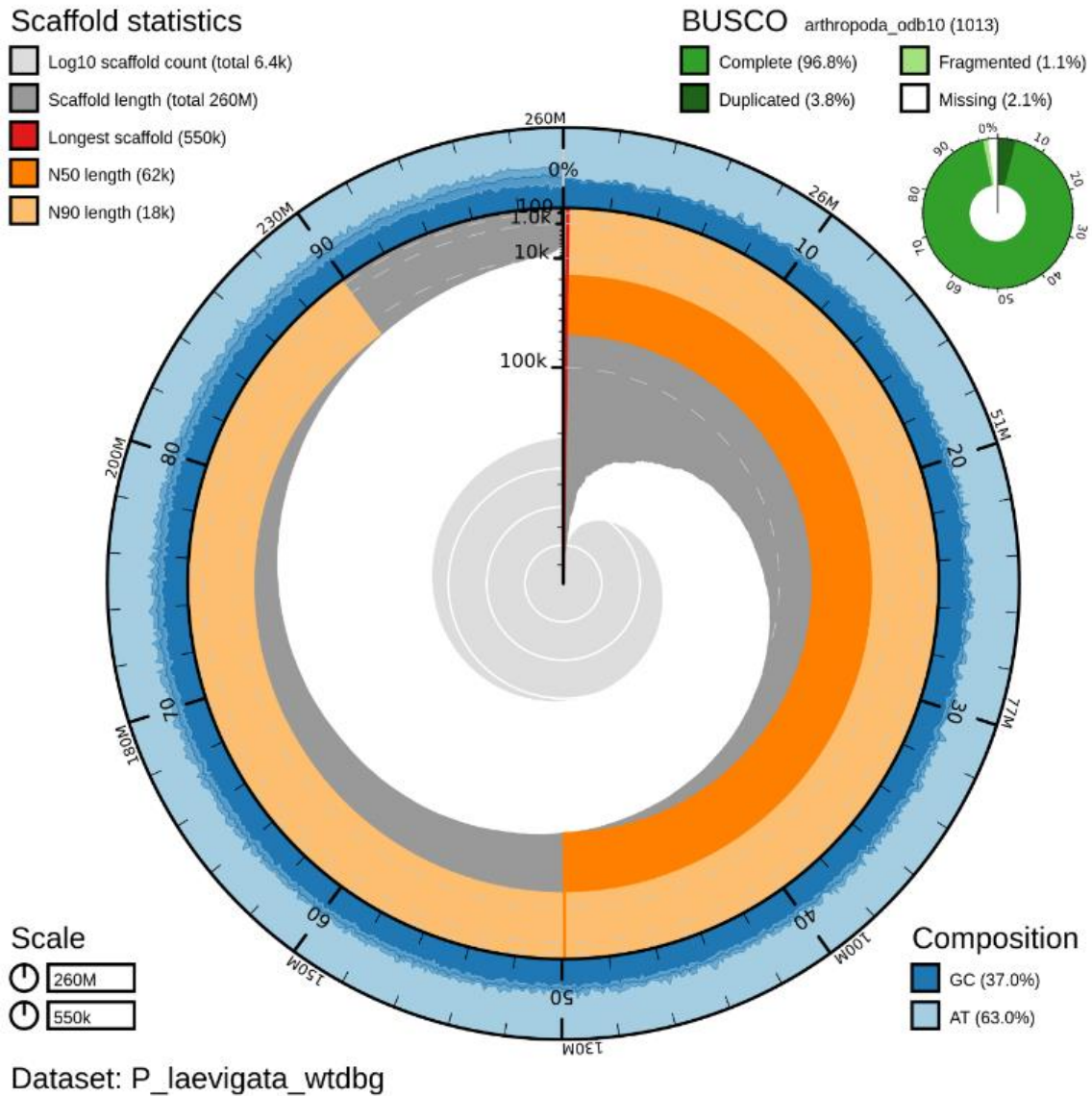


Figure 3.2 Graphical representation of filtered genome assembly of *Philidris laevigata* produced by Blobtoolkit2 (Challis et al. 2020). The main centre plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 234,525,867 bp assembly. The distribution of scaffold lengths is shown as blocks moving clockwise with the plot radius scaled to the longest contig present in the assembly (550 Kb, denoted by the first radial line). The N50 and N90 record lengths (67,264 and 18,711 bp) are denoted by the second and third radial lines, respectively. The spiral shows the cumulative record count on a log scale with dashed lines showing successive orders of magnitude. The outside of the plot shows the distribution of GC, AT, and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the arthropoda_odb10 set is shown in the top right (see Tables 3.6-8 for further details).

Table 3.3 Assembly statistics for *Philidris* reference genomes before filtering. Both species were assembled from PacBio HiFi reads using both HiFiasm version 0.1.6 (Cheng et al. 2021) and wtdbg version 2.5 (Ruan and Li 2020) to compare performance.

Species	No of contigs		N50		L50		Size of assembly (bp)		GC (%)	
	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg
<i>P. nagasau</i>	2228	2936	186778	127513	355	489	234525867	215169452	36.46	36.14
<i>P. laevigata</i>	7715	6392	69394	61754	1537	1135	378189735	255943723	37.04	37.03

Table 3.4 BUSCO completeness of for *Philidris* reference genomes before filtering, using the Arthropoda odb10 dataset (Manni et al. 2021). Both species were assembled from PacBio HiFi reads using both HiFiasm version 0.1.6 (Cheng et al. 2021) and wtdbg version 2.5 (Ruan and Li 2020) to compare performance.

Species	Completeness		Single copy		Duplicated		Fragmented		Missing	
	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg
<i>P. nagasau</i>	99	97	97.9	96.2	1.1	0.8	0.4	0.9	0.6	2.1
<i>P. laevigata</i>	96.2	96.8	62.3	93	33.9	3.8	1.1	1.1	2.7	2.1

Table 3.5 BUSCO completeness of for *Philidris* reference genomes before filtering using the Hymenoptera odb10 dataset (Manni et al. 2021). Both species were assembled from PacBio HiFi reads using both HiFiasm version 0.1.6 (Cheng et al. 2021) and wtdbg version 2.5 (Ruan and Li 2020) to compare performance.

Species	Completeness		Single copy		Duplicated		Fragmented		Missing	
	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg	HiFiasm	wtdbg
<i>P. nagasau</i>	93	90.5	92.7	90.3	0.3	0.2	1.8	1.6	5.2	9.9
<i>P. laevigata</i>	88.7	86.9	58	86.1	30.7	0.8	2.3	2.8	9	10.3

Table 3.6 Assembly statistics for *Philidris* reference genomes after filtering with Blobtoolkit2 (Challis et al. 2020) to remove mitochondrial and non-arthropod sequences (both genomes) and with Funannotate Clean (Palmer and Stajich 2019) to remove contigs under 1000 bp (*P. laevigata* only). Both species were assembled from PacBio HiFi reads. *Philidris nagasau* was assembled using HiFiasm version 0.1.6 (Cheng et al. 2021) and *P. laevigata* was assembled using wtdbg version 2.5 (Ruan and Li 2020) following a comparison of the performance of the two methods.

Species	No of contigs	N50	L50	Size of assembly	GC
<i>P. nagasau</i>	2205	186826	352	228239541	36.5
<i>P. laevigata</i>	5143	67264	905	218827041	36.09

Table 3.7 BUSCO completeness (using the Arthropoda odb10 dataset (Manni et al. 2021)) of *Philidris* reference genomes after filtering with Blobtoolkit2 (Challis et al. 2020) to remove mitochondrial and non-arthropod sequences (both genomes) and with Funannotate Clean (Palmer and Stajich 2019) to remove contigs under 1000 bp (*P. laevigata* only). Both species were assembled from PacBio HiFi reads. *P. nagasau* was assembled using HiFiasm version 0.1.6 (Cheng et al. 2021) and *P. laevigata* was assembled using wtdbg version 2.5 (Ruan and Li 2020) following a comparison of the performance of the two methods.

Species	Completeness	Single Copy	Duplicated	Fragmented	Missing
<i>P. nagasau</i>	97.7	96.6	1.1	0.5	1.8
<i>P. laevigata</i>	96.3	95.5	0.8	0.8	2.9

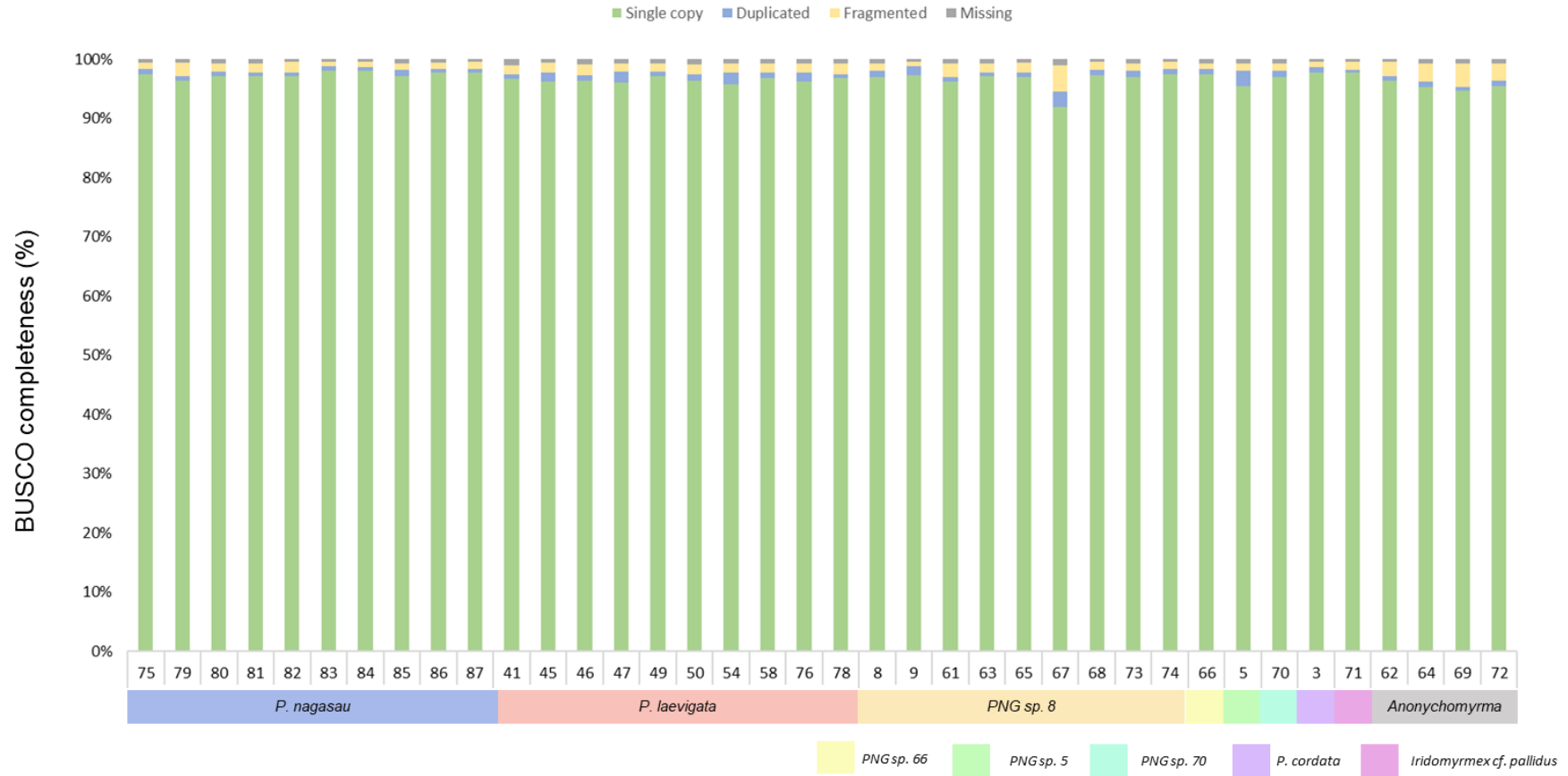
Table 3.8 BUSCO completeness (using the Hymenoptera odb10 dataset (Manni et al. 2021)) of *Philidris* reference genomes after filtering with Blobtoolkit2 (Challis et al. 2020) to remove mitochondrial and non-arthropod sequences (both genomes) and with Funannotate Clean (Palmer and Stajich 2019) to remove contigs under 1000 bp (*P. laevigata* only). Both species were assembled from PacBio HiFi reads. *P. nagasau* was assembled using HiFiasm version 0.1.6 (Cheng et al. 2021) and *P. laevigata* was assembled using wtdbg version 2.5 (Ruan and Li 2020) following a comparison of the performance of the two methods.

Species	Completeness	Single Copy	Duplicated	Fragmented	Missing
<i>P. nagasau</i>	91.5	91.2	0.3	1.8	6.7
<i>P. laevigata</i>	87	86.7	0.3	2.3	10.7

3.1.2 Resequencing genomes

The samples were sequenced with an average coverage of 94X, see Table S3.3 for details on resequencing data output. The 38 resequencing genomes have a high average BUSCO completeness (Arthropoda: C = 97.72 S = 96.44, D = 1.01 F = 1.64, M = 0.67, Hymenoptera: C = 90.33, S = 90.087, D = 0.50, F = 4.62, M = 5.03), see Tables 3.9 and 3.10 and Figure 3.3 for values for each genome. As expected for short read sequencing, the contiguousness is markedly lower with the assemblies consisting of an average of 377,187 contigs, an average N50 of 27,635 and an average L50 of 2,732 (see Table 3.11). The average assembly size of 215 Mb which is similar to the reference *Philidris* genomes as well as the only other existing Dolichoderinae genome assembly (*Linepithema humile*, see

Supplementary Table S3.1 and Smith et al. 2011a), see Table 3.11 and Figure 3.4 for assembly statistics for each genome.



Sample number and species delimitation

Figure 3.3 BUSCO completeness (Arthropoda odb10 dataset [Manni et al. 2021]) of the genomes assembled from resequencing data after filtering out mitochondrial and non-arthropod sequences using Blobtoolkit2 (Challis et al. 2020), species delimitations are based of the genome-wide molecular phylogeny (see Figures 3.6 and 3.7).

Table 3.9 BUSCO completeness of resequencing genomes, using the Arthropoda odb10 dataset (Manni et al. 2021). Genomes were assembled using SPAdes version 3.15.5 (Prijbelski et al. 2020), then filtered using Blobtoolkit2 (Challis et al. 2020) to remove non-arthropod and mitochondrial sequences and then cleaned in preparation for annotation using Funannotate Clean (Palmer and Stajich 2019) to remove sequences under 1000 bp.

Sample	Species	Completeness			Single Copy			Duplication			Fragmented			Missing		
		Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned
75	<i>nagasau</i>	98.3	98.3	96.1	97.5	97.5	95.3	0.8	0.8	0.8	1.1	1.1	1	0.6	0.6	2.9
79	<i>nagasau</i>	98.1	98.1	96.6	97.3	97.3	96	0.8	0.8	0.6	1.3	2.3	1.5	0.6	0.6	1.9
80	<i>nagasau</i>	98	97.9	96	97.3	97.2	95.2	0.7	0.7	0.8	1.3	1.3	1.3	0.7	0.8	2.7
81	<i>nagasau</i>	97.7	97.7	95	97.1	97.1	94.4	0.6	0.6	0.6	1.6	1.6	1.5	0.7	0.7	3.5
82	<i>nagasau</i>	97.8	97.8	94.6	97.2	97.2	94.2	0.6	0.6	0.4	1.8	1.8	1.9	0.4	0.4	3.5
83	<i>nagasau</i>	98.8	98.8	96.8	98.1	98.1	96.3	0.7	0.7	0.5	0.7	0.7	1	0.5	0.5	2.2
84	<i>nagasau</i>	98.6	98.6	96.8	98	98	96.2	0.6	0.6	0.6	1	1	2.3	0.4	0.4	0.9
85	<i>nagasau</i>	98.2	98.2	96.6	97.2	97.2	95.7	1	1	0.9	1.1	1.1	1	0.7	0.7	2.4
86	<i>nagasau</i>	98.3	98.3	96	97.7	97.7	95.5	0.6	0.6	0.5	1.1	1.1	1	0.6	0.6	3
87	<i>nagasau</i>	98.4	98.4	97.1	97.7	97.7	96.5	0.7	0.7	0.6	1.2	1.2	0.9	0.4	0.4	2
41	<i>laevigata</i>	97.4	97.4	93.5	96.6	96.6	92.9	0.8	0.8	0.6	1.6	1.6	1.5	1	1	5
45	<i>laevigata</i>	97.7	97.7	93.8	96.2	96.2	92.7	1.5	1.5	1.1	1.7	1.7	1.4	0.6	0.6	4.8
46	<i>laevigata</i>	97.3	97.3	90	96.3	96.3	89.3	1	1	0.7	1.8	1.8	1.7	0.9	0.9	8.3
47	<i>laevigata</i>	97.9	97.9	92.6	96.1	96.1	91.2	1.8	1.8	1.4	1.3	1.3	1.7	0.8	0.8	5.7
49	<i>laevigata</i>	97.9	97.9	93.4	97.2	97.2	93	0.7	0.7	0.4	1.3	1.3	1.4	0.8	0.8	5.2
50	<i>laevigata</i>	97.4	97.4	92.8	96.4	96.4	92.1	1	1	0.7	1.7	1.7	1.2	0.9	0.9	6
54	<i>laevigata</i>	97.8	97.8	91.2	95.7	95.7	89.4	2.1	2.1	1.8	1.5	1.5	1.5	0.7	0.7	7.3
58	<i>laevigata</i>	97.8	97.8	93.4	96.8	96.8	92.8	1	1	0.6	1.4	1.4	1.5	0.8	0.8	5.1
76	<i>laevigata</i>	97.8	97.8	93.5	96.2	96.2	92.1	1.6	1.6	1.4	1.4	1.4	1.4	0.8	0.8	5.1
78	<i>laevigata</i>	97.4	97.4	93.1	96.8	96.8	92.7	0.6	0.6	0.4	1.9	1.9	1.8	0.7	0.7	5.1
61	<i>PNG spp8</i>	97	97	88.5	96.2	96.2	87.9	0.8	0.8	0.6	2.2	2.2	2	0.8	0.8	9.5
63	<i>PNG spp8</i>	97.7	97.7	93.8	97.2	97.2	93.5	0.5	0.5	0.3	1.5	1.5	1.6	0.8	0.8	4.6
65	<i>PNG spp8</i>	97.7	97.7	92.4	97	97	91.9	0.7	0.7	0.5	1.7	1.7	1.3	0.6	0.6	6.3
67	<i>PNG spp8</i>	94.6	94.6	82.1	91.9	91.9	79.7	2.7	2.7	2.4	4.3	4.3	3	1.1	1.1	14.9
68	<i>PNG spp8</i>	98.2	98.2	94.6	97.3	97.3	93.9	0.9	0.9	0.7	1.4	1.4	1.6	0.4	0.4	3.8
73	<i>PNG spp8</i>	98	98	94.6	97	97	93.8	1	1	0.8	1.3	1.3	1.4	0.7	0.7	4

74	<i>PNG spp8</i>	98.3	98.3	94.6	97.4	97.4	93.9	0.9	0.9	0.7	1.2	1.2	1.2	0.5	0.5	4.2
8	<i>PNG spp8</i>	98.1	98.1	92.8	97	97	92.1	1.1	1.1	0.7	1.2	1.2	1.5	0.7	0.7	5.7
9	<i>PNG spp8</i>	98.8	98.8	95.3	97.3	97.3	94	1.5	1.5	1.3	0.7	0.7	0.8	0.5	0.5	3.9
66	<i>PNG spp66</i>	98.5	98.4	95.1	97.5	97.4	94.4	1	1	0.7	0.9	0.9	0.9	0.6	0.7	4
5	<i>PNG spp5</i>	98.2	98.1	95.8	95.5	95.4	93.3	2.7	2.7	2.5	1.1	1.1	1.1	0.7	0.8	3.1
70	<i>PNG spp70</i>	98	98	94.9	97	97	94	1	1	0.9	1.3	1.3	1.6	0.7	0.7	3.5
3	<i>cordata</i>	98.6	98.6	96.2	97.8	97.8	95.7	0.8	0.8	0.5	1	1	1.1	0.4	0.4	2.7
71	<i>Iridomyrmex</i>	98.2	98.2	95.7	97.7	97.7	95.2	0.5	0.5	0.5	1.4	1.4	1.1	0.4	0.4	3.2
62	<i>Anonychomyrm a</i>	97.2	97.2	88.2	96.4	96.4	87.6	0.8	0.8	0.6	2.4	2.4	2.2	0.4	0.4	9.6
64	<i>Anonychomyrm a</i>	96.2	96.2	86.6	95.3	95.3	85.9	0.9	0.9	0.7	3.1	3.1	2.2	0.7	0.7	11.2
69	<i>Anonychomyrm a</i>	95.3	95.3	87.3	94.7	94.7	86.9	0.6	0.6	0.4	4	4	3	0.7	0.7	9.7
72	<i>Anonychomyrm a</i>	96.4	96.4	81	95.5	95.5	80.2	0.9	0.9	0.8	2.9	2.9	1.7	0.7	0.7	17.3

Table 3.10 BUSCO completeness of resequencing genomes, using the Hymenoptera odb10 dataset (Manni et al. 2021). Genomes were assembled using SPAdes version 3.15.5 (Prjibelski et al. 2020), then filtered using Blobtoolkit2 (Challis et al. 2020) to remove non-arthropod and mitochondrial sequences and then cleaned in preparation for annotation using Funannotate Clean (Palmer and Stajich 2019) to remove sequences under 1000 bp.

Sample	Species	Completeness			Single Copy			Duplication			Fragmented			Missing		
		Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned	Unfiltered	Filtered	Filtered and cleaned
75	<i>nagasau</i>	92.4	92.4	90.8	92.1	92.1	90.6	0.3	0.3	0.2	3.4	4.8	3.1	4.2	4.2	6.1
79	<i>nagasau</i>	93	93	91.8	92.8	92.8	91.6	0.2	0.2	0.2	3	3.5	2.9	4	4	5.3
80	<i>nagasau</i>	93.2	93.1	91.8	92.8	92.7	91.4	0.4	0.4	0.4	2.9	4.4	2.6	3.9	4	5.6
81	<i>nagasau</i>	92.4	92.2	90.3	92	91.8	90	0.4	0.4	0.3	3.5	2.7	3.2	4.1	4.3	6.5
82	<i>nagasau</i>	91.1	91.1	88.8	90.8	90.8	88.5	0.3	0.3	0.3	4.4	2.9	3.9	4.5	4.5	7.3
83	<i>nagasau</i>	93.6	93.5	92.4	93.2	93.1	92	0.4	0.4	0.4	2.7	2.9	2.4	3.7	3.8	5.2
84	<i>nagasau</i>	93.3	93.3	91.9	92.9	92.9	91.6	0.4	0.4	0.3	2.9	3.2	2.6	3.8	3.8	5.5
85	<i>nagasau</i>	93.4	93.4	92.1	92.8	92.8	91.6	0.6	0.6	0.5	2.9	2.6	2.7	3.7	3.7	5.2
86	<i>nagasau</i>	92.6	92.6	91.2	92.3	92.3	90.9	0.3	0.3	0.3	3.2	2.9	2.9	4.2	4.2	5.9
87	<i>nagasau</i>	93.6	93.5	92.3	93.2	93.1	92	0.4	0.4	0.3	2.6	3	2.4	3.8	3.9	5.3
41	<i>laevigata</i>	90.6	90.6	87.4	90	90	86.9	0.6	0.6	0.5	4.3	6.1	3.6	5.1	5.1	9
45	<i>laevigata</i>	91.1	91.1	88.1	90.6	90.6	87.7	0.5	0.5	0.4	4.2	5.5	3.7	4.7	4.7	8.2
46	<i>laevigata</i>	87.8	87.8	82.5	87.4	97.4	82.1	0.4	0.4	0.4	6.1	4.4	5	6.1	6.1	12.5
47	<i>laevigata</i>	89.2	89.2	85.5	88.6	88.6	85	0.6	0.6	0.5	5.5	4.8	4.6	5.3	5.3	9.9
49	<i>laevigata</i>	90.4	90.3	87.3	90	89.9	87	0.4	0.4	0.3	4.5	6	3.8	5.1	4.3	8.9
50	<i>laevigata</i>	90.3	90.3	87	90	90	86.8	0.3	0.3	0.2	4.8	4.2	3.9	4.9	4.9	9.1
54	<i>laevigata</i>	88.3	88.3	83.8	87.2	87.2	82.8	1.1	1.1	1	6	7.1	5	5.7	5.7	11.2
58	<i>laevigata</i>	91	91	87.7	90.6	90.6	87.4	0.4	0.4	0.3	4.2	6.2	3.6	4.8	4.8	8.7
76	<i>laevigata</i>	90.6	90.6	87.4	89.9	89.9	86.8	0.7	0.7	0.6	4.6	3	3.8	4.8	4.8	8.8
78	<i>laevigata</i>	90.1	90	86.6	89.8	89.7	86.4	0.3	0.3	0.2	4.8	2.9	4.2	5.1	5.2	9.2
61	<i>PNG spp8</i>	85.8	85.8	79.3	85.6	85.6	79.1	0.2	0.2	0.2	7.1	4.7	5.4	7.1	7.1	15.3
63	<i>PNG spp8</i>	90.5	90.5	87.8	90.3	90.3	87.6	0.2	0.2	0.2	4.7	6.3	4	4.8	4.8	8.2
65	<i>PNG spp8</i>	87.6	87.6	83.1	87.4	87.4	83	0.2	0.2	0.1	6.3	9.9	5	6.1	6.1	11.9
67	<i>PNG spp8</i>	80.8	80.8	72.1	79.1	79.1	70.4	1.7	1.7	1.7	9.9	6.9	6.9	9.3	9.3	21
68	<i>PNG spp8</i>	90.7	90.7	87.5	90.3	90.3	87.2	0.4	0.4	0.3	4.7	3.1	4	4.6	4.6	8.5

73	<i>PNG spp8</i>	90.2	90.2	88	89.9	89.9	87.7	0.3	0.3	0.3	4.6	3.4	4.1	5.2	5.2	7.9
74	<i>PNG spp8</i>	90.7	90.7	87.9	90.4	90.4	87.6	0.3	0.3	0.3	4.6	4.6	3.8	4.7	4.7	8.3
8	<i>PNG spp8</i>	90.8	90.7	87.8	89.6	89.5	86.7	1.2	1.2	1.1	4.8	4.8	3.9	4.4	4.5	8.3
9	<i>PNG spp8</i>	92.2	92.2	90.2	91.3	91.3	89.3	0.9	0.9	0.9	3.5	3.5	2.8	4.3	4.3	7
66	<i>PNG spp66</i>	92.1	92	89.7	91.6	91.5	89.2	0.5	0.5	0.5	3.6	4.7	3	4.3	4.4	7.3
5	<i>PNG spp5</i>	92.8	92.5	91.1	90.8	90.5	89.1	2	2	2	3	4.8	2.7	4.2	4.5	6.2
70	<i>PNG spp70</i>	92.6	92.6	90.3	92.2	92.2	89.9	0.4	0.4	0.4	3.1	8.4	2.6	4.3	4.3	7.1
3	<i>cordata</i>	92.9	92.9	91.1	92.5	92.5	90.7	0.4	0.4	0.4	2.9	3.5	2.4	4.2	4.2	6.5
71	<i>Iridomyrmex</i>	90.5	90.5	88.3	90.3	90.3	88.1	0.2	0.2	0.2	4.5	4.6	4	5	5	7.7
62	<i>Anonychomyrma</i>	87.8	87.8	81.7	87.5	87.5	81.5	0.3	0.3	0.2	6.2	6.2	5.1	6	6	13.2
64	<i>Anonychomyrma</i>	87.6	87.6	80.9	87.3	87.3	80.7	0.3	0.3	0.2	6.3	3.6	4.8	6.1	6.2	14.3
69	<i>Anonychomyrma</i>	86.6	86.6	79.8	86.3	86.3	79.6	0.3	0.3	0.2	6.9	4.5	5.4	6.5	6.5	14.8
72	<i>Anonychomyrma</i>	83.4	83.4	73.4	83.1	83.1	73.2	0.3	0.3	0.2	8.4	4.6	5.8	8.2	8.2	20.8

Table 3.11 Assembly statistics for resequencing genomes. Genomes were assembled using SPAdes version 3.15.5 (Prjibelski et al. 2020), then filtered using Blobtoolkit2 (Challis et al. 2020) to remove contamination and mitochondrial sequences and then cleaned in preparation for annotation using Funannotate Clean (Palmer and Stajich 2019) to remove sequences under 1000 bp. Unfiltered (U), Filtered (F) and Filtered and Cleaned (F&C). Coverage (Cov.) was calculated by dividing the sum of the length of the reads by the assembly size after the filtering with Blobtoolkit2 (see column F) number of reads is available in Table S3.3.

Sample	Species	No of contigs			N50			L50			Size of assembly (Mb)			GC %			Cov.
		U	F	F & C	U	F	F & C	U	F	F & C	U	F	F & C	U	F	F & C	
75	<i>nagasau</i>	376379	376364	8808	32754	32754	28976	1836	1836	1825	207.06	207.05	186.98	36.78	36.79	37.09	102
79	<i>nagasau</i>	392463	392453	7383	35348	35352	40145	1717	1716	1427	209.02	209.01	187.12	36.82	36.84	37.15	102
80	<i>nagasau</i>	298564	298553	6356	45370	45317	49445	1330	1330	1141	206.80	206.63	188.65	36.78	36.81	37.13	102
81	<i>nagasau</i>	329311	329301	7782	32155	32115	36285	1813	1814	1525	205.75	205.48	185.80	36.84	36.82	37.12	99
82	<i>nagasau</i>	359787	359777	9111	25153	25153	28610	2412	2412	1975	207.00	206.99	183.61	36.81	36.82	37.08	91
83	<i>nagasau</i>	336679	336670	6128	48761	48751	54067	1238	1238	1066	206.86	206.72	189.08	36.78	36.78	37.09	100
84	<i>nagasau</i>	343770	343759	6841	38779	38779	43917	1509	1509	1270	207.68	207.66	187.99	36.78	36.79	37.11	100
85	<i>nagasau</i>	322398	322388	6440	44686	44686	49455	1323	1323	1134	207.29	207.26	189.47	36.79	36.78	37.08	98
86	<i>nagasau</i>	331732	331725	7297	35426	35440	39865	1675	1674	1414	207.26	207.25	187.58	36.82	36.79	37.08	101
87	<i>nagasau</i>	329668	329660	6063	48611	48604	54313	1211	1211	1042	206.77	206.63	189.21	36.79	36.79	37.08	100
41	<i>laevigata</i>	386010	385943	8415	24025	24032	31292	2520	2519	1731	224.13	224.07	180.95	37.86	37.87	37.55	93
45	<i>laevigata</i>	348156	348038	8233	27222	27248	34286	2317	2316	1663	229.65	229.59	189.82	38.64	38.64	38.53	92
46	<i>laevigata</i>	1041582	1041575	12859	10268	10263	22309	7041	7039	2779	340.90	340.83	211.94	40.21	40.21	38.43	58
47	<i>laevigata</i>	393543	393493	10126	18758	18767	22902	3196	3193	2353	211.16	211.04	176.21	37.07	37.08	37.42	87
49	<i>laevigata</i>	309356	309285	8928	23654	23647	28085	2521	2521	1936	210.68	210.47	180.29	36.91	36.91	37.37	100
50	<i>laevigata</i>	315370	315340	8584	25820	25824	29946	2332	2330	1827	207.83	207.71	179.72	36.85	36.85	37.39	100
54	<i>laevigata</i>	423516	423371	11172	15359	15359	19440	4002	4000	2746	215.93	215.87	172.62	37.03	37.03	37.4	97
58	<i>laevigata</i>	462865	462819	8752	21522	21525	30826	2909	2908	1796	241.75	241.68	184.54	38.57	38.57	37.8	85

76	<i>laevigata</i>	328829	328731	8808	23809	23812	28976	2422	2421	1825	211.59	211.55	180.20	37.06	37.06	37.37	99
78	<i>laevigata</i>	445490	445456	9786	19828	19833	24285	3041	3039	2244	211.48	211.39	176.51	37.2	37.2	37.41	99
61	<i>PNG spp8</i>	424406	424365	12258	10901	10901	15069	5257	5256	3167	210.19	210.16	156.73	36.93	36.93	37.32	86
63	<i>PNG spp8</i>	322047	322013	8640	26189	26194	30723	2344	2343	1835	210.40	210.35	181.61	36.83	36.83	37.36	86
65	<i>PNG spp8</i>	439844	439816	11683	12587	12586	17571	4654	4651	2845	219.42	219.35	165.75	37.46	37.46	37.35	67
67	<i>PNG spp8</i>	502710	502683	13457	8335	8334	11962	7135	7134	3884	211.05	211.02	146.32	36.87	36.87	37.16	98
68	<i>PNG spp8</i>	319487	319472	8300	26602	26596	31939	2165	2166	1658	210.29	210.14	180.62	36.83	36.82	37.33	90
73	<i>PNG spp8</i>	337617	337600	9104	23063	23069	27388	2576	2575	1961	210.43	210.38	179.57	36.84	36.84	37.29	98
74	<i>PNG spp8</i>	347404	347384	8183	28051	28068	33251	2091	2090	1621	211.06	211.00	182.42	36.86	36.86	37.36	92
08	<i>PNG spp8</i>	354515	354502	8411	27410	27410	33063	2137	2136	1654	211.86	211.81	182.82	36.82	36.85	37.36	98
09	<i>PNG spp8</i>	313893	313872	6756	38995	38995	45981	1534	1534	1217	211.96	211.92	185.23	36.84	36.82	37.37	66
66	<i>PNG spp66</i>	337053	337041	7431	33785	33754	40345	1839	1841	1416	220.42	220.15	188.87	37.68	37.68	37.85	96
05	<i>PNG spp5</i>	327494	327482	5770	53406	53301	61478	1126	1127	919	214.69	214.40	190.56	37.02	37.02	37.53	84
70	<i>PNG spp70</i>	326266	326251	6906	36592	36612	43201	1575	1574	1252	209.77	209.75	184.18	37.09	37.09	37.4	99
03	<i>cordata</i>	348573	348569	5920	49006	49006	56024	1166	1166	976	205.10	205.10	185.20	37.14	37.14	37.57	88
71	<i>Iridomyrmex</i>	194962	194957	9004	23398	23398	27016	2335	2335	1880	197.77	197.77	174.88	36.43	36.43	36.73	105
62	<i>Anonychomyrma</i>	399258	399252	11384	15098	15098	18697	3835	3835	2756	206.60	206.58	170.38	36.68	36.68	37.1	94
64	<i>Anonychomyrma</i>	327419	327413	10957	16164	16164	19794	3486	3486	2502	206.72	206.71	171461 335	36.67	36.67	37.17	101
69	<i>Anonychomyrma</i>	360716	360707	11927	13701	13701	16999	4200	4200	2945	205.75	205.75	167.40	36.68	36.68	37.09	102
72	<i>Anonychomyrma</i>	475013	475007	13048	9679	9683	13106	6000	5999	3603	206.09	206.09	152.30	36.71	36.71	37.16	102

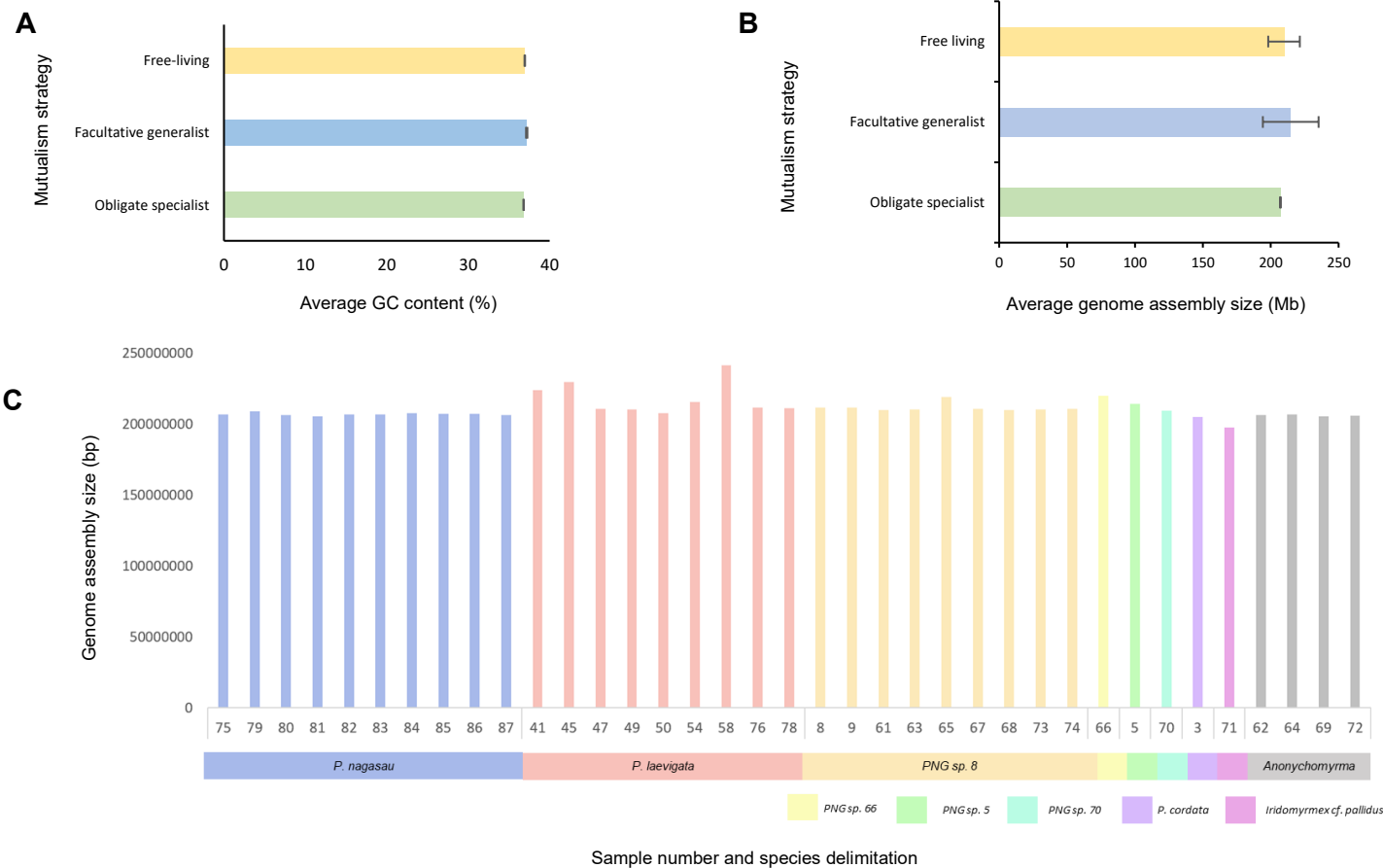


Figure 3.4 (A) Average GC content (%) of resequencing genomes, categorised by mutualism strategy (obligate specialist [i.e. obligate farming of *Squamellaria*], facultative generalist [i.e. facultative farming of various epiphytes] and free living). Error bars represent standard error. (B) Average genome assembly size in base pairs (bp) for resequencing genomes, categorised by mutualism strategy (obligate specialist [i.e. obligate farming of *Squamellaria*], facultative generalist [i.e. facultative farming of various epiphytes] and free living). Error bars represent standard error. (C) genome assembly size in base pairs (bp) categorised by species delimitation. Species delimitation is based of the genome-wide molecular wide phylogeny (see Figures 3.6 and 3.7). Values were taken from the genomes after they have been filtered by Blobtoolkit2 to remove mitochondrial and non-arthropod sequences, but before the Funannotate Clean step that removes contigs under 1000 bp. Sample 46 was excluded from these comparisons as it appears to have an inflated genome size (340.83) and an unusually higher GC compared to the others (40.21), indicating that not all contaminated reads were removed during the first filtering step. This appears to have been resolved by the Funannotate Clean step (See Table 3.11) and therefore it was included in downstream analyses.

3.2 Genome annotations

3.2.1 Traditional annotation software (Funannotate vs Braker3)

When comparing Braker3 and Funannotate we found that Braker3 was better suited for the reference genomes, assembled from PacBio HiFi long reads, producing annotations with higher BUSCO completeness (Table 3.12) and a more sensible gene count compared to Funannotate (Table 3.13). In contrast when testing one resequencing genome (Sample 41), Funannotate appeared to be more appropriate for genomes assembled from Illumina short reads, Funannotate and Braker3 gave a similar BUSCO completeness score (90.9 and 91.2 respectively). However, the BUSCO duplication rate was considerably higher in the annotation produced with Braker, 9.5% compared to 0.5% from Funannotate. For this reason, I did not proceed to annotate the rest of the resequencing genomes with Braker3.

Table 3.12 A comparison of BUSCO completeness (Arthropoda odb10 (Manni et al. 2021)) for annotations produced using Funannotate version 1.8.16 (Palmer and Stajich 2019) (F), Braker3 (Gabriel et al. 2023) (B) and Helixer (Holst et al. 2023) (H) using the reference genomes of *Philidris nagasau* and *P. laevigata*.

Species	Completeness			Single copy			Duplicated			Fragmented			Missing		
	F	B	H	F	B	H	F	B	H	F	B	H	F	B	H
<i>P. nagasau</i>	80.5	95.0	98.7	79.7	87.4	97.6	0.8	7.6	1.1	5.2	0.2	0.7	13.8	4.8	0.6
<i>P. laevigata</i>	73.6	85.2	96.2	73.0	79.6	95.1	0.6	5.6	1.0	10.9	0.7	1.3	15.5	14.1	2.6

Table 3.13 Genome annotation statistics for annotations produced using Funannotate version 1.8.16 (Palmer and Stajich 2019) (F), Braker3 (Gabriel et al. 2023) (B) and Helixer (Holst et al. 2023) (H) using the reference genomes of *Philidris nagasau* and *P. laevigata*. Table is continued below.

Species	Number of gene models			Min. gene length			Max. gene length			Average gene length			Number of gene models less than 200bp in length		
	F	B	H	F	B	H	F	B	H	F	B	H	F	B	H
<i>P. nagasau</i>	32220	15813	13813	68	31	104	45747	307216	359534	1885.3	3636.3	8014	444	67	62
<i>P. laevigata</i>	25636	13244	15231	65	76	104	41618	162011	337202	1809.6	2975.2	6135	675	49	92

Table 3.13 (continued). Genome annotation statistics for annotations produced using Funannotate version 1.8.16 (Palmer and Stajich 2019) (F), Braker3 (Gabriel et al. 2023) (B) and Helixer (Holst et al. 2023) (H) using the reference genomes of *Philidris nagasau* and *P. laevigata*.

Species	Average number of exons per gene model			Average exon length			Number of transcripts			Average number of transcripts per gene model		
	F	B	H	F	B	H	F	B	H	F	B	H
<i>P. nagasau</i>	3.9	3.6	6.8	203.9	273.8	319.8	32038	17860	13813	1	1.1	1
<i>P. laevigata</i>	3.6	3.4	6.4	225.9	280	308.5	25326	14078	15231	1	1.1	1

Table 3.14 BUSCO completeness (Arthropoda odb10, (Manni et al. 2021)) for annotations produced using Funannotate and Helixer using *Philidris* genomes assembled from short-read Illumina resequencing data.

Sample	Species	Completeness		Single copy		Duplicated		Fragmented		Missing	
		Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer
75	<i>nagasau</i>	90.7	96.5	90.2	95.8	0.5	0.7	4.3	1	5	2.5
79	<i>nagasau</i>	94.5	96.9	93.9	96.2	0.6	0.7	2.2	1.3	3.3	1.8
80	<i>nagasau</i>	93.8	96.8	93	96	0.8	0.8	2.5	1.2	3.7	2
81	<i>nagasau</i>	89.9	95.7	89.4	95.1	0.5	0.6	4.1	1.3	6	3
82	<i>nagasau</i>	88.7	94.8	88.4	94.1	0.3	0.7	5.5	1.8	5.8	3.4
83	<i>nagasau</i>	94.3	97.1	93.7	96.5	0.6	0.6	2.2	1.1	3.5	1.8
84	<i>nagasau</i>	94.1	96.9	93.5	96.3	0.6	0.6	2.4	1.1	3.5	2
85	<i>nagasau</i>	91.9	96.9	91	96	0.9	0.9	3.8	1	4.3	2.1
86	<i>nagasau</i>	90.6	96.2	90.2	95.7	0.4	0.5	4.3	1.1	5.1	2.7
87	<i>nagasau</i>	94.9	97.5	94.3	96.9	0.6	0.6	2	1.1	3.1	1.4
41	<i>laevigata</i>	91.1	93.9	90.6	93.3	0.5	0.6	3.5	2	5.4	4.1
45	<i>laevigata</i>	91	93.6	90	92.5	1	1.1	2.9	1.6	6.1	4.8
46	<i>laevigata</i>	83.5	89.7	83.1	89	0.4	0.7	6.7	2.4	9.8	7.9
47	<i>laevigata</i>	90.1	93.1	89	91.8	1.1	1.3	3.6	1.8	6.3	5.1
49	<i>laevigata</i>	91.6	93.8	90.7	93	0.9	0.8	2.4	1.5	6	4.7
50	<i>laevigata</i>	90.3	93.4	89.6	92.7	0.7	0.7	3.1	1.6	6.6	5
54	<i>laevigata</i>	86.6	91.5	85.2	89.4	1.4	2.1	4.6	1.8	8.8	6.7
58	<i>laevigata</i>	90.6	93.3	89.9	92.6	0.7	0.7	2.9	1.7	6.5	5
76	<i>laevigata</i>	88.3	93.5	87	92.2	1.3	1.3	4.6	1.5	7.1	5
78	<i>laevigata</i>	90.7	93.4	90.1	92.9	0.6	0.5	3.1	2.1	6.2	4.5
8	<i>PNG spp8</i>	90.5	93	89.7	92.2	0.8	0.8	3.1	1.8	6.4	5.2
9	<i>PNG spp8</i>	92.6	95.5	91.4	94.1	1.2	1.4	2.1	0.8	5.3	3.7
61	<i>PNG spp8</i>	86.7	88.8	86.3	88.3	0.4	0.5	3.4	1.9	9.9	9.3
63	<i>PNG spp8</i>	88.8	94.4	88.5	94	0.3	0.4	4.7	1.4	6.5	4.2

65	<i>PNG spp8</i>	87.1	92.7	86.7	92.2	0.4	0.5	5.1	1.3	7.8	6
67	<i>PNG spp8</i>	78.4	83.4	75.6	80.3	2.8	3.1	5.3	3.1	16.3	13.5
68	<i>PNG spp8</i>	92	94.6	91.1	93.8	0.9	0.8	2.9	1.8	5.1	3.6
73	<i>PNG spp8</i>	89.6	94.5	88.9	93.5	0.7	1	4.1	1.6	6.3	3.9
74	<i>PNG spp8</i>	91.3	94.3	90.8	93.7	0.5	0.6	3	1.4	5.7	4.3
66	<i>PNG spp66</i>	92.8	95.3	91.6	94.6	1.2	0.7	2.1	1.1	5.1	3.6
5	<i>PNG spp5</i>	94.1	96	91.3	93.2	2.8	2.8	2	1.4	3.9	2.6
70	<i>PNG spp70</i>	92	95	91	93.9	1	1.1	2.6	1.4	5.4	3.6
3	<i>cordata</i>	91.3	96.6	90.6	96	0.7	0.6	3.9	1	4.8	2.4
71	<i>Iridomyrmex</i>	93.5	95.8	92.9	95.1	0.6	0.7	1.9	1	4.6	3.2
62	<i>Anonychomyrma</i>	83.5	88.8	83	88.2	0.5	0.6	5.5	2.1	11	9.1
64	<i>Anonychomyrma</i>	81.7	87.1	80.7	86.3	1	0.8	5	2.2	13.3	10.7
69	<i>Anonychomyrma</i>	82.2	87.4	81.5	86.7	0.7	0.7	6.1	2.9	11.7	9.7
72	<i>Anonychomyrma</i>	78.4	80.7	77.3	79.9	1.1	0.8	3.2	1.9	18.4	17.4

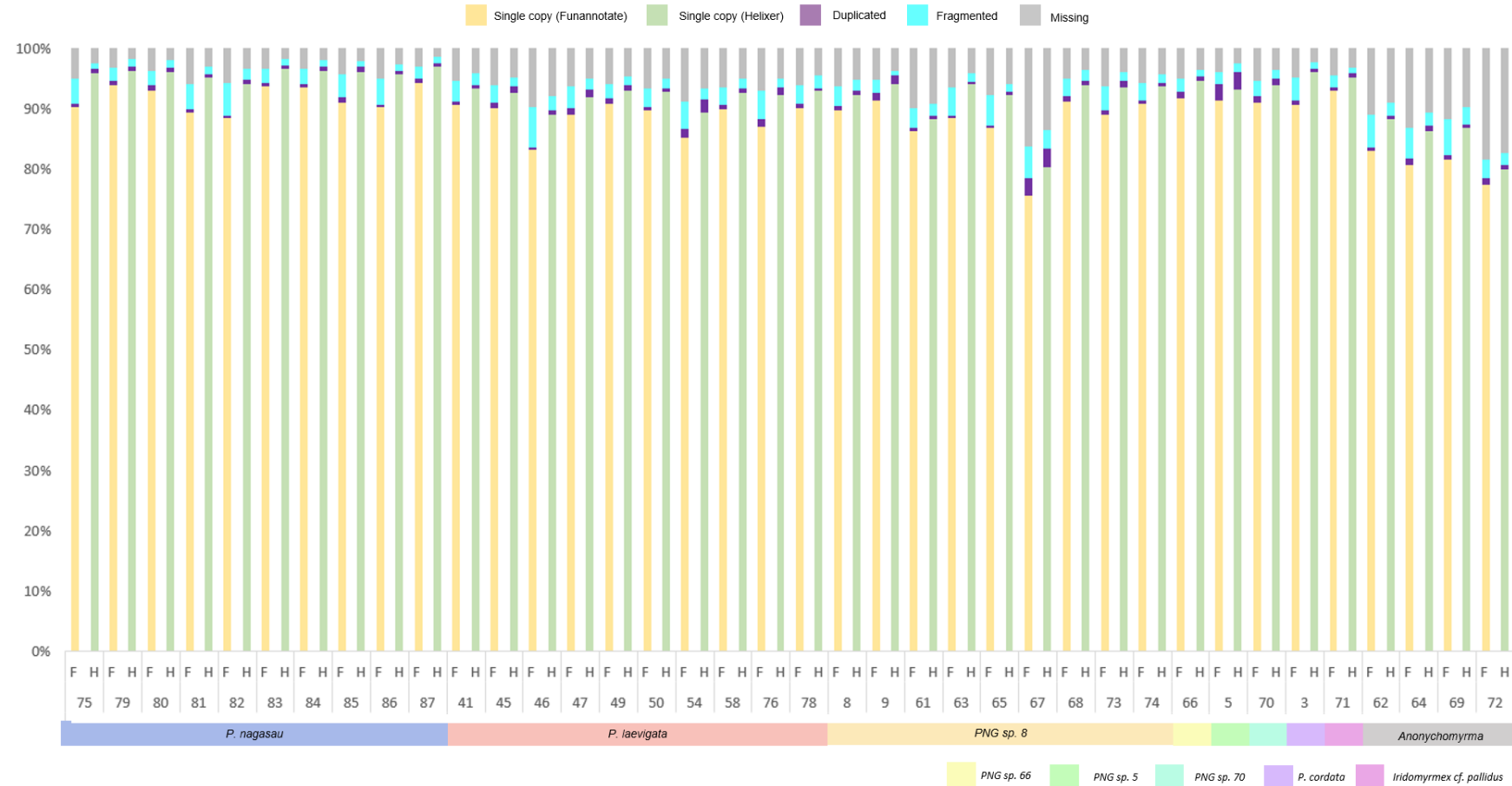


Figure 3.5 BUSCO completeness for Illumina resequencing annotations, comparing Funannotate version 1.8.16 (Palmer and Stajich 2019) (F) and Helixer (Holst et al. 2023) (H) using the Arthropoda odb10 dataset (Manni et al. 2021). Annotations produced with Helixer consistently have a higher BUSCO single copy completeness average (3.7% improvement) and lower BUSCO duplication (2.1% improvement) compared to Funannotate.

Table 3.15 Genome annotation statistics for annotations produced using Funannotate version 1.8.16 (Palmer and Stajich 2019) and Helixer (Holst et al. 2023) of *Philidris* genomes assembled from short-read Illumina resequencing data. Table is continued below.

Sample number	Species	Number of gene models		Min. gene length		Max. gene length		Average gene length		Number of gene models less than 200bp length	
		Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer
75	<i>nagasau</i>	26610	13411	58	107	30601	134979	1674.3	5904.8	634	38
79	<i>nagasau</i>	27443	13457	58	107	47730	155102	1833.9	6045.6	547	51
80	<i>nagasau</i>	27776	13332	71	107	47724	155193	1829.2	6454.6	496	35
81	<i>nagasau</i>	22396	13256	58	105	40226	140983	1821.2	5976.8	563	44
82	<i>nagasau</i>	25881	13409	56	111	31953	146676	1652.1	5486	660	33
83	<i>nagasau</i>	26423	13228	58	104	47039	182301	1883.2	6696.9	487	45
84	<i>nagasau</i>	28669	13369	58	107	47678	162827	1778.2	6128	533	34
85	<i>nagasau</i>	26639	13422	58	106	33308	149520	1738.2	6443.6	571	56
86	<i>nagasau</i>	26551	13257	58	104	41730	158042	1657.4	6086.3	596	56
87	<i>nagasau</i>	27015	13336	68	104	47727	155267	1867.7	6571.7	491	48
41	<i>laevigata</i>	34939	13291	69	106	41204	117026	1459.6	5747.9	655	59
45	<i>laevigata</i>	27328	14894	55	104	47723	143255	1772.5	5446.5	564	72
46	<i>laevigata</i>	48435	16938	61	102	37499	104656	1322.4	4390.7	1156	154
47	<i>laevigata</i>	32715	13247	67	110	34110	119280	1425.4	5154.5	753	49
49	<i>laevigata</i>	26071	13286	67	112	47742	100617	1792.5	5593.3	576	62
50	<i>laevigata</i>	28882	13130	65	113	37373	104253	1658.9	5700.3	587	49
54	<i>laevigata</i>	25048	13288	68	113	33747	124150	1573.4	4939.5	684	60
58	<i>laevigata</i>	31703	13578	69	104	35992	132279	1633	5633.2	570	73
76	<i>laevigata</i>	25644	13300	67	107	25170	141811	1613.8	5655.8	666	58
78	<i>laevigata</i>	26849	13161	67	110	37353	84585	1700	5317.4	638	51
8	<i>PNG spp8</i>	27974	13405	67	108	35559	146098	1722.3	5831.4	512	44
9	<i>PNG spp8</i>	26248	13328	67	110	41189	168947	1867.9	6459.6	476	54
61	<i>PNG spp8</i>	29781	12865	69	107	22121	67752	1438.4	4461.8	822	62
63	<i>PNG spp8</i>	25327	13478	68	106	29505	150805	1672.3	5618.8	651	52

65	<i>PNG spp8</i>	24169	13276	68	104	24025	91380	1569.6	4694.8	690	58
67	<i>PNG spp8</i>	22220	12715	71	101	22590	48596	1514.5	4040	712	49
68	<i>PNG spp8</i>	26583	13167	67	107	35986	159356	1727.2	5822.5	534	42
73	<i>PNG spp8</i>	25679	13481	71	107	33005	134511	1654.1	5472.6	630	53
74	<i>PNG spp8</i>	35300	13401	67	107	45825	135125	1470.6	5818.1	625	57
66	<i>PNG spp66</i>	25023	13405	67	105	33603	132469	1913	6088.5	512	50
5	<i>PNG spp5</i>	26449	13350	68	108	46929	220615	1936.9	6970.5	488	33
70	<i>PNG spp70</i>	26005	13072	68	105	47807	158867	1857.5	6384.2	509	49
3	<i>cordata</i>	23833	13028	67	104	34579	176547	1786.2	6803	492	48
71	<i>Iridomyrmex</i>	25488	13044	66	119	30937	204006	1732	5527.9	605	28
62	<i>Anonychomyrma</i>	23027	12998	68	111	35124	83227	1571.8	4772	680	62
64	<i>Anonychomyrma</i>	24097	12782	71	104	28682	206672	1511.1	5027.6	660	48
69	<i>Anonychomyrma</i>	23039	12917	59	104	21566	88361	1519.8	4653.3	725	60
72	<i>Anonychomyrma</i>	21949	12200	66	104	20413	64460	1607.2	4195.7	734	43

Table 3.15 (continued) Genome annotation statistics for annotations produced using Funannotate version 1.8.16 (Palmer and Stajich 2019) and Helixer (Holst et al. 2023) of *Philidris* genomes assembled from short-read Illumina resequencing data.

Sample number	Species	Number of exons		Average number of exons per gene model		Average exon length		Number of transcripts		Average number of transcripts per gene model	
		Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer	Funannotate	Helixer
75	<i>nagasau</i>	91871	88038	3.5	6.6	250.1	322.8	26434	13411	1	1
79	<i>nagasau</i>	112098	88541	4.1	6.6	219.2	323.4	27286	13457	1	1
80	<i>nagasau</i>	112497	88488	4.1	6.6	220.2	326.5	27627	13332	1	1
81	<i>nagasau</i>	81296	87956	3.6	6.6	258.6	319.7	22247	13256	1	1
82	<i>nagasau</i>	89483	87469	3.5	6.5	248.8	317.8	25723	13409	1	1
83	<i>nagasau</i>	110543	88527	4.2	6.7	218.4	326.3	26282	13228	1	1
84	<i>nagasau</i>	115426	88611	4	6.6	218.5	325	28525	13369	1	1
85	<i>nagasau</i>	93879	89148	3.5	6.6	247.5	325.2	26463	13422	1	1
86	<i>nagasau</i>	91395	88089	3.4	6.6	249.8	322.9	26382	13257	1	1
87	<i>nagasau</i>	112061	88879	4.1	6.7	219	326.7	26869	13336	1	1
41	<i>laevigata</i>	121396	86010	3.5	6.5	222.4	321.6	34828	13291	1	1
45	<i>laevigata</i>	99208	88072	3.6	5.9	289.8	342.5	27105	14894	1	1
46	<i>laevigata</i>	117749	90509	2.4	5.3	390.6	348.3	47851	16938	1	1
47	<i>laevigata</i>	113639	85081	3.5	6.4	219.5	318.2	32600	13247	1	1
49	<i>laevigata</i>	103931	86006	4	6.5	228.7	321.6	25937	13286	1	1
50	<i>laevigata</i>	110584	86082	3.8	6.6	219.8	319.9	28778	13130	1	1
54	<i>laevigata</i>	83829	84230	3.3	6.3	256.9	315.1	24915	13288	1	1
58	<i>laevigata</i>	115333	85957	3.6	6.3	246.7	328	31553	13578	1	1
76	<i>laevigata</i>	87362	86249	3.4	6.5	256.9	322.3	25482	13300	1	1
78	<i>laevigata</i>	104184	85174	3.9	6.5	223.8	318	26729	13161	1	1
8	<i>PNG spp8</i>	109497	87246	3.9	6.5	225.8	324	27850	13405	1	1
9	<i>PNG spp8</i>	107406	88283	4.1	6.6	225	328.2	26112	13328	1	1
61	<i>PNG spp8</i>	105339	80214	3.5	6.2	217.3	314	29679	12865	1	1
63	<i>PNG spp8</i>	86691	87126	3.4	6.5	255.9	322.1	25172	13478	1	1

65	<i>PNG spp8</i>	81043	83031	3.4	6.3	258	316.5	24023	13276	1	1
67	<i>PNG spp8</i>	74076	76981	3.3	6.1	255.2	311.7	22117	12715	1	1
68	<i>PNG spp8</i>	105908	86389	4	6.6	221.4	322.2	26473	13167	1	1
73	<i>PNG spp8</i>	88602	87620	3.5	6.5	248.6	320.3	25552	13481	1	1
74	<i>PNG spp8</i>	125151	86924	3.5	6.5	215.7	324.1	35173	13401	1	1
66	<i>PNG spp66</i>	98995	87866	4	6.6	263.1	325.8	24849	13405	1	1
5	<i>PNG spp5</i>	109009	89507	4.1	6.7	237.3	330	26263	13350	1	1
70	<i>PNG spp70</i>	108163	87226	4.2	6.7	217.1	324.4	25895	13072	1	1
3	<i>cordata</i>	85952	87592	3.6	6.7	257.2	330.1	23669	13028	1	1
71	<i>Iridomyrmex</i>	103328	84985	4.1	6.5	219	320.7	25370	13044	1	1
62	<i>Anonychomyrma</i>	76540	81525	3.3	6.3	272.4	318.8	22841	12998	1	1
64	<i>Anonychomyrma</i>	77218	80654	3.2	6.3	273.8	320.9	23917	12782	1	1
69	<i>Anonychomyrma</i>	75121	80546	3.3	6.2	266.3	316.3	22868	12917	1	1
72	<i>Anonychomyrma</i>	82977	74679	3.8	6.1	240.6	314.7	21811	12200	1	1

3.2.2 Deep-learning approach (Helixer)

Helixer produced higher quality annotations for the reference genomes and the resequencing genomes with higher BUSCO completeness and more reasonable gene counts (more similar to the genome annotation of *Linepithema humile*, the only other currently published Dolichoderine ant genome), than Funannotate (Tables 3.12-15). BUSCO completeness improved by 3.7% in *P. nagasau* and a staggering 11% in *P. laevigata*. Additionally, BUSCO duplication decreased by 6.5% in *P. nagasau* and 4.6% in *P. laevigata* compared to Braker3. The Illumina annotations saw an average increase in BUSCO completeness of 3.8%. BUSCO duplication was roughly consistent between Funannotate and Helixer (on average, Helixer annotations had a 0.06% higher BUSCO duplication score). The BUSCO fragmentation reduced on average by 2.1% for Helixer annotations compared to Funannotate (see Tables 3.14 and 3.15 and Figure 3.5).

A noteworthy result is the increase in annotation quality for the reference genomes, as Helixer does not utilise RNAseq data, unlike Braker3 and Funannotate. To verify whether valuable information was being lost due to the lack of RNAseq input I aligned the RNAseq data to both the Braker and Helixer annotations, and then extracted the unmapped reads and aligned the unmapped Helixer reads to the Braker annotation and vice versa, the overall mapping rate (%) was comparable between the two annotators, with Helixer having a slightly higher rate (Table 3.16).

Table 3.16 Alignment rate of RNAseq data (produced from a worker and male *Philidris nagasau* specimen and a pool of workers and one queen for *P. laevigata*) to genome annotations (CDS proteomes) produced by Braker3 and Helixer. Unlike Braker3, Helixer does not take RNAseq data as an input. To evaluate whether data was missing from the Helixer annotations RNAseq data was aligned to the genome annotations of *P. nagasau* and *P. laevigata*. First the RNAseq data was aligned the annotation, then the unaligned reads were extracted. Reads that were not aligned to the Braker annotation were aligned to Helixer and vice versa.

Species	Caste	Overall alignment rate (%)		Alignment rate of unmapped reads (%)	
		Braker	Helixer	Braker	Helixer
<i>P. nagasau</i>	Worker	23.27	24.72	0.92	3.05
<i>P. nagasau</i>	Male	20.01	21.13	0.71	2.28
<i>P. laevigata</i>	Worker	38.08	44.86	1.73	13.35
<i>P. laevigata</i>	Queen	41.59	48.6	1.81	14.51

3.3 Genome-wide phylogeny of *Philidris*

A previous phylogeny of *Philidris* based on five nuclear genes had little statistical support (Chomicki et al. 2017). I now present a well resolved phylogeny, with a maximally supported backbone produced from 2,100 single copy orthologous genes, using both a coalescent approach (using the software ASTRAL (Zhang et al. 2018)) and a maximum likelihood tree

produced from a concatenated matrix (Figures 3.6 and 3.7). These two approaches produced highly congruent results, with a completely congruent backbone. This exceptional level of congruence, and lack of phylogenetic conflicts in the coalescent tree appears to point to a lack of gene flow between *Philidris* species. Based on this phylogeny, we can see seven *Philidris* potential species: *P. nagasau*, *P. laevigata*, *P. cordata*, *P. sp. 8* [PNG], *P. sp. 66* [PNG], *P. sp. 5* [PNG], and *P. sp. 70* [PNG]. It is not clear which of (if any) of the current accepted *Philidris* species these four Papua New Guinean species represent, since there is no obvious match between the locality of our Papua New Guinean samples and the type localities of *P. brunnea* or *P. pubescens* (Bolton 2024).

Both the concatenated (Figure 3.6) and coalescent phylogenetic tree (Figure 3.7) are well supported with high bootstrap and posterior probability support, respectively, for the deeper nodes. The topologies of the concatenated and the posterior probability tree backbones were entirely congruent. The facultative ant-garden farming ant, Australian *Philidris cordata* is the earliest diverging species, followed by the obligate Fijian *P. nagasau*. The Fijian *Philidris nagasau* clade is well supported, and specimens from the two islands where this species occurs (Vanua Levu and Taveuni) separate into two clades. Then, four Papua New Guinean lineages split, forming a paraphyletic group including (*P. sp. 8* [PNG], *P. sp. 66* [PNG], *P. sp. 5* [PNG], and *P. sp. 70* [PNG]), which consist in a mixture of facultative ant-garden farming ants and non-ant associated. Finally, all South-East Asian (Thailand, peninsular Malaysia, Borneo) specimens group together with *Philidris laevigata*; all of these are facultative ant-garden farmers.

3.4 Amino acid biosynthesis pathways

Philidris nagasau and *P. laevigata* had identical abilities to produce amino acids with the ability to produce glutamine, glycine, serine, cysteine, proline, asparagine, aspartate and glutamate, see Table 3.17. The two *Philidris* species lacked the ability to produce arginine, alanine, threonine, methionine, valine, leucine, isoleucine, lysine, histidine, tyrosine, phenylalanine, and tryptophan. *Anonychomyrma sp.* had the ability to produce fewer amino acids than the two *Philidris* species investigated, lacking the pathways to produce glycine, serine and cysteine. It is a possibility that the lower completeness and contiguity of the *Anonychomyrma* genome and annotation compared to the *P. nagasau* and *P. laevigata* reference genomes, contributed to this result.

3.5 Genome size and GC content

There were no significant differences in genome assembly size between genomes of obligate specialists, facultative generalists or non-mutualists (Tukey's HSD adjusted p value: non-mutualist - facultative generalist 0.1979818, obligate specialist- facultative generalist

0.0331823 and obligate specialist – non-mutualist 0.6758711). Similarly, there was no significant difference in GC content between genome assemblies of obligate specialists, facultative generalists and non-mutualists mutualists (Tukey's HSD adjusted p value: non-mutualist - facultative generalist 0.4057894, obligate specialist- facultative generalist 0.1026065 and obligate specialist – non-mutualist 0.7510953).

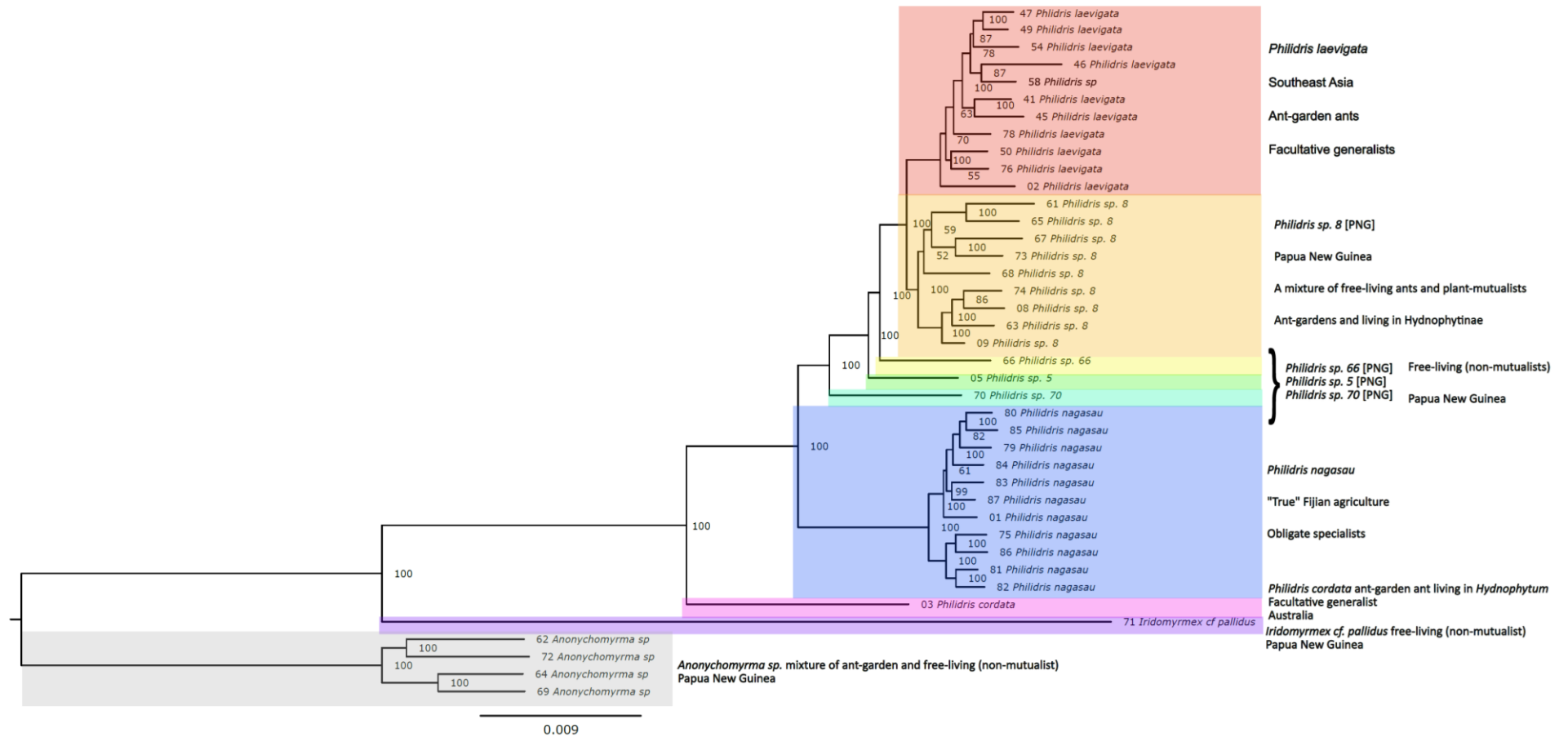


Figure 3.6 Genome-wide maximum likelihood phylogeny of *Philidris*, calculated from a concatenated matrix of 2,100 single copy orthologous genes. Node labels represent bootstrap values. Scale bar represents average number of changes per site.

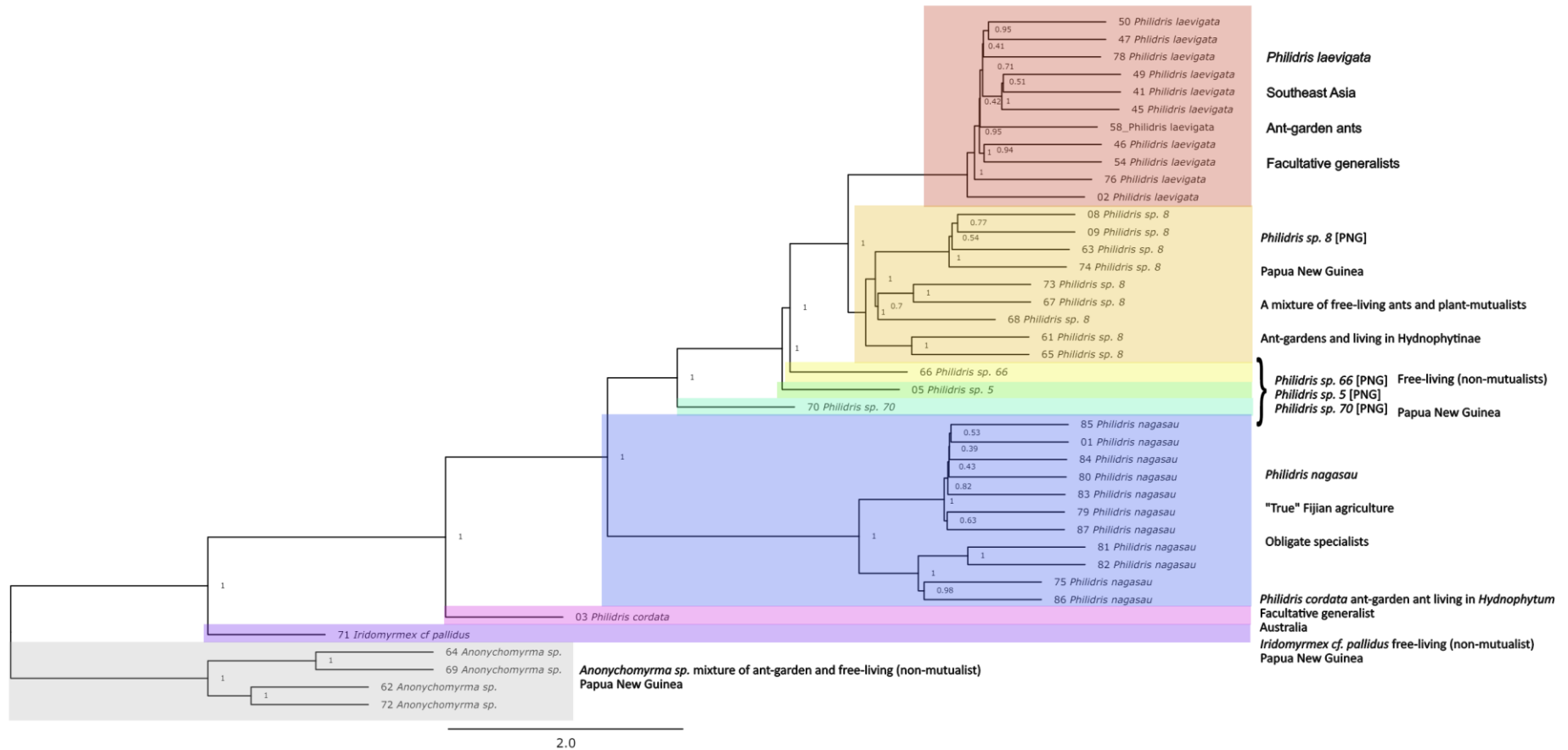


Figure 3.7 Genome wide coalescent phylogeny of *Philidris*, constructed using 2100 gene trees calculated using ASTRAL version 5.7.8 (Zhang et al. 2018). Node labels represent posterior probabilities. ASTRAL measures branch lengths in coalescent units (the scale bar shown corresponds to two coalescent units) for internal branches and not terminal branches (branch lengths of terminal branches are therefore arbitrary and meaningless).

Table 3.17 Amino acid biosynthesis capabilities of *Philidris nagasau*, *P. laevigata*, *Anonychomyrma* sp. and plant mutualists *Squamellaria imberbis* and *S. wilkinsonii**, analysis performed using KofamKOALA (Aramaki et al. 2020). If the pathway to produce the amino acid is complete it is marked with “yes” and if it is incomplete it is marked with “no”. *Analysis of plant genomes performed by PhD student Noah Bourne (Sheffield University). **Amino acids that are essential in the diets of *Drosophila*.

Amino Acid	<i>P. nagasau</i>	<i>P. laevigata</i>	<i>Anonychomyrma</i> sp.	<i>S. imberbis</i>	<i>S. wilkinsonii</i>
Arginine**	No	No	No	Yes	Yes
Alanine	No	No	No	Yes	Yes
Glutamine	Yes	Yes	Yes	Yes	Yes
Glycine	Yes	Yes	No	Yes	Yes
Serine	Yes	Yes	No	Yes	Yes
Threonine**	No	No	No	Yes	Yes
Cysteine	Yes	Yes	No	Yes	Yes
Methionine**	No	No	No	Yes	Yes
Valine**	No	No	No	Yes	Yes
Leucine**	No	No	No	Yes	Yes
Isoleucine**	No	No	No	Yes	Yes
Lysine**	No	No	No	Yes	Yes
Proline	Yes	Yes	Yes	Yes	Yes
Histidine**	No	No	No	Yes	Yes
Tyrosine	No	No	No	Yes	Yes
Phenylalanine**	No	No	No	Yes	Yes
Tryptophan**	No	No	No	Yes	Yes
Asparagine	Yes	Yes	Yes	Yes	Yes
Aspartate	Yes	Yes	Yes	Yes	Yes
Glutamate	Yes	Yes	Yes	Yes	Yes

4. Discussion

4.1 Building a genome-wide evolutionary framework for *Philidris* farming ants

This study contributes two high quality reference genomes and an additional 38 resequencing genomes, thus building the genomic framework for studying the genus *Philidris*. Both the reference genomes and resequencing genomes had high levels of BUSCO completeness, despite the expected low levels of contiguity in the resequencing genomes, giving a strong basis for further analyses. *Philidris* is particularly useful for the study of mutualistic dependence, due to the great variation seen in mutualistic strategy, varying from obligate specialist plant-farmer *Philidris nagasau* (Chomicki and Renner 2016a) to the facultative generalist ant-garden ants in Southeast Asia and Australasia (Kaufmann 2002, Chomicki et al. 2017, Campbell et al. 2023) and populations that appear to not engage in any plant mutualism at all (Milan Janda, pers. comm. March 2022). This phylogenomic and comparative genomic framework for *Philidris* is pivotal and is the basis for Chapters 4 and 5 which explore gene family expansions and contractions in response to the evolution of agriculture and shifts in signatures of selection relating to mutualism strategy.

4.2 Comparisons of long-read assemblers for *Philidris* ants

The field of bioinformatics is quick moving, with tools being released and updated constantly. This can provide challenges as well as great opportunities. HiFiasm is the most widely used genome assembler for HiFi reads, and appears to perform well in most cases (Cheng et al. 2021). However, as seen in our study, performance can vary. I found that HiFiasm produced a poor result when assembling HiFi reads for *P. laevigata*, giving a high level of BUSCO duplication (33.9% Arthropoda, 30.7% Hymenoptera) and a genome that is much larger than expected (398Mb). When the genome was reassembled with another software, wtdbg (Ruan and Li 2020), the genome had higher levels of BUSCO completeness, low levels of BUSCO duplication and a genome size in line with what we expect for Dolichoderine ants. In contrast, when comparing HiFiasm and wtdbg for *P. nagasau* HiFiasm produced a more contiguous and complete assembly (although the wtdbg was not a poor assembly).

4.3 Comparisons of annotation software for long and short reads in *Philidris* ants

Similarly, there are many genome annotation methods to choose from. For this study I compared Braker3 (Gabriel et al. 2024), Funannotate (Palmer and Stajich 2019) and a machine learning based, Helixer (Holst et al. 2023). Initially I compared Braker3 and Funannotate, Braker3 performed best for the reference genomes assembled from PacBio

HiFi reads whereas Funannotate performed best for the resequencing genomes assembled from Illumina reads. This is not surprising given that Funannotate is an older software, developed at a time when most genomes were assembled from short reads. Helixer is a genome annotation program that utilises deep neural networks (Holst et al. 2023). We found that Helixer outperformed both Braker and Funannotate in terms of BUSCO completeness as well as producing more sensible gene counts compared to Funannotate, which seems to give an inflated gene count. Machine learning approaches such as Helixer have promise to increase accessibility of genome annotations for researchers working on species wherein obtaining high quality RNAseq data is not feasible, or for researchers with budget constraints.

4.4 A phylogenomic framework for *Philidris*

The phylogeny presented here is the first well supported and resolved phylogeny of *Philidris*. Two approaches were used, a coalescent approach calculated from 2,100 gene trees and a maximum likelihood analysis of a concatenated alignment of 2,100 genes. The two approaches produced highly congruent results, with a completely congruent backbone.

Based on this phylogeny, we can see seven *Philidris* potential species: *P. nagasau*, *P. laevigata*, *P. cordata*, *P. sp. 8* [PNG], *P. sp. 66* [PNG], *P. sp. 5* [PNG], and *P. sp. 70* [PNG]. It is not clear which of (if any) of the current accepted *Philidris* species these four Papua New Guinean species represent, since there is no obvious match between the locality of our Papua New Guinean samples and the type localities of *P. brunnea* or *P. pubescens* (Bolton 2024). Biogeographic analyses of the subfamily Dolichoderinae (Ward et al. 2010) and an earlier 5-gene phylogeny for *Philidris* (Chomicki et al., 2017) indicated that *Philidris* likely originated in Australia in the mid Miocene (~10 Ma) when Southern New Guinea was still submerged (Hall 2009). Following the decrease in sea level Australia was connected to Southern New Guinea and *Philidris* was enabled to expand its range, this provided a stepping stone for further range expansion into what is now Northern New Guinea, but at the time was several disconnected landmasses (around 10-5Ma). *Philidris* seems to have colonised Sundaland at around 10Ma from Australia-Southern New Guinea. *Philidris nagasau* also appears to have colonised Fiji from Australia-Southern New Guinea, there are two hypotheses as to when this occurred. First, if *P. nagasau* directly colonised the islands in which it is endemic (Vanua Levu and Taveuni) then this can only have happened as early as 4Ma (the age of Vanua Levu), Taveuni only emerged 0.8 Ma. Alternatively, *P. nagasau* may have colonised Fiji as early as 6.5 +/- 3Ma (the stem age of *P. nagasau*), colonising the largest and oldest Fijian island (Viti Levu), subsequently colonising Vanua Levu and Taveuni and going extinct on the main island (Chomicki et al. 2017).

My phylogenomic backbone is consistent with this hypothesis. Hence, the colonization of Fiji occurred from this area as a long-distance dispersal, while that of South-East Asia involved more 'island-hopping' throughout the Sunda shelf.

Shattuck erected *Philidris* as a genus in 1992 transferring the species *P. cordatus*, *P. cordatus fuscus*, *P. cordatus protensus*, *P. cordatus stewartii*, *P. crudus*, *laevigatus*, *myrmecodiae*, *myrmecodiae andamanensis*, *myrmecodiae mandibularis*, *myrmecodiae nigriventris*, *nagasau*, *nagasau agnatus*, *nagasau alticola*, *pubescens* from the genus *Iridomyrmex* to *Philidris*. Additionally, the species *Pseudolasius brunneus* was transferred to *Philidris* (Shattuck 1992a). Mann first described *Philidris nagasau* (at the time, *Iridomyrmex nagasau*) in 1921. In addition to the standard *P. nagasau nagasau*, Mann described two additional subspecies of *P. nagasau*, *alticola* and *agnatus* described from Taveuni and Vanua Levu, respectively (Mann 1921). *P. nagasau alticola* was differentiated from *P. nagasau nagasau* in that the basal portion of the epinotum is less elevated and that the head is reddish in colour and the thorax slightly lighter. *P. nagasau agnatus* is differentiated from *P. nagasau nagasau* as being smaller and having a lower, more sloping epinotum (as seen in *P. nagasau alticola*) and being brown in colour, with a slightly darker gaster and white tarsi (Mann 1921). Sarnat and Economo (2012) examined numerous *P. nagasau* colonies and found that the variation in colour and shape separating these three taxa "bleed into a near seamless continuum" with colour seeming to be conserved within nest series but varies between islands and localities and found that there are no other characters that correlated with colour. Therefore, Sarnat and Economo (2012) synonymised the subspecies to be one species. Our molecular phylogeny separates into two clades, one containing the specimens from Vanua Levu and the other from Taveuni. The *P. nagasau* samples in this phylogeny clearly separate into two clades, one clade containing samples collected from Vanua Levu and another clade containing samples from Taveuni, suggested that the geographic separation has also led to reproductive isolation of these two populations. However, given the short branch lengths between these two populations, the lack of any useful morphological characteristics and identical farming behaviours I agree that synonymising the *P. nagasau* subspecies is appropriate.

Philidris is in great need of a taxonomic revision. There are currently 9 accepted species and 7 valid subspecies (Bolton 2024). Our sampling covers a most of *Philidris*' range although is missing samples from China and India. There is currently no evidence of ant-plant mutualism within *Philidris* in China or India, and therefore the inclusion of South Asian *Philidris* would be particularly interesting for the future study of mutualism strategy. Due to the lack of a clear key, as well as variation in morphology within our 'species' (Kaufmann, 2002; M. Janda, pers. comm., 2023), it is not clear which of the described species are sampled in our study.

Nevertheless, our widespread geographic coverage, thanks to field collections by Eva Kauffmann (Thailand, Malaysia, Borneo), Milan Janda (Papua New Guinea, Australia), and Guillaume Chomicki (Fiji) covers most of the range, and all mutualistic strategies.

4.5 Comparisons to other obligate symbioses

This dataset represents a variation in mutualistic dependence varying from obligate specialists (*P. nagasau*) to facultative generalists (*P. laevigata*, *P. cordata*, some of *P. sp. 8* and some of the outgroup *Anonychomyrma* sp.) and free-living non-mutualists (*P. sp. 66*, *P. sp. 5*, *P. sp. 70* and *Iridomyrmex cf. pallidus*) (see Table S3.3 for details per sample). This variation in dependence allows the opportunity to investigate the consequences of shifts in mutualism strategy on genome evolution. One hypothesis is that shifts to obligate specialist lifestyle would mirror the changes seen in bacterial symbionts, which experience significant genome size reduction (Moran and Bennett 2014) as well as an AT nucleotide composition bias (McCutcheon and Moran 2010), based on our 40 genome assemblies, there is no evidence that mutualism strategy leads to a reduction in genome size or GC content (Figure 3.4), indicating that the shifts in genome size and GC content are a consequence of endosymbiotic life specifically (i.e. the mode of interdependence *sensu* Lewis (1985) rather than the degree of interdependence). The lack of difference of genome size of the obligately dependent mutualist *P. nagasau* suggests that obligate dependency is mediated by very distinct mechanisms or at least has different consequences on genome evolution. So far, our knowledge of mutualistic dependence is largely restricted to microbial endosymbioses, where the symbiont is obligately dependent via functional gene loss and further undergoes genome streamlining (McCutcheon and Moran 2010, Bennett and Moran 2015).

It has been proposed that attine ants became irreversibly obligately dependent on farming fungi following the loss of the arginine biosynthesis pathway (Nygaard et al. 2016). While the primary function of the mutualism between *P. nagasau* and farmed *Squamellaria* is the provisioning of nesting space, five of the six farmed species provide food rewards to *P. nagasau*, whereas most southeast Asian ant-garden plants do not provide any food rewards (Campbell et al. 2023 and Chapter 2). *Philidris nagasau* and *P. laevigata* both had identical amino acid biosynthesis repertoires, suggesting that the shift from facultative generalist mutualism to obligate specialist was not accompanied with a loss in amino acid biosynthesis pathways. *Anonychomyrma* sp. (sample 62) was also analysed as an outgroup, and to represent non-mutualist lifestyle. *Anonychomyrma* had fewer complete amino acid biosynthesis pathways than *P. nagasau* and *P. laevigata*, further indicating that plant cultivation mutualisms in ants are not accompanied with losses of amino acid biosynthesis, although this result may be due to the lower completeness and contiguity rather than an actual loss of these genes. Although there is no evidence that amino acid provisioning is the

primary mechanism leading to *P. nagasau* being obligately dependent on *Squamellaria*, it is possible that *Squamellaria* is provisioning amino acids to *P. nagasau*, given that *Squamellaria* has complete biosynthesis pathways for all twenty amino acids (Table 3.17). This provisioning could therefore be acting as an additional benefit of the mutualism, and strengthen the dependence even if it is not the primary mechanism.

Philidris nagasau, attine ants and bacterial endosymbionts are all obligate specialist mutualists. However, the mechanisms that led these species to be obligately dependent on their mutualist partners differ greatly. A framework for describing species interactions proposed by Lewis (1985) captures some of these differences well. Of relevance here are the criteria integration and dependence. Integration refers to the range from independent to integrated, making a distinction between species that are independent organisms from the species they are interacting with, in contrast to endosymbionts which are not. Endosymbiotic parasites also experience a reduction in genome size compared to free-living relatives (Moran and Plague 2004) suggesting that this particular genomic change is related to endosymbiosis rather than obligate mutualism and therefore it is not surprising that obligate mutualisms in non-endosymbionts do not experience similar genomic streamlining. Next, the criterion dependence differentiates between obligate and facultative interactions, and has the subcriterion of “mode”. The mode of dependence refers to the mechanism of dependence. *Philidris nagasau* depends on farming *Squamellaria* for nesting space, not food, evidenced by the fact that one of the six species of farmed *Squamellaria* (*S. grayi*) does not provide food rewards and yet *P. nagasau* displays no preference for the food reward bearing species (Chomicki et al. 2016). *Philidris nagasau* has become dependent on farmed *Squamellaria* due to losing the ability to build their own carton nests, the dependence is mediated by a loss in behaviour. In contrast, the mutualism between attine ants and their farmed fungi is nutritional.

5. Conclusion

Ant genomics is a quickly expanding field, with just under 100 genomes already published. However, the vast majority of the 14,000 described ant species do not currently have a genome. This study contributes two reference quality genomes, assembled from HiFi reads and a further 38 resequencing genomes assembled from short Illumina reads. This is a large contribution to the study of Dolichoderinae, which prior to this study had one genome, and to the understanding of ant-plant mutualism. We present a well-resolved phylogeny, which was not possible before when using five genes. This work creates the foundation for comparative

work, including gene family expansion and contraction analysis, signatures of selection and population genetics work.

Supplementary material (Chapter 3)

Table S3.1 Ant genome assemblies available on 16/04/2024 from the NCBI Genbank database.

Genus	Species	Sex	Assembly size (bp)	Assembly level	Date of publication
Ponerinae					
<i>Dinoponera</i>	<i>quadriceps</i>	male	259665865	Scaffold	13/10/2015
<i>Harpegnathos</i>	<i>saltator</i>		294465601	Scaffold	17/09/2010
<i>Harpegnathos</i>	<i>saltator</i>	female	334161684	Scaffold	14/06/2018
<i>Odontomachus</i>	<i>brunneus</i>	male	393036571	Scaffold	13/02/2020
Dolichoderinae					
<i>Linepithema</i>	<i>humile</i>		219500750	Scaffold	10/06/2011
Dorylinae					
<i>Eciton</i>	<i>burchellii</i>		192453053	Scaffold	07/10/2021
<i>Ooceraea</i>	<i>biroi</i>		212825769	Scaffold	08/04/2014
<i>Ooceraea</i>	<i>biroi</i>		223876465	Chromosome	23/10/2018
Formicinae					
<i>Anoplolepis</i>	<i>gracilipes</i>		234485207	Scaffold	16/02/2023
<i>Anoplolepis</i>	<i>gracilipes</i>		235423743	Scaffold	17/02/2023
<i>Anoplolepis</i>	<i>gracilipes</i>		243919194	Scaffold	08/09/2023
<i>Camponotus</i>	<i>fellah</i>		266031460	Contig	02/08/2023
<i>Camponotus</i>	<i>floridanus</i>		232685334	Scaffold	17/09/2010
<i>Camponotus</i>	<i>floridanus</i>	female	284009182	Scaffold	14/06/2018
<i>Camponotus</i>	<i>pennsylvanicus</i>	female	306426343	Contig	02/06/2022
<i>Camponotus</i>	<i>vicinus</i>	male	302730088	Scaffold	03/10/2022
<i>Cataglyphis</i>	<i>hispanica</i>	male	198942706	Scaffold	15/02/2019
<i>Cataglyphis</i>	<i>hispanica</i>	male	206388925	Chromosome	19/01/2022
<i>Cataglyphis</i>	<i>hispanica</i>	male	208734006	Chromosome	19/01/2022
<i>Cataglyphis</i>	<i>niger</i>	male	295977620	Scaffold	06/03/2019
<i>Formica</i>	<i>aquilonia</i> x <i>F.polyctena</i>		272015305	Contig	15/05/2021
<i>Formica</i>	<i>aserva</i>	female	310433589	Scaffold	07/03/2024

<i>Formica</i>	<i>exsecta</i>	male	277633851	Scaffold	15/10/2018
<i>Formica</i>	<i>selysi</i>	male	290452129	Chromosome	14/01/2020
<i>Lasius</i>	<i>fuliginosus</i>		256203092	Chromosome	08/04/2023
<i>Lasius</i>	<i>niger</i>	female	236236391	Scaffold	02/07/2015
<i>Lasius</i>	<i>platythorax</i>		288673857	Chromosome	07/03/2024
<i>Nylanderia</i>	<i>fulva</i>		369947649	Scaffold	13/05/2019
<i>Nylanderia</i>	<i>fulva</i>		375170133	Chromosome	15/07/2022
<i>Polyergus</i>	<i>mexicanus</i>	male	252309906	Scaffold	13/07/2023
<i>Prenolepis</i>	<i>imparis</i>	male	327265477	Scaffold	14/07/2023
Myrmicinae					
<i>Acromyrmex</i>	<i>charruanus</i>		296539234	Scaffold	29/03/2021
<i>Acromyrmex</i>	<i>echinator</i>	male	295944863	Scaffold	03/05/2011
<i>Acromyrmex</i>	<i>echinator</i>		313947481	Scaffold	19/08/2022
<i>Acromyrmex</i>	<i>heyeri</i>		295598610	Scaffold	29/03/2021
<i>Acromyrmex</i>	<i>insinuator</i>		294656391	Scaffold	29/03/2021
<i>Aphaenogaster</i>	<i>ashmeadi</i>		310333780	Scaffold	23/04/2018
<i>Aphaenogaster</i>	<i>floridana</i>		382861007	Scaffold	23/04/2018
<i>Aphaenogaster</i>	<i>fulva</i>		346134187	Scaffold	23/04/2018
<i>Aphaenogaster</i>	<i>miamiana</i>		342642954	Scaffold	23/04/2018
<i>Aphaenogaster</i>	<i>picea</i>		386044929	Scaffold	23/04/2018
<i>Aphaenogaster</i>	<i>rudis</i>		395412201	Scaffold	23/04/2018
<i>Aphaenogaster</i>	<i>rudis</i>		429703865	Scaffold	23/04/2018
<i>Atta</i>	<i>cephalotes</i>	male	317671980	Scaffold	06/07/2010
<i>Atta</i>	<i>colombica</i>		291257934	Scaffold	25/03/2016
<i>Atta</i>	<i>texana</i>	male	286872587	Scaffold	27/03/2019
<i>Cardiocondyla</i>	<i>obscurior</i>	pooled male and female	193051228	Scaffold	29/07/2021
<i>Cephalotes</i>	<i>varians</i>	male	291692201	Scaffold	14/03/2024
<i>Crematogaster</i>	<i>levior</i>		326199551	Scaffold	16/07/2019
<i>Cyphomyrmex</i>	<i>costatus</i>		299845939	Scaffold	25/03/2016

<i>Harpagoxenus</i>	<i>sublaevis</i>		341523168	Contig	17/08/2023
<i>Leptothorax</i>	<i>acervorum</i>		344696344	Contig	08/01/2024
<i>Monomorium</i>	<i>pharaonis</i>		257976923	Scaffold	22/04/2015
<i>Monomorium</i>	<i>pharaonis</i>	male	262528503	Scaffold	19/09/2018
<i>Monomorium</i>	<i>pharaonis</i>		259040147	Scaffold	06/02/2019
<i>Monomorium</i>	<i>pharaonis</i>	female	325506644	Chromosome	14/08/2020
<i>Pogonomyrmex</i>	<i>barbatus</i>		235645958	Scaffold	04/02/2011
<i>Pogonomyrmex</i>	<i>californicus</i>	male	241054575	Scaffold	02/03/2021
<i>Pogonomyrmex</i>	<i>californicus</i>	female	252297138	Scaffold	20/07/2022
<i>Pseudoatta</i>	<i>argentina</i>		280199293	Scaffold	29/03/2021
<i>Solenopsis</i>	<i>fugax</i>	male	335323959	Scaffold	25/09/2018
<i>Solenopsis</i>	<i>invicta</i>	male	398979080	Scaffold	01/08/2018
<i>Solenopsis</i>	<i>invicta</i>		384494608	Chromosome	28/10/2019
<i>Solenopsis</i>	<i>invicta</i>		349309009	Chromosome	28/10/2019
<i>Solenopsis</i>	<i>invicta</i>	male	409712810	Chromosome	14/11/2019
<i>Solenopsis</i>	<i>invicta</i>	female	413978255	Chromosome	12/02/2020
<i>Solenopsis</i>	<i>invicta</i>	male	378101515	Chromosome	03/02/2021
<i>Solenopsis</i>	<i>invicta</i>		384929089	Chromosome	03/06/2021
<i>Temnothorax</i>	<i>americanus</i>		277741648	Contig	17/08/2023
<i>Temnothorax</i>	<i>curvispinosus</i>		303539295	Scaffold	24/04/2018
<i>Temnothorax</i>	<i>longispinosus</i>	male	260681563	Scaffold	15/04/2019
<i>Temnothorax</i>	<i>longispinosus</i>		298285805	Contig	22/08/2023
<i>Temnothorax</i>	<i>nylanderii</i>		313490928	Contig	22/08/2023
<i>Temnothorax</i>	<i>ravouxi</i>		362408415	Contig	17/08/2023
<i>Temnothorax</i>	<i>rugatulus</i>		335735536	Contig	08/01/2024
<i>Temnothorax</i>	<i>unifasciatus</i>		314503407	Contig	17/08/2023
<i>Tetramorium</i>	<i>alpestre</i>	male	245724784	Scaffold	13/01/2022
<i>Tetramorium</i>	<i>bicarinatum</i>	male	229238156	Scaffold	23/03/2020
<i>Tetramorium</i>	<i>bicarinatum</i>		257967262	Chromosome	02/02/2022
<i>Tetramorium</i>	<i>immigrans</i>	male	227078691	Scaffold	23/03/2020

<i>Tetramorium</i>	<i>parvispinum</i>	male	229641016	Scaffold	23/03/2020
<i>Tetramorium</i>	<i>simillimum</i>	male	232155731	Scaffold	23/03/2020
<i>Trachymyrmex</i>	<i>cornetzi</i>		369078778	Scaffold	25/03/2016
<i>Trachymyrmex</i>	<i>septentrionalis</i>		291747019	Scaffold	25/03/2016
<i>Trachymyrmex</i>	<i>zeteki</i>		267973152	Scaffold	31/03/2016
<i>Vollenhovia</i>	<i>emeryi</i>		287900827	Scaffold	06/03/2015
<i>Wasmannia</i>	<i>auropunctata</i>		324120201	Scaffold	17/03/2015
Pseudomyrmecinae					
<i>Pseudomyrmex</i>	<i>concolor</i>	female	196807752	Scaffold	30/09/2020
<i>Pseudomyrmex</i>	<i>cubaensis</i>	female	204624832	Scaffold	30/09/2020
<i>Pseudomyrmex</i>	<i>dendroicus</i>	female	206595161	Scaffold	30/09/2020
<i>Pseudomyrmex</i>	<i>elongatus</i>	female	244058880	Scaffold	30/09/2020
<i>Pseudomyrmex</i>	<i>flavicornis</i>	female	198472643	Scaffold	30/09/2020
<i>Pseudomyrmex</i>	<i>gracilis</i>		282776121	Scaffold	23/02/2017
<i>Pseudomyrmex</i>	<i>pallidus</i>	female	200367487	Scaffold	30/09/2020
<i>Pseudomyrmex</i>	<i>sp.PSW-54</i>	female	194520232	Scaffold	30/09/2020

Table S3.2 Estimated genome sizes predicted from PacBio HiFi reads analysed by Jellyfish version 2.3.0.

Species	Estimated minimum length (bp)	Estimated maximum length (bp)
<i>P. nagasau</i>	250,950,036	297,573,254
<i>P. laevigata</i>	136,688,951	139,395,329

Table S3.3 Number of reads and clean bases for the Illumina sequencing data for this project. Species delimitation is based of phylogenies (see Figures 3.6 and 3.7).

Sample ID	Species	Voucher	Geography	Presence and details of mutualistic association with plants	Clean Reads	Clean Base
41	<i>Philidris laevigata</i>	Kfm A85-813	Thailand	Yes, ant-garden	208453236	20845323600
45	<i>Philidris laevigata</i> .	Kfm A37-830	Malaysia	Yes, ant-garden	210625562	21062556200
46	<i>Philidris laevigata</i> .	Kfm A37-1187	Thailand	Yes, ant-garden	196812840	19681284000
47	<i>Philidris laevigata</i> .	Kfm A160-876	Borneo	Yes, ant-garden	183104526	18310452600
49	<i>Philidris laevigata</i>	Kfm A37-42	Malaysia	Yes, ant-garden	209638594	20963859400
50	<i>Philidris laevigata</i>	Kfm A160-735	Borneo	Yes, ant-garden	208576828	20857682800
54	<i>Philidris laevigata</i> .	Kfm A209-724	Borneo	Yes, ant-garden	209080312	20908031200
58	<i>Philidris laevigata</i>	Kfm A208-479	Borneo	Yes, ant-garden	206503032	20650303200
61	<i>Philidris sp.8</i>	MJ 13263	Papua New Guinea	No, free living	147722802	14772280200
62	<i>Anonychomyrma sp.</i>	MJ 6332	Papua New Guinea	No, free living	206840096	20684009600
63	<i>Philidris sp.</i>	MJ 13265	Papua New Guinea	Yes, Myrmecodia schlechteri	193344668	19334466800
64	<i>Anonychomyrma sp.</i>	MJ 6322	Papua New Guinea	No, free living	209308696	20930869600
65	<i>Philidris sp.8</i>	MJ 13251	Papua New Guinea	No, free living	189441078	18944107800
66	<i>Philidris sp.66</i>	MJ 13274	Papua New Guinea	No, free living	211357720	21135772000
67	<i>Philidris sp.8</i>	MJ 13272	Papua New Guinea	Yes, Hydnophytum moseleyanum	206908442	20690844200
68	<i>Philidris sp.8</i>	WEAM 10/0233	Papua New Guinea	Yes, ant-garden (Myrmecodia, Dischidia, Hydnophytum, Lecanopteris)	194691530	19469153000
69	<i>Anonychomyrma sp.</i>	D04	Papua New Guinea	No, free living	209514548	20951454800
70	<i>Philidris sp.70</i>	MJ 18416	Papua New Guinea	No, free living	207983718	20798371800
71	<i>Iridomyrmex cf. pallidus</i>	MJ 13256	Papua New Guinea	No, free living	208486384	20848638400
72	<i>Anonychomyrma sp.</i>	MJ 6308	Papua New Guinea	Yes, ant-garden	210510372	21051037200
73	<i>Philidris sp.8</i>	MJ 18060	Papua New Guinea	No, free living	208727072	20872707200
74	<i>Philidris sp.8</i>	EY-YAW 12/0900	Papua New Guinea	Yes, AG <i>Myrmecodia</i>	140194594	14019459400
75	<i>Philidris nagasau</i>	EMS 1946	Fiji, Taveuni	Yes, True agriculture	210198488	21019848800

76	<i>Philidris laevigata</i>	Kfm A37-983	Malaysia	Yes, ant-garden	210184124	21018412400
78	<i>Philidris laevigata</i>	Kfm A209-729	Malaysia	Yes, ant-garden	189386920	18938692000
79	<i>Philidris nagasau</i>	Chomicki 158	Fiji, Vanua Levu	Yes, True agriculture	208528322	20852832200
80	<i>Philidris nagasau</i>	Chomicki 147	Fiji, Vanua Levu	Yes, True agriculture	207099700	20709970000
81	<i>Philidris nagasau</i>	Chomicki 145	Fiji, Taveuni	Yes, True agriculture	209297158	20929715800
82	<i>Philidris nagasau</i>	Chomicki 143	Fiji, Taveuni	Yes, True agriculture	212097114	21209711400
83	<i>Philidris nagasau</i>	Chomicki 153	Fiji, Vanua Levu	Yes, True agriculture	207259940	20725994000
84	<i>Philidris nagasau</i>	Chomicki 139	Fiji, Vanua Levu	Yes, True agriculture	203494430	20349443000
85	<i>Philidris nagasau</i>	Chomicki 146	Fiji, Vanua Levu	Yes, True agriculture	209860856	20986085600
86	<i>Philidris nagasau</i>	Chomicki 144	Fiji, Taveuni	Yes, True agriculture	204209018	20420901800
87	<i>Philidris nagasau</i>	Chomicki 162	Fiji, Vanua Levu	Yes, True agriculture	206098414	20609841400
03	<i>Philidris cordata</i>	MJ21345, Janda et al., IRNP14/0143	Australia	Yes, ant-garden, <i>Hydnophytum moseleyanum</i>	205103011	20510301100
08	<i>Philidris sp.8</i>	EY-YAW12-0855, M. Janda	Papua New Guinea	Yes, nesting in <i>Myrmecodia schlechteri</i>	211855814	21185581400
09	<i>Philidris sp.8</i>	EY-YAW12-0901, M. Janda	Papua New Guinea	Yes, nesting in <i>Myrmecodia schlechteri</i>	211955770	21195577000
05	<i>Philidris sp. 5</i>	MJ15475, Janda & Ibalim, MJPNG08/0819	Papua New Guinea	No, Free living	214691628	21469162800

Chapter 4: Gene family evolution of plant farming ants



Abstract

Gene duplications are a major source of novel evolutionary material. Genome sequencing projects have found that gene family expansions and contractions are widespread, and often associated with major evolutionary transitions. Here, I investigate gene family expansions and contractions in the ant genus *Philidris*. *Philidris* is most well-known for its mutualistic interactions with plants, particularly the Fijian farming ant *Philidris nagasau*, which is obligately dependent on farming six species of *Squamellaria* due to having lost the ability to build its own nests. Within the same genus, there are ant-garden ants that engage in plant farming in a 'facultative generalist' manner and populations that are non-mutualist. We find that the Fijian farming ant has highest number of both gene family expansions and contractions. Amongst the significantly expanding and contracting gene families include olfactory receptors (ORs), gustatory receptors (GRs) and genes relating to the neurotransmitter glutamate. Subsequently we investigate signatures of selection amongst ORs and find that 35 OR gene families are experiencing diversifying selection in *P. nagasau* as well as 21 experiencing intensified selection and 5 experiencing relaxed selection. These results support the hypothesis that the Fijian farming system is mediated by olfactory signals, as had been found in plant-cultivation mutualisms in the Neotropics, although more functional work is needed.

1. Introduction

The evolution of farming is a major evolutionary transition. Humans started farming around 12,000 years ago (Carey 2023) with as many as 24 origins (Fuller and Denham 2022), although this number is debated (Carey 2023), fundamentally changing human evolution and culture. Many non-human species have evolved to farm too, albeit with varying degree of specialisation (Aanen et al. 2002, Mueller et al. 2005, Hata and Kato 2006, Brock et al. 2011, Pauli et al. 2014, Schultz 2022). Previously, authors have made distinctions between farming-like systems and “true” agriculture which was proposed as having four criteria (i) habitual planting, (ii) cultivation, (iii) harvest and (iv) obligate dependency on the crop for food (Mueller et al. 2005). In Chapter 2 (Campbell et al. 2023) I propose expanding on this framework to create a more inclusive eco-evolutionary framework, focused on evolutionary processes. My framework has three stages (i) resource expansion, (ii) adaptive cultivation and (iii) obligate agriculture. The first stage, resource expansion, refers to a pre-adapted species behaving in a way that encourages the growth a resource (usually food), however there is no coevolutionary adaptation yet. The second stage, adaptive cultivation describes systems that are usually facultative and generalist and show some adaptation to farming yet lack reciprocal obligate dependence. The last stage, obligate agriculture, we retain the four criteria proposed by Mueller et al. but expand the last criterion to include obligate dependency including services beyond nutrition (see Chapter 2). The aim of this framework was to focus on stages in the evolution of agriculture and to capture all farming mutualisms, including those that had sometimes been relegated to being called “farming-like” species as well as the highly coevolved farming mutualisms considered “true” agriculture in Mueller (2005)’s framework. One of the main advantages of this approach is that it gives researchers more data points to study and allows opportunities to identify traits that are pre-adaptions to agriculture. However, our framework maintains the category of obligate agriculture with a modified version of Mueller et al.’s four criteria as these rare, highly specialised systems do represent major transitions which are evolutionarily interesting. For example, the shift to fungus farming in *Attine* ants has led to the group being ecologically dominant in the Neotropics, with colonies in the millions (Price et al. 2003) and a large adaptive radiation with over 200 species (Mueller et al. 2001).

We expect major phenotypic changes to be accompanied by major genomic changes. One major source of raw genetic material is gene duplication (Zhang 2003), suggested as early as 1936 (Bridges 1936), but the extent to which gene duplications occur, and their impact on species evolution was not appreciated until genome sequencing became widely available. Early genome sequencing efforts seem to confirm this hypothesis, with gene family expansions and contractions being widespread (Rubin et al. 2000, Mouse Genome

Consortium 2002, Rhesus Macaque Genome Sequencing and Analysis Consortium 2007). Gene family evolution appears to have been particularly important to human evolution, as gene losses and gains accelerated in primate lineages compared to non-primates whereas nucleotide substitution rates declined (Hahn et al. 2007, Demuth and Hahn 2009). The genome of the leafcutter ant, *Atta cephalotes*, experienced many gene family expansions, including an expansion in a gene family containing Δ -9-desaturases; cuticular hydrocarbon (CHC) synthesis involves Δ -9-desaturases, and CHCs mediate nestmate recognition which is vital to the eusocial lifestyle (Suen et al. 2011). Peptidase gene families were found to have expanded in the leafcutter species *Acromyrmex echinator* (Nygaard et al. 2011). Both *Atta* and *Acromyrmex* have lost the arginine biosynthesis pathway (Nygaard et al. 2011, 2016, Suen et al. 2011), which occurred early Attine evolution and is hypothesised to have made the shift to from hunter gatherer to fungus farmer irreversible (Nygaard et al. 2016), thus making the farmer obligately dependent on its fungal crops.

Philidris nagasau, like leafcutter ants, is an obligate agriculturalist and fulfils the four criteria (i) habitual planting, (ii) cultivation, (iii) harvest and (iv) obligate dependency on the crop. A major difference between Attines and *P. nagasau* is that *P. nagasau* obligately depends on farming *Squamellaria* for nesting space, in the form of domatia (Chomicki and Renner 2016a) whereas Attines are obligately depend on farming fungi for food. The level of dependency is the same, but the mode, sensu Lewis (1985), differs. The loss of Arginine biosynthesis has made Attines biochemically dependent on their fungal crops, whereas *P. nagasau* has lost the ability to build its own carton nests, a behavioural change. I hypothesise that *P. nagasau* will experience a greater amount of gene family expansions and contractions given the dramatic changes in its ecology and evolution compared to the rest of the genus. Specifically, changes related to the nervous system and sensory perception seem highly likely to mediate this mutualism, give the association between changes in the nervous system and behavioural change (Rubin and Moreau 2016) and the high specificity of seed selection by ants. Previous work on Neotropical ant-garden ants suggests that the chemical methyl 6-methylsalicylate (6-MMS) mediates seed collection preferences (Youngsteadt et al. 2008).

Philidris is a useful model for studying the evolution of mutualism due to the variety of mutualistic strategies it engages in, varying from the obligate-agriculturalist Fijian farming ant, *Philidris nagasau* to “resource expansion” farmers, known as ant-garden ants in Southeast Asia and Australasia (see Chapter 2 and Campbell et al. 2023) to populations of ants that appear to not be engaging in ant plant-mutualism at all (see Table 4.1, Figure 4.1 and Chapter 2). Here we investigate gene family expansions and contractions within the genus. One major finding is that there have been both expansions and contractions of

olfactory receptor (OR) genes at the genus and species level, subsequently we investigate signatures of selection in the full OR repertoire of *Philidris*.

Table 4.1 Genomes used in this study, species delimitations are based on the genome wide 2,100 gene phylogeny (see Figure 4.1), for more information on the genome assemblies and annotations refer to Chapter 3.

Sample ID	Species	Geography	Mutualism	Type of sequencing
01	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	PacBio HiFi
75	<i>Philidris nagasau</i>	Fiji, Taveuni	Yes, True agriculture	Illumina
79	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	Illumina
80	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	Illumina
81	<i>Philidris nagasau</i>	Fiji, Taveuni	Yes, True agriculture	Illumina
82	<i>Philidris nagasau</i>	Fiji, Taveuni	Yes, True agriculture	Illumina
83	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	Illumina
84	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	Illumina
85	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	Illumina
86	<i>Philidris nagasau</i>	Fiji, Taveuni	Yes, True agriculture	Illumina
87	<i>Philidris nagasau</i>	Fiji, Vanua Levu	Yes, True agriculture	Illumina
02	<i>Philidris laevigata</i>	Thailand	Yes, ant-garden ¹	PacBio HiFi
41	<i>Philidris laevigata</i>	Thailand	Yes, ant garden	Illumina
45	<i>Philidris laevigata</i>	Malaysia	Yes, ant garden	Illumina
46	<i>Philidris laevigata</i>	Thailand	Yes, ant garden	Illumina
47	<i>Philidris laevigata</i>	Borneo	Yes, ant garden	Illumina
49	<i>Philidris laevigata</i>	Malaysia	Yes, ant garden	Illumina
50	<i>Philidris laevigata</i>	Borneo	Yes, ant garden	Illumina
54	<i>Philidris laevigata</i>	Borneo	Yes, ant garden	Illumina
58	<i>Philidris laevigata</i>	Borneo	Yes, ant garden	Illumina
76	<i>Philidris laevigata</i>	Malaysia	Yes, ant garden	Illumina
78	<i>Philidris laevigata</i>	Malaysia	Yes, ant garden	Illumina
08	<i>Philidris sp.8</i>	Papua New Guinea	Yes, inhabiting <i>Myrmecodia schlechteri</i>	Illumina
09	<i>Philidris sp.8</i>	Papua New Guinea	Yes, inhabiting <i>Myrmecodia schlechteri</i>	Illumina
61	<i>Philidris sp.8</i>	Papua New Guinea	No, free living	Illumina
63	<i>Philidris sp.8</i>	Papua New Guinea	Yes, <i>Myrmecodia schlechteri</i>	Illumina
65	<i>Philidris sp.8</i>	Papua New Guinea	No, free living	Illumina
67	<i>Philidris sp.8</i>	Papua New Guinea	Yes, <i>Hydnophytum moseleyanum</i>	Illumina
68	<i>Philidris sp.8</i>	Papua New Guinea	Yes, ant garden (<i>Myrmecodia</i> + <i>Dischidia</i>)	Illumina
73	<i>Philidris sp.8</i>	Papua New Guinea	No, free living	Illumina
74	<i>Philidris sp.8</i>	Papua New Guinea	Yes, <i>Myrmecodia</i>	Illumina
66	<i>Philidris sp.66</i>	Papua New Guinea	No, free living	Illumina
05	<i>Philidris sp.5</i>	Papua New Guinea	No, free living	Illumina
70	<i>Philidris sp.70</i>	Papua New Guinea	No, free living	Illumina
03	<i>Philidris cordata</i>	Australia	Yes, nesting in <i>Hydnophytum moseleyanum</i>	Illumina
71	<i>Iridomyrmex cf. pallidus</i>	Papua New Guinea	No, free living	Illumina
62	<i>Anonychomyrma sp.</i>	Papua New Guinea	No, free living	Illumina
64	<i>Anonychomyrma sp.</i>	Papua New Guinea	No, free living	Illumina
69	<i>Anonychomyrma sp.</i>	Papua New Guinea	No, free living	Illumina
72	<i>Anonychomyrma sp.</i>	Papua New Guinea	Yes, ant-garden	Illumina

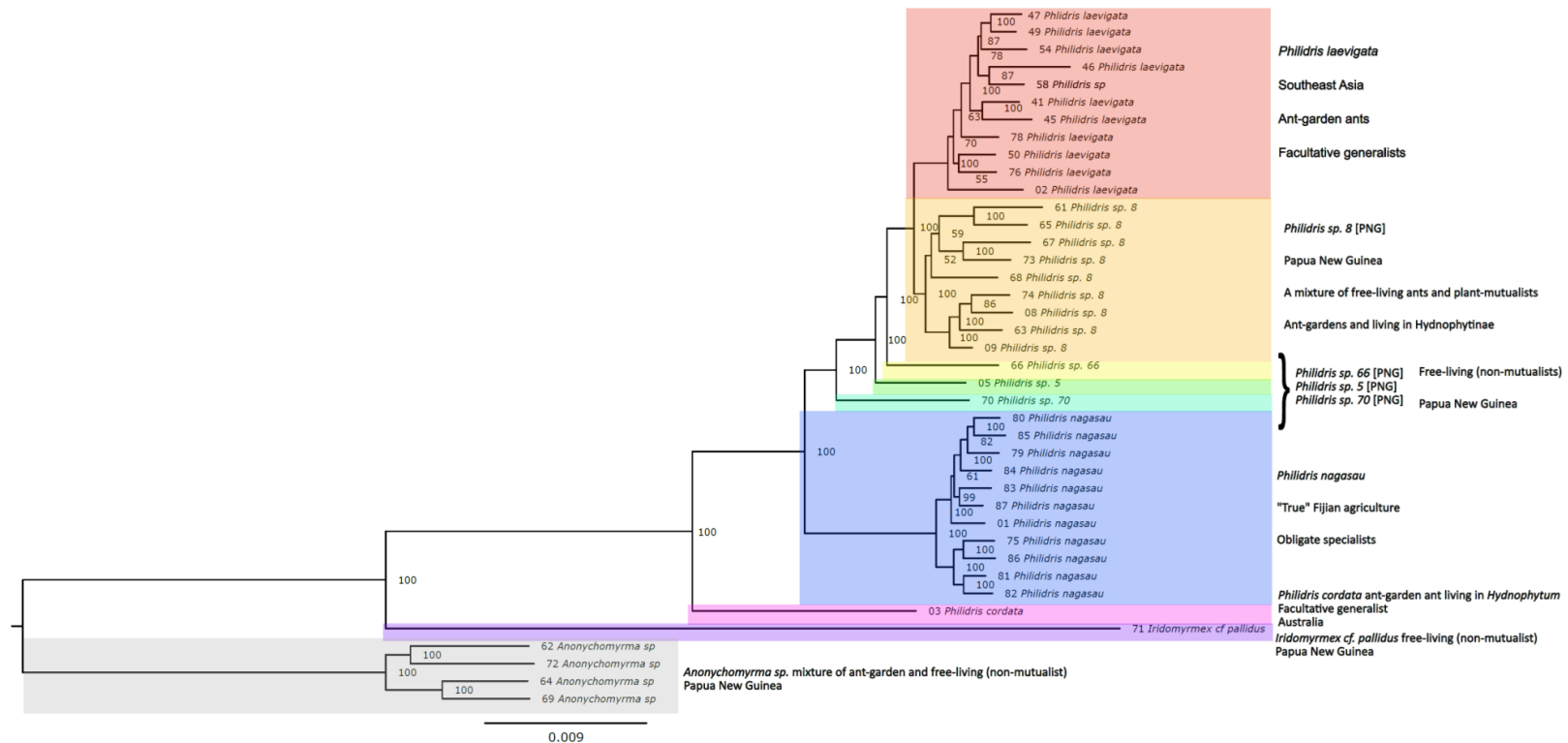


Figure 4.1 Genome-wide maximum likelihood phylogeny of *Philidris*, calculated from a concatenated matrix of 2,100 single copy orthologues. Node labels represent bootstrap values, see Chapter 3 for more details. In this study we refer to nine “species” based on phylogenetic grouping: *P. nagasau*, *P. laevigata*, *P. cordata*, *P. sp. 8*, *P. sp. 5*, *P. sp. 66*, *P. sp. 70* (all of which are within the genus *Philidris* Shattuck, 1992), *Iridomyrmex cf. pallidus* and *Anonychomyrma sp.* *Philidris laevigata* occurs in Southeast Asia, and nest in ant-gardens wherein ants initially build a carton nest (a structure made of macerated plant material and ant secretions) and then introduce epiphyte seeds, the growing roots provide a scaffolding which increases nest stability and enables the building of larger nests. *Philidris sp. 8* occurs in Papua New Guinea and displays a variation in behaviour relating to plants, ranging from ant-garden ants (including nesting in domatium-bearing ant garden species) to free-living, non-mutualist ants. *Philidris sp. 66*, *P. sp. 5* and *P. sp. 70* all occur in Papua New Guinea, and are free-living, non-mutualist ants. *Philidris nagasau*, the Fijian farming ant, is an obligate specialist, relying on the farming of six species of *Squamellaria* for nesting space. *Philidris cordata* is a mutualist ant, that forms ant-gardens, sometimes including the domatium-bearing *Myrmecodia beccarii* and *Hydnophytum moseleyanum* in northern Queensland, Australia. *Iridomyrmex cf. pallidus* is a free-living, non-mutualist ant.

2. Materials and methods

See Chapter 3 for details on genome assembly (section 2.2) and annotation (section 2.3) and details on phylogenetic constructions (section 2.4).

2.1 Orthogroup prediction

Amino acid sequences were clustered into orthogroups using OrthoFinder version 2.5.5 using standard settings (Emms and Kelly 2015, 2019). Helixer outputs only the longest isoforms for each gene, so there was no need to filter to alternate isoforms (Holst et al. 2023).

2.2 Gene family expansion and contraction analysis

CAFE 5 (Mendes et al. 2020) was used to generate lambda models for (i) the whole tree, (ii) the genus *Philidris* and (iii) a separate lambda for each “species” (see Figure 4.1, Table 4.1 and discussion of species in Chapter 3). Repeated runs of CAFE gave similar numbers of significantly expanding and contracting orthogroups, therefore it was assumed that the global lambda model was representative, and was used for all downstream analysis.

2.2.1 Annotation and GO enrichment of significantly expanding and contracting orthogroups

A custom Bash script was used to extract significantly expanding and contracting orthogroups ($p > 0.05$). Significantly expanding and contracting orthogroups were annotated with InterProScan version 5.47-82.0 (Jones et al. 2014) using the option `-goterms`. GO terms were extracted and then summarised by performing a GO enrichment using REVIGO (Supek et al. 2011).

2.3 Signatures of selection analysis of olfactory receptor (OR) genes

OrthoFinder was used to group CDS sequences into orthogroups. Olfactory receptor (OR) genes were identified by annotated all of the 40 proteomes with InterProScan version 5.47-82.0 using options `-goterms` and application options (`-appl`) *CDD*, *COILS*, *Gene3D*, *HAMAP*, *MobiDBLite*, *PANTHER*, *Pfam*, *PIRSF*, *PRINTS*, *PROSITEPATTERNS*, *PROSITEPROFILES*, *SFLD*, *SMART*, *SUPERFAMILY*, *TIGRFAM* (Jones et al. 2014) and using the command ‘grep’ to search for “Olfactory receptor, insect” to identify a list of OR sequences in all of the 40 proteomes. This list of sequences was used to identify Orthogroups containing OR genes.

Some 289 CDS orthogroups were identified as containing OR sequences. This is similar to published number of OR genes in the only other sequenced Dolichoderine ant, *Linepithema humile* (320) and to the number of OR sequences identified in our two reference (PacBio

HiFi) genomes (311 *P. nagasau* and 304 *P. laevigata*). Orthogroup sequences were cleaned using a custom Python script (check_aa_and_cds_trans.py available from <https://github.com/NatJWalker-Hale/sequitils>) that compares nucleotide sequences with translated amino acid sequences (translated using seqkit translate (Shen et al. 2016)) to prune the sequences to multiples of three, allowing for codon alignments to be produced. Then, codon alignments were produced using Prank (Löytynoja 2014, -codon). Gene trees were produced for each orthogroup using the codon alignments as inputs to IQTree version 2.2.3 (Minh et al. 2020) using the options -m GTR+F+G. The genomes assembled, annotated and analysed in this thesis represent a combination of species level and population level sampling, with some species represented by many samples and some species with just one. While it was possible to utilise this population-level sampling in the gene family evolution analysis, it was not sensible for signatures of selection analysis since there would be too many identical or near-identical sequences, which could lead to spurious inferences due to the lack of information (Yang 2014) and worst, selection analysis on intraspecific samples can be actively misleading (Kryazhimskiy and Plotkin 2008).

A custom Python script was used to collapse monophyletic and single-node paraphyletic tips so that each species was only represented with one genome, following the approach of Yang and Smith (2014) maintaining intraspecific duplications using the species overlap algorithm (collapse_monophyly_scripts.py, available from https://github.com/NatJWalker-Hale/alignment_and_tree_tools). For *P. nagasau* and *P. laevigata* the HiFi genomes were prioritised over resequencing genomes and otherwise the longest sequence was kept. Each gene tree had every *P. nagasau* sequence labelled as foreground using custom Python scripts (label_for_RELAX.py and label_multicopy_foreground.py, available from https://github.com/NatJWalker-Hale/alignment_and_tree_tools). aBSREL, part of the HyPhy package (version 2.5.60) (Smith et al. 2015, Kosakovsky Pond et al. 2020) was used to identify branches experiencing diversifying selection. aBSREL is a test for positive selection that models both site-level and branch-level dN/dS (ω). One aBSREL has fitted the full adaptive model a Likelihood Ratio Test (LRT) is performed to assess each branch, comparing to a null model where branches are constrained to have an ω below 1. Here, aBSREL is used to test the hypothesis that *P. nagasau* branches are evolving differently compared to the background (Smith et al. 2015).

RELAX, also part of the HyPhy package (version 2.5.60) (Wertheim et al. 2015, Kosakovsky Pond et al. 2020) was used to identify orthogroups experiencing either intensifying or relaxed selection. The RELAX model compares the ratio of non-synonymous to synonymous substitution rates (dN/dS, ω) in three site classes representing purifying ($\omega_0 < 1$), purifying-to-neutral ($\omega_1 \leq 1$) and positively selected ($\omega_2 > 1$) sites between foreground and reference

branch partitions. Relaxed selection corresponds to an elevated dN/dS in the purifying site class and reduced dN/dS in the positively selected class in foreground branches, while intensified selection corresponds to a decrease in dN/dS in the purifying class and an increase in the positively selected class in foreground branches (Wertheim et al. 2015). This is controlled by the addition of a parameter, K , indicating relaxed selection when $K < 1$ and intensified selection when $K > 1$ (Wertheim et al. 2015). Statistical support for the RELAX model is tested by comparing the RELAX alternative with K free to vary with the RELAX null where $K = 1$.

3. Results

There were more contracting than expanding orthogroups for every taxa investigated in this study. The Fijian farming ant, *Philidris nagasau*, node had the greatest number of significantly expanding orthogroups (41) compared to the *P. laevigata* node and the *Iridomyrmex cf. pallidus* node which had the lowest number of significantly expanding orthogroups (8). The *Philidris nagasau* node also had the greatest number of significantly contracting orthogroups (562) compared the *Philidris sp. 5 node* which had the least (8), see Table 4.2, and Figure 4.2 for all results. *Philidris nagasau* is endemic to two Fijian islands (Vanua Levu and Taveuni), and based on the phylogeny these are isolated populations. Therefore, I also tested if the Vanua Levu and Taveuni clade underwent specific expansions or contractions. On Vanua Levu clade node, there were 15 orthogroups with significant evidence for expansion and 177 orthogroups for significant evidence for contraction. In contrast, the on Taveuni clade node had only one orthogroup with evidence for expansion and 4 with evidence for contraction.

Table 4.2 Total number and number of significantly ($p < 0.05$) expanding and contracting orthogroups per node. Analysis performed using CAFE 5 (Mendes et al. 2020).

Taxa	Expansions		Contractions	
	Total	$p < 0.05$	Total	$p < 0.05$
<i>Philidris</i>	7	5	124	12
<i>P. nagasau</i>	100	41	866	562
<i>P. laevigata</i>	17	8	323	191
<i>P. sp. 8</i>	56	15	423	264
<i>P. sp. 66</i>	434	23	2110	10
<i>PNG_spp70</i>	402	13	2229	18
<i>PNG_spp5</i>	515	34	2172	8
<i>P. cordata</i>	390	16	2463	11
<i>Iridomyrmex cf. pallidus</i>	550	8	2604	23
<i>Anonychomyrma sp.</i>	174	30	86	7
<i>P. nagasau</i> (Vanua Levu clade)	28	15	274	177
<i>P. nagasau</i> (Taveuni clade)	302	1	524	4

3.1 Annotation of significantly expanding orthogroups

The biological function GO terms axon guidance, sensory perception of smell, sensory perception of taste, cell adhesion, cell surface receptor signalling pathway, DNA replication, endoplasmic reticulum tubular network organisation and proteolysis were all expanded in *P. nagasau*. The cellular component GO terms membrane and plasma membrane were enriched amongst the significantly expanding orthogroups in *P. nagasau*. The molecular function GO terms heme binding, peptidyl-prolyl cis-trans isomerase activity, alcohol-forming very long-chain fatty acyl-CoA reductase activity, DNA-directed DNA polymerase activity, zinc ion binding, olfactory receptor activity, odorant binding, protein binding and serine-type endopeptidase inhibitor activity were enriched amongst orthogroups significantly expanding in *P. nagasau*.

Amongst the significantly contracting orthogroups the enriched biological function GO terms include: endocytosis, malate metabolic process, fructose 6-phosphate metabolic process, G protein-coupled receptor signalling pathway, response to oxidative stress, gluconeogenesis and sensory perception of smell. The cellular component GO terms ribosome and proton-transporting ATP synthase were enriched amongst the significantly contracting orthogroups in *P. nagasau*. Molecular function GO terms malate dehydrogenase (decarboxylating) (NAD⁺) activity, heme transmembrane transported activity, glucose binding, olfactory receptor activity and odorant binding were enriched amongst orthogroups that were significantly contracting in *P. nagasau*. This list is not exhaustive, see Tables 4.3-4.5 for full list of all enriched GO terms for each of the species in this study.

3.1.1 Olfactory receptor gene families

At the genus level, there was significant expansions relating to the sensory perception of smell, odorant binding and olfactory receptor activity, coming from orthogroups AAOG0000039, AAOG0000066 and AAOG0000092. There was also one contracting olfactory receptor orthogroup, AAOG0010576.

At the species level there were many expansions and contractions in orthogroups containing OR genes, with the exception of *P. sp. 8* which had neither expansions or contractions of OR orthogroups. *P. sp. 5* had the greatest number of significantly expanding orthogroups containing OR genes (5) and *Iridomyrmex cf. pallidus* had the greatest number of contracting orthogroups (4). The *P. nagasau* node had two significantly expanding OR orthogroups and 2 significantly contracting orthogroups. The *P. nagasau* Vanua Levu node had one significantly expanding OR orthogroup and three significantly contracting OR orthogroups, see Table 4.6. The *P. nagasau* Taveuni clade had no significantly expanding or contracting OR orthogroups.

3.1.2 Gustatory receptor gene families

At the genus level, there were no significantly expanding or contracting orthogroups containing gustatory receptor (GR) genes. However, there were some significant expansions and contractions at the species level: *Philidris nagasau* and *Philidris cordata* had one significantly expanding orthogroup and *P. sp. 5* had two. *Philidris laevigata* and *Iridomyrmex cf. pallidus* had each had one significantly contracting orthogroup containing GR genes, see Table 4.7.

3.1.3 Glutamate receptor gene families

At the genus level there was one significantly contracting orthogroup containing genes relating to glutamate, with the annotation of *ionotropic glutamate receptor* and no significantly expanding orthogroups containing genes relating to glutamate. At the species level *P. nagasau* had the greatest number of contracting orthogroups containing glutamate (4). Additionally, *P. laevigata*, *P. sp. 70* and *I. cf. pallidus* had one significantly contracting orthogroup. The species *P. sp. 66*, *P. sp. 5* and *P. sp. 70* all have one significantly expanding orthogroup containing gene relating to glutamate, see Table 4.8.

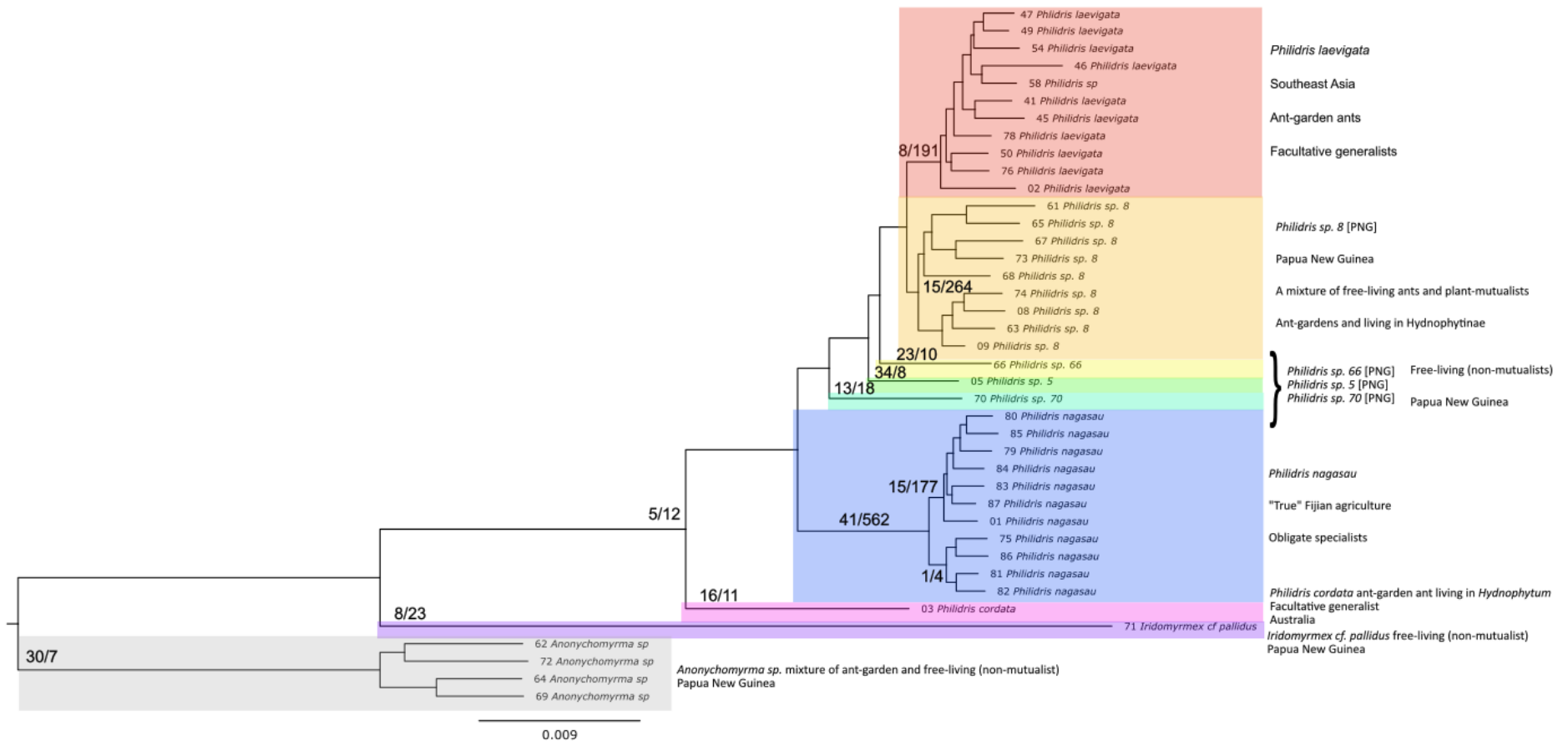


Figure 4.2 Phylogeny of *Philidris* with the number of significantly expanding and contracting gene families labelled on each of the tested branches (expansions followed by contractions). Gene family expansion and contraction analysis was performed using CAFE 5 (Mendes et al. 2020). Bootstraps of this phylogeny were removed but are visible in Figure 4.1.

Table 4.3 Enriched GO terms (biological process) for significantly expanding and contracting gene families for *Philidris*. Enriched GO terms for significantly expanding gene families are represented with a + sign and highlighted in green and enrich GO terms for significantly contracting gene families are represented with a - symbol and highlighted in peach. Gene expansion and contraction analysis was performed using CAFE5 (Mendes et al. 2020) and GO enrichment was performed using REVIGO (Supek et al. 2011).

Description	GO term	Philidris		nagasau		laevigata		P. sp. 8		P. sp. 66		P. sp. 5		P. sp. 70		P. cordata		Iridomyrmex cf. pallidus		Anonychomyrma sp.		P. nagasau (Vanua Levu)		P. nagasau (Taveuni)	
		Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
acetyl-CoA biosynthetic process from pyruvate	GO:0006086				-																				
activation of GTPase activity	GO:0090630				-																				
aerobic electron transport chain	GO:0019646				-																				
alanine transport	GO:0032328				-																				
amino acid biosynthetic process	GO:0008652							-																	
amino acid metabolic process	GO:0006520							-										+							
amino acid transmembrane transport	GO:0003333									+															
antigen processing and presentation of endogenous peptide antigen via MHC class I	GO:0019885				-		-																		
arginine biosynthetic process	GO:0006526				-																				
aspartate family amino acid biosynthetic process	GO:0009067							-																	
ATP metabolic process	GO:0046034				-																				
autophagy	GO:0006914													+											
axon guidance	GO:0007411			+	-			-		-		-											-		
bacterial-type flagellum organization	GO:0044781							-																	
bacteriocin transport	GO:0043213							-																	
biosynthetic process	GO:0009058				-			-					-							+					
branched-chain amino acid biosynthetic process	GO:0009082							-															-		
branched-chain amino acid catabolic process	GO:0009083				-																				
branched-chain amino acid metabolic process	GO:0009081							-															-		
butanediol metabolic process	GO:0034077							-															-		
calcium ion import	GO:0070509																						-		
calcium ion transmembrane transport	GO:0070588				-															+			-		
calcium ion transport	GO:0006816		-																						

[illegible]

[illegible]

IMP salvage	GO:0032264		-					
insulin receptor signalling pathway	GO:0008286		-					
interstrand cross-link repair	GO:0036297		-					
intracellular glucose homeostasis	GO:0001678		-	-				
intracellular iron ion homeostasis	GO:0006879					+		+
intracellular phosphate ion homeostasis	GO:0030643		-					
intracellular protein transport	GO:0006886		-		-			
intracellular signal transduction	GO:0035556	-	-	-	-	+		
intracellular transport	GO:0046907		-		-			
isoleucine biosynthetic process	GO:0009097				-			
isoleucyl-tRNA aminoacylation	GO:0006428		-		-			
isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway	GO:0019288		-		-			
lipid catabolic process	GO:0016042			-				
lipid metabolic process	GO:0006629		-	-				-
lipid transport	GO:0006869		-					
lipoprotein biosynthetic process	GO:0042158				-			
long-term synaptic potentiation	GO:0060291				-			
lysine biosynthetic process via diaminopimelate	GO:0009089				-			
lysosome organization	GO:0007040		-		-			
lysyl-tRNA aminoacylation	GO:0006430				-			
magnesium ion transport	GO:0015693				-			
malate metabolic process	GO:0006108		-					
metal ion transport	GO:0030001				-			
methionine biosynthetic process	GO:0009086		-		-			
methionine metabolic process	GO:0006555		-		-			
methionine transport	GO:0015821					+		
methylation	GO:0032259		-		-			
microtubule cytoskeleton organization	GO:0000226				-			
microtubule nucleation	GO:0007020				-			

microtubule-based movement	GO:0007018	-	-
mismatch repair	GO:0006298	-	-
mitochondrial ADP transmembrane transport	GO:0140021	-	-
mitochondrial ATP transmembrane transport	GO:1990544	-	-
monoatomic cation transport	GO:0006812	-	-
monoatomic ion transmembrane transport	GO:0034220	-	-
monoatomic ion transport	GO:0006811	-	-
monoatomic ion transport	GO:0006811	-	-
mRNA catabolic process	GO:0006402	-	-
N-4 methylation of cytosine	GO:0090124	-	-
negative regulation of phosphate metabolic process	GO:0045936	-	-
negative regulation of TOR signalling	GO:0032007	-	-
negative regulation of translational elongation	GO:0045900	-	-
neurogenesis	GO:0022008	-	-
neuronal action potential	GO:0019228	-	-
nitrogen compound transport	GO:0071705	-	-
non-motile cilium assembly	GO:1905515	-	-
nuclear transport	GO:0051169	-	-
nucleobase-containing compound metabolic process	GO:0006139	-	-
nucleoside metabolic process	GO:0009116	-	-
nucleoside transmembrane transport	GO:1901642	-	-
nucleotide-excision repair	GO:0006289	-	-
organic phosphonate transport	GO:0015716	-	-
organic substance metabolic process	GO:0071704	-	-
pantothenate catabolic process	GO:0015941	-	-
peptide transport	GO:0015833	-	-
peptidyl-L-beta-methylthioaspartic acid biosynthetic process from peptidyl-aspartic acid	GO:0018339	-	-
phenylalanyl-tRNA aminoacylation	GO:0006432	-	-
phosphate ion transmembrane transport	GO:0035435	-	-

[illegible]

Biological Process	GO ID	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5	Condition 6	Condition 7	Condition 8	Condition 9	Condition 10	Condition 11	Condition 12
proton-transporting ATP synthase complex assembly	GO:0043461	-				-							
pseudouridine synthesis	GO:0001522	-											
pteridine-containing compound metabolic process	GO:0042558	-				-							
purine nucleotide biosynthetic process	GO:0006164	-											
purine ribonucleotide biosynthetic process	GO:0009152	-											
pyruvate metabolic process	GO:0006090	-											
regulation of cell shape	GO:0008360					-							
regulation of DNA-templated transcription	GO:0006355	-	-	-	+	-	+	-		-	-	+	-
regulation of gene expression	GO:0010468	-											
regulation of neurotransmitter secretion	GO:0046928					-							
regulation of response to reactive oxygen species	GO:1901031					-							
regulation of signal transduction	GO:0009966	-											
regulation of small GTPase mediated signal transduction	GO:0051056		-										
regulation of stress fiber assembly	GO:0051492			+									
regulation of termination of DNA-templated transcription	GO:0031554					-							
regulation of transcription by RNA polymerase II	GO:0006357	-				-							
regulation of translational fidelity	GO:0006450	-											
respiratory electron transport chain	GO:0022904	-											
response to heat	GO:0009408									+			
response to iron(II) ion	GO:0010040						+				+		
response to oxidative stress	GO:0006979	-	-	-									
response to xenobiotic stimulus	GO:0009410											-	
riboflavin biosynthetic process	GO:0009231					-							
ribosomal large subunit assembly	GO:0000027					-							
ribosome biogenesis	GO:0042254	-											
ribosome biogenesis	GO 0042254	+	-	-									
RNA methylation	GO:0001510	-											
RNA modification	GO:0009451	-											
RNA processing	GO:0006396					-		-					-
rRNA catabolic process	GO:0016075					-							

rRNA processing	GO:0006364			+																	
S-adenosylmethionine biosynthetic process	GO:0006556					-															
secretion by the type IV secretion system	GO:0044097					-															
sensory perception of light stimulus	GO:0050953							+													
sensory perception of smell	GO:0007608	+	-	+	-	+		+	-	+	-	+		+	-		-	+	-	+	-
sensory perception of sound	GO:0007605							+													
sensory perception of taste	GO:0050909			+					+	-			+				-	+	-	+	
signal peptide processing	GO:0006465							-													
signal transduction	GO:0007165				-	-	+			-	+					+					
small GTPase mediated signal transduction	GO:0007264				-			-													
sodium ion transport	GO:0006814				-																-
sphingolipid metabolic process	GO:0006665				-																
SRP-dependent cotranslational protein targeting to membrane	GO:0006614							-													
sulphate transport	GO:0008272				-																
teichoic acid biosynthetic process	GO:0019350				-			-													
telomere maintenance	GO:0000723				-																
thiamine transmembrane transport	GO:0071934							+													
thiamine transport	GO:0015888							+													
threonine biosynthetic process	GO:0009088							-													
toll-like receptor signalling pathway	GO:0002224					+				-	+										
transcription antitermination	GO:0031564							-													
translation	GO:0006412				-			-													-
translational initiation	GO:0006413							-													
translational termination	GO:0006415				-			-													
transmembrane transport	GO:0055085		-		-		-	-	+		-			-		+					-
trehalose catabolic process	GO:0005993								+		-			-							
tricarboxylic acid cycle	GO:0006099				-																
tRNA aminoacylation for protein translation	GO:0006418				-			-													
tRNA methylthiolation	GO:0035600				-																
tRNA modification	GO:0006400				-			-													

tRNA processing	GO:0008033	-	-			
tRNA wobble uridine modification	GO:0002098		-			
tRNA-guanine transglycosylation	GO:0101030		-			
UDP-glucose metabolic process	GO:0006011		-			
UDP-N-acetylgalactosamine biosynthetic process	GO:0019277		-			
V(D)J recombination	GO:0033151			+		
xenobiotic metabolic process	GO:0006805					-

Table 4.4 Enriched GO terms (cellular component) for significantly expanding and contracting gene families for *Philidris*. Enriched GO terms for significantly expanding gene families are represented with a + sign and highlighted in green and enrich GO terms for significantly contracting gene families are represented with a - symbol and highlighted in peach. Gene expansion and contraction analysis was performed using CAFE5 (Mendes et al. 2020) and GO enrichment was performed using REVIGO (Supek et al. 2011).

Description	GO term	<i>Philidris</i>		<i>nagasau</i>		<i>laevigata</i>		<i>P. sp. 8</i>		<i>P. sp. 66</i>		<i>P. sp. 5</i>		<i>P. sp. 70</i>		<i>P. cordata</i>		<i>Iridomyrmex cf. pallidus</i>		<i>Anonychomyrma sp.</i>		<i>P. nagasau (Vanua Levu)</i>		<i>P. nagasau (Taveuni)</i>	
		Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
aryl hydrocarbon receptor complex	GO:0034751																						-		
ATP-binding cassette (ABC) transporter complex	GO:0043190				-					+															
BBSome	GO:0034464				-		-																		
chromosome	GO:0005694								-											+					
collagen-containing extracellular matrix	GO:0062023				-																				
cytoplasm	GO:0005737				-		-		-	+			-	+			-			+			-		
cytoskeleton	GO:0005856																						-		
cytosol	GO:0005829				-				-																
dynein complex	GO:0030286				-																				
egg chorion	GO:0042600				-				-																
endoplasmic reticulum	GO:0005783				-		-																		
exodeoxyribonuclease V complex	GO:0009338								-																
extracellular region	GO:0005576				-		-																		
Fanconi anaemia nuclear complex	GO:0043240				-																				
glutamyl-tRNA(Gln) amidotransferase complex	GO:0030956				-																				
Golgi cisterna membrane	GO:0032580																						-		
heterotrimeric G-protein complex	GO:0005834																						-		
HslUV protease complex	GO:0009376						-																		
intracellular membrane-bounded organelle	GO:0043231				-																				
junctional membrane complex	GO:0030314																						-		

[illegible]

voltage-gated calcium channel complex	GO:0005891	-	-			+	-
voltage-gated potassium channel complex	GO:0008076					+	-

Table 4.5 Enriched GO terms (molecular function) for significantly expanding and contracting gene families for *Philidris*. Enriched GO terms for significantly expanding gene families are represented with a + sign and highlighted in green and enrich GO terms for significantly contracting gene families are represented with a - symbol and highlighted in peach. Gene expansion and contraction analysis was performed using CAFE5 (Mendes et al. 2020) and GO enrichment was performed using REVIGO (Supek et al. 2011).

Description	GO term	<i>Philidris</i>		<i>nagasau</i>		<i>laevigata</i>		<i>P. sp. 8</i>		<i>P. sp. 66</i>		<i>P. sp. 5</i>		<i>P. sp. 70</i>		<i>P. cordata</i>		<i>Iridomyrmex cf. pallidus</i>		<i>Anonychomyrma sp.</i>		<i>P. nagasau (Vanua Levu)</i>		<i>P. nagasau (Taveuni)</i>	
		Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
3',5'-cyclic-nucleotide phosphodiesterase activity	GO:0004114																			+					
15-oxoprostaglandin 13-oxidase activity	GO:0047522									+					+										
2-alkenal reductase [NAD(P)+] activity	GO:0032440									+					+										
1-pyrroline-5-carboxylate dehydrogenase activity	GO:0003842				-																				
2 iron, 2 sulfur cluster binding	GO:0051537				-																				
ABC-type amino acid transporter activity	GO:0015424									+															
3'-5' exonuclease activity	GO:0008408																						-		
3-deoxy-manno-octulosonate	GO:0008690								-																
cytidyltransferase activity																									
3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring) activity	GO:0003863				-																				
3-oxoacyl-[acyl-carrier-protein] synthase activity	GO:0004315					+							-						+			+			
4 iron, 4 sulfur cluster binding	GO:0051539				-							+							+						
4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase activity	GO:0051745				-				-																
4-hydroxy-tetrahydrodipicolinate reductase	GO:0008839								-																
5'-3' DNA helicase activity	GO:0043139								-																
6-phosphofructokinase activity	GO:0003872				-																				
7S RNA binding	GO:0008312								-																

GO term	GO ID	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5	Condition 6	Condition 7	Condition 8	Condition 9
ABC-type phosphonate transporter activity	GO:0015416					+				
alpha,alpha-phosphotrehalase activity	GO:0008788					+	-		-	
acetolactate synthase activity	GO:0003984				-					-
acetylglactosaminyltransferase activity	GO:0008376									-
acetylglutamate kinase activity	GO:0003991		-							
acid phosphatase activity	GO:0003993		-		-					
aconitate hydratase activity	GO:0003994					+			+	
actin binding	GO:0003779		-	-						
acyl-CoA dehydrogenase activity	GO:0003995		-		-					
acyltransferase activity	GO:0016746		-	+	-		-		+	+
acyltransferase activity, acyl groups converted into alkyl on transfer	GO:0046912		-							
acyltransferase activity, transferring groups other than amino-acyl groups	GO:0016747							-		
adenylate kinase activity	GO:0004017				-					
ADP binding	GO:0043531		-							
alanine:sodium symporter activity	GO:0015655		-							
alcohol-forming very long-chain fatty acyl-CoA reductase activity	GO:0080019		+		-			-		
aldehyde-lyase activity	GO:0016832					-				
ATP binding	GO:0005524	-	-	-	+	-	+		+	-
alpha-amylase activity	GO:0004556		-	-						
aminoacyl-tRNA editing activity	GO:0002161		-			-				
aminoacyl-tRNA ligase activity	GO:0004812		-			-				
aminopeptidase activity	GO:0004177		+	-	-					

GO term	GO ID	Cell type	Condition
AMP deaminase activity	GO:0003876	-	
antiporter activity	GO:0015297	-	
aspartate kinase activity	GO:0004072	-	
aspartate-semialdehyde dehydrogenase activity	GO:0004073	-	
aspartic-type endopeptidase activity	GO:0004190	-	+ - +
ATP hydrolysis activity	GO:0016887	- - + - +	+
ATPase-coupled phosphate ion transmembrane transporter activity	GO:0015415		+
ATP:ADP antiporter activity	GO:0005471	- -	
ATPase activator activity	GO:0001671	-	
ATPase-coupled transmembrane transporter activity	GO:0042626	- -	+ -
branched-chain-amino-acid transaminase activity	GO:0004084	-	-
calcium ion binding	GO:0005509	- -	- + -
calcium, potassium:sodium antiporter activity	GO:0008273		-
calcium:sodium antiporter activity	GO:0005432	-	
carbohydrate binding	GO:0030246	-	
carbohydrate derivative binding	GO:0097367		-
carbon-nitrogen ligase activity, with glutamine as amido-N-donor	GO:0016884	-	
cardiolipin synthase activity	GO:0008808		-
cargo receptor activity	GO:0038024		-
catalytic activity	GO:0003824	- -	- + - - + +
cation binding	GO:0043169	- -	
chitin binding	GO:0008061	- -	-

[illegible]

G protein-coupled receptor activity	GO:0004930	-	-	-		-	+			-	-
gamma-tubulin binding	GO:0043015				-						
gluconate transmembrane transporter activity	GO:0015128		-		-						
glucose binding	GO:0005536		-	-							
glucose-6-phosphate dehydrogenase activity	GO:0004345				-						
glucosylceramidase activity	GO:0004348		-								
glutamate dehydrogenase (NAD+) activity	GO:0004352		-	-							
glutamate-gated receptor activity	GO:0004970	-				+	+	+	-	-	+
glutamyl-tRNA synthase (glutamine-hydrolyzing) activity	GO:0050567		-								
glutathione synthase activity	GO:0004363				-						
glycerol-3-phosphate cytidyltransferase activity	GO:0047348		-		-						
glycosyltransferase activity	GO:0016757										-
GMP synthase (glutamine-hydrolyzing) activity	GO:0003922		-								
G-protein beta/gamma-subunit complex binding	GO:0031683										-
G-protein beta-subunit binding	GO:0031681										-
GTP binding	GO:0005525		-	-	-				+		
GTPase activator activity	GO:0005096			-	-						
GTPase activity	GO:0003924		-	-	-				+		-
guanyl nucleotide binding	GO:0019001										-
guanyl-nucleotide exchange factor activity	GO:0005085		-		-		+				
helicase activity	GO:0004386		-		-						
heme binding	GO:0020037	+	+	-	+	-	-	+	-	+	-
heme transmembrane transporter activity	GO:0015232		-								

[illegible]

magnesium ion binding	GO:0000287	-		-			-			-
magnesium ion transmembrane transporter activity	GO:0015095						-			
malate dehydrogenase (-arboxylating) (NAD+) activity	GO:0004471			-						
malic enzyme activity	GO:0004470			-						
MAP-kinase scaffold activity	GO:0005078			-			-			
membrane insertase activity	GO:0032977						-			
metal ion binding	GO:0046872			-		-	-			
metal ion transmembrane transporter activity	GO:0046873						-			
metalloaminopeptidase activity	GO:0070006			-		-				
metallocarboxypeptidase activity	GO:0004181							+		
metallodipeptidase activity	GO:0070573	-		-						
metalloendopeptidase activity	GO:0004222		+	-	+	-	-		+ -	+
metallopeptidase activity	GO:0008237		+	-	+	-	- +		+ - + - +	
methionine adenosyltransferase activity	GO:0004478			-						
methylenetetrahydrofolate reductase (NAD(P)H) activity	GO:0004489			-			-			
methylthiotransferase activity	GO:0035596			-						
methyltransferase activity	GO:0008168			-			-			
microtubule binding	GO:0008017			-						
microtubule motor activity	GO:0003777			-						
minus-end-directed microtubule motor activity	GO:0008569			-		-				
mismatched DNA binding	GO:0030983			-		-				

monoatomic cation channel activity	GO:0005261					-					-				+			-	
monoatomic cation transmembrane transporter activity	GO:0008324			-															
monoatomic ion channel activity	GO:0005216			-		-		-		+	-		-		-	+		+	-
monooxygenase activity	GO:0004497	+		+	-			-				-	+		+	-		-	-
N6-(1,2-dicarboxyethyl)AMP AMP-lyase (fumarate-forming) activity	GO:0004018			-															
N6-threonylcarbamyladenosine methylthiotransferase activity	GO:0035598			-															
N-acetyltransferase activity	GO:0008080																		-
NAD binding	GO:0051287			-		-		-											
NADH dehydrogenase activity	GO:0003954			-				-											
NADP binding	GO:0050661			-		-		-											
nitronate monooxygenase activity	GO:0018580			-															
N-methyltransferase activity	GO:0008170			-				-											
nuclease activity	GO:0004518							-											
nucleic acid binding	GO:0003676		+	-		-	+	-	+	+		+	+	-		+			-
nucleobase-containing compound kinase activity	GO:0019205							-											
nucleoside transmembrane transporter activity	GO:0005337			-				-											
nucleotide binding	GO:0000166		+	-		-		-	+										
nucleotidyltransferase activity	GO:0016779							-											
odorant binding	GO:0005549	+	-	+	-	+			+	-	+	-	+		+	-	-	+	-
olfactory receptor activity	GO:0004984	+	-	+	-	+			+	-	+	-	+		+	-	-	+	-
oxidoreductase activity	GO:0016491				-			-	+			-	+		+			+	

protein binding	GO:0005515	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	+
protein dimerization activity	GO:0046983			-		-		-	+					-	+		-
protein kinase activity	GO:0004672	-		-		-		-									-
protein serine/threonine kinase activity	GO:0004674	-															
protein tyrosine kinase activity	GO:0004713			-													-
protein tyrosine kinase collagen receptor activity	GO:0038062												+				
protein tyrosine phosphatase activity	GO:0004725			-													
protein-transporting ATPase activity	GO:0015450			-				-									
proton-transporting ATP synthase activity, rotational mechanism	GO:0046933			-													
pseudouridine synthase activity	GO:0009982			-													
purine nucleotide binding	GO:0017076			-				-			+			+			
pyridoxal phosphate binding	GO:0030170			-													
pyrophosphatase activity	GO:0016462			-													
pyruvate dehydrogenase (acetyl-transferring) activity	GO:0004739			-													
pyruvate, phosphate dikinase activity	GO:0050242			-													
queuine tRNA-ribosyltransferase activity	GO:0008479																
quinone binding	GO:0048038			-													
riboflavin kinase activity	GO:0008531							-									
ribonuclease III activity	GO:0004525							-									
ribonuclease P activity	GO:0004526							-									
RNA binding	GO:0003723	+	-		-			-		-							-
RNA helicase activity	GO:0003724							-									
RNA-DNA hybrid ribonuclease activity	GO:0004523							-									
rRNA binding	GO:0019843															-	

[illegible]

GO term	GO ID	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12	Cell 13	Cell 14	Cell 15
translation initiation factor activity	GO:0003743						-									
translation release factor activity	GO:0003747		-				-									
translation release factor activity, codon specific	GO:0016149		-				-									
transmembrane signaling receptor activity	GO:0004888	+	-		+			-	+			-			-	
transmembrane transporter activity	GO:0022857		-	-		-	+								-	
transposase activity	GO:0004803		-	+		-		-	+	+	-	+	-	+	-	-
tRNA (guanine(37)-N(1))-methyltransferase activity	GO:0052906		-													
tRNA binding	GO:0000049		-			-										
tRNA-guanosine(34) queuine transglycosylase activity	GO:0008479					-										
ubiquinol-cytochrome-c reductase activity	GO:0008121		-													
ubiquitin protein ligase activity	GO:0061630									+						
ubiquitin-protein transferase activity	GO:0004842		-													
UDP-glucose 6-dehydrogenase activity	GO:0003979					-										
UDP-glycosyltransferase activity	GO:0008194	+			+	-										
UDP-N-acetylglucosamine 1-carboxyvinyltransferase activity	GO:0008760					-										
UTP:glucose-1-phosphate uridylyltransferase activity	GO:0003983					-										
voltage-gated calcium channel activity	GO:0005245		-									+			-	
voltage-gated potassium channel activity	GO:0005249			-								+			-	
xanthine phosphoribosyltransferase activity	GO:0000310					-										
zinc ion binding	GO:0008270	+	-		+	-	+	+		+	-	+	+	-	-	

Table 4.6 Significantly expanding and contracting orthogroups containing olfactory receptor (OR) genes.

Orthogroup	Node(s) experiencing expansion/contraction	No. of sequences
AA OG0000011	<i>Anonychomyrma</i> contraction	194
AA OG0000039	<i>Philidris</i> expansion, <i>P. cordata</i> expansion	133
AA OG0000066	<i>Philidris</i> expansion, <i>Iridomyrmex</i> contraction	106
AA OG0000073	<i>P. cordata</i> expansion and <i>P. sp. 66</i> contraction	102
AA OG0000092	<i>Philidris</i> expansion	94
AA OG0000118	<i>P. cordata</i> contraction	87
AA OG0000134	<i>P. sp. 5</i> expansion, <i>P. cordata</i> expansion, <i>Iridomyrmex</i> contraction	83
AA OG0000230	<i>Iridomyrmex</i> contraction	73
AA OG0000591	<i>P. sp. 5</i> expansion	57
AA OG0000647	<i>P. sp. 5</i> contraction, <i>Iridomyrmex</i> contraction	56
AA OG0000734	<i>P. nagasau</i> expansion, <i>P. laevigata</i> expansions	54
AA OG0000748	<i>P. laevigata</i> expansion	53
AA OG0001135	<i>P. nagasau</i> expansion	48
AA OG0001143	<i>P. sp. 5</i> expansion, <i>Anonychomyrma</i> expansion	48
AA OG0001320	<i>P. nagasau</i> Vanua Levu clade expansion	46
AA OG0001677	<i>P. sp. 5</i> expansion	44
AA OG0010576	<i>Philidris</i> and <i>P. sp. 5</i> contractions	33
AA OG0011168	<i>P. sp. 66</i> expansion	29
AA OG0012415	<i>P. nagasau</i> Vanua Levu clade contraction	16
AA OG0012445	<i>P. nagasau</i> contraction	16
AA OG0013156	<i>P. nagasau</i> Vanua Levu clade contraction	11
AA OG0013355	<i>P. nagasau</i> Vanua Levu clade contraction	10
AA OG0015976	<i>P. nagasau</i> contraction	3

Table 4.7 Significantly expanding and contracting orthogroups containing gustatory receptor (GR) genes.

Orthogroup	Node(s) experiencing expansion/contraction	No. of sequences
AA OG0000002	<i>P. nagasau</i> Vanua Levu clade expansion, <i>Anonychomyrma</i> contraction	390
AAOG0000123	<i>P. sp. 5</i> expansion	86
AA OG0000128	<i>P. nagasau</i> expansion	85
AA OG0000130	<i>P. sp. 5</i> expansion	85
AA OG0001298	<i>Anonychomyrma</i> expansion	46
AA OG0001840	<i>Iridomyrmex</i> contraction	43
AA OG0003257	<i>P. nagasau</i> Vanua Levu clade expansion	41
AA OG0003257	<i>P. cordata</i> expansion	41
AA OG0013180	<i>P. laevigata</i> contraction	11

Table 4.8 Significantly expanding or contracting orthogroups containing genes related to glutamate.

Orthogroup	Node(s) experiencing expansion/contraction	Annotation	No. of sequences
AA OG0000008	<i>P. sp. 66</i> expansion, <i>P. sp. 5</i> contraction, <i>Anonychomyrma</i> contraction	Ionotropic glutamate receptor	206
AA OG0000199	<i>P. sp. 70</i> contraction	Ionotropic glutamate receptor	76
AA OG0000229	<i>P. laevigata</i> expansion, <i>P. nagasau</i> (Vanua Levu) expansion, <i>Iridomyrmex</i> contraction	Ionotropic glutamate receptor	73
AA OG0000675	<i>Philidris</i> contraction	Ionotropic glutamate receptor	55
AA OG0012420	<i>P. nagasau</i> Vanua Levu clade contraction	Ionotropic glutamate receptor	16
AA OG0012649	<i>P. nagasau</i> Vanua Levu clade contraction	Metabolic glutamate receptor	14
AA OG0012935	<i>P. nagasau</i> contraction	Acetylglutamate kinase	12
AA OG0013369	<i>P. nagasau</i> contraction	Metabotropic glutamate receptor	10
AA OG0015282	<i>P. nagasau</i> contraction	Metabotropic glutamate receptor	4
AA OG0015972	<i>P. nagasau</i> contraction	Ionotropic glutamate receptor	3

3.2 Signatures of selection in olfactory receptor (OR) genes

In every species, apart from *P. sp. 8*, there were significantly expanding and/or contracting orthogroups containing olfactory receptor (OR) genes, including at the genus-level *Philidris* node. In light of these results, we investigated signatures of selection in OR orthogroups. 289 CDS orthogroups were identified as containing OR genes. One of these orthogroups contained only three sequences, meaning it was not possible to produce a gene tree using this orthogroup and therefore it was excluded from signatures of selection analysis. Of the remaining 288 orthogroups tested, 35 had *P. nagasau* branches which had significant evidence for diversifying selection ($p > 0.05$), see Table 4.9 and S4.2. There was significant evidence of intensified selection in 21 orthogroups and 5 orthogroups had evidence for relaxed natural selection, see and Figures 4.3-4 and Table S4.3.

Table 4.9 Olfactory receptor (OR) orthogroups with significant evidence for diversifying selection on at least one *Philidris nagasau* branch as determined with an aBSREL test (Smith et al. 2015), part of the HyPhy package. For each gene tree every *P. nagasau* branch was tested. Monophyletic and single-node paraphyletic nodes were collapsed so that each species was only represented with one genome, meaning that the branches represent true duplications rather than being a bioinformatic artefact (see Methods section 2.3). See Table S4.1 for full model results.

Gene	No. of significant branches	No. of <i>P. nagasau</i> branches tested
OG0000042.fa	2	9
OG0000048.fa	2	4
OG0000058.fa	2	6
OG0000175.fa	1	2
OG0000228.fa	1	7
OG0000234.fa	1	5
OG0000357.fa	1	2
OG0000535.fa	1	2
OG0000552.fa	1	7
OG0001104.fa	1	5
OG0001404.fa	1	1
OG0002188.fa	1	1
OG0002254.fa	1	1
OG0002294.fa	1	3
OG0002769.fa	1	4
OG0005578.fa	1	3
OG0007863.fa	1	1
OG0008887.fa	1	2
OG0009273.fa	1	4
OG0009417.fa	1	1
OG0009596.fa	1	1
OG0009654.fa	1	1
OG0010134.fa	1	2
OG0010275.fa	1	1
OG0010362.fa	1	1
OG0010793.fa	1	1
OG0011090.fa	2	3
OG0011111.fa	1	1
OG0011122.fa	1	5
OG0011137.fa	1	2
OG0012530.fa	1	1
OG0012633.fa	1	2
OG0012698.fa	1	2
OG0013683.fa	2	3
OG0016602.fa	1	2

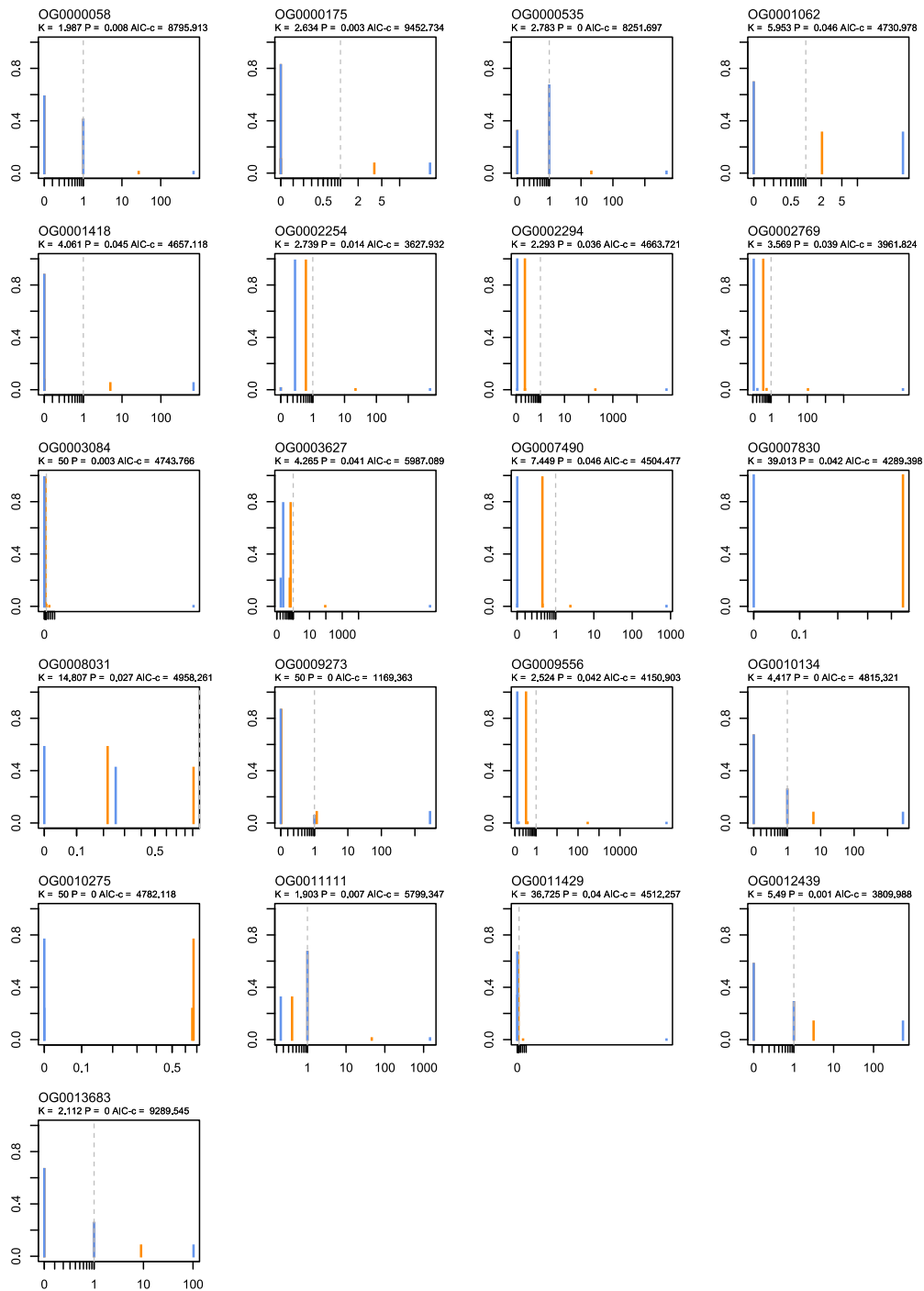


Figure 4.3 Olfactory receptor (OR) orthogroups with significant evidence for intensified selection in *Philidris nagasau*. Plots of model fits from the RELAX alternative model (Wertheim et al. 2015). Each pair of bars represents the site proportion assigned to that site class for reference (orange) and test (blue) branch partitions, while the x-axis position gives the inferred dN/dS (omega) of that site partition. The grey dotted line represents a neutral omega value (1). Note that site proportions are constrained to be equal between reference and test groups. In each case, K gives the inferred intensification/relaxation parameter, which corresponds to relaxed selection if $K < 1$ and intensified selection if $K > 1$. The P-value of the likelihood ratio test on one degree of freedom of the RELAX alternative against the RELAX null which constrains $K = 1$ is shown. AIC-c gives the small sampled-corrected Akaike Information Criterion for the RELAX alternative model. See Table S4.2 for full model output.

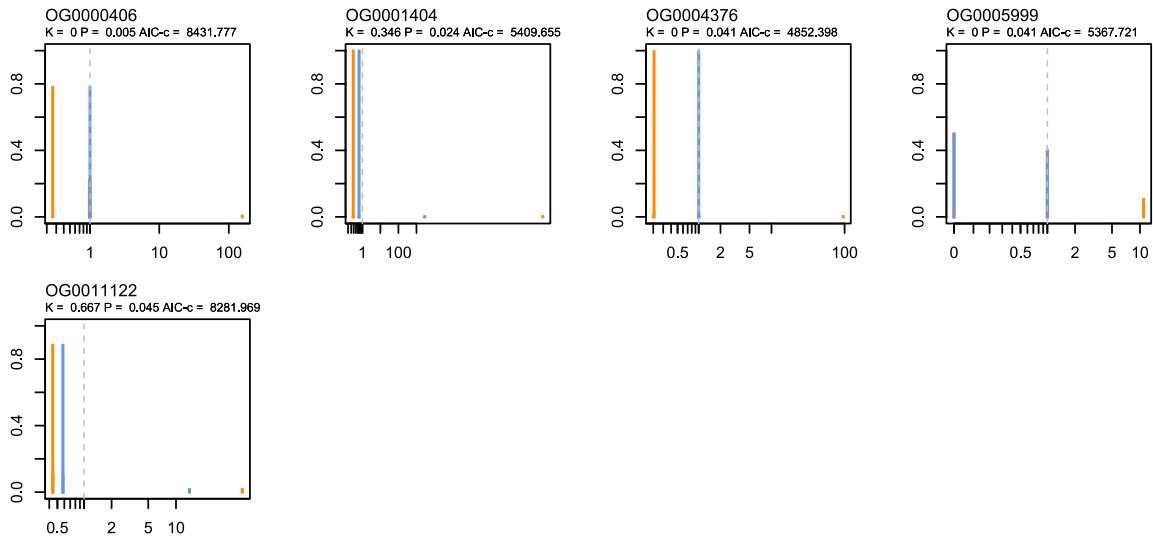


Figure 4.4 Olfactory receptor (OR) orthogroups with significant evidence for relaxed selection in *Philidris nagasau*. Plots of model fits from the RELAX alternative model (Wertheim et al. 2015). Each pair of bars represents the site proportion assigned to that site class for reference (orange) and test (blue) branch partitions, while the x-axis position gives the inferred dN/dS (omega) of that site partition. The grey dotted line represents a neutral omega value (1). Note that site proportions are constrained to be equal between reference and test groups. In each case, K gives the inferred intensification/relaxation parameter, which corresponds to relaxed selection if $K < 1$ and intensified selection if $K > 1$. The P-value of the likelihood ratio test on one degree of freedom of the RELAX alternative against the RELAX null which constrains $K = 1$ is shown. AIC-c gives the small sampled-corrected Akaike Information Criterion for the RELAX alternative model. See Table S4.2 for full model output.

4. Discussion

Gene family expansion and contraction appears to be widespread across the genus *Philidris*. Contractions were more common than expansions (see Table 4.2 and Figure 4.2). The Fijian farming ant, *Philidris nagasau*, experienced the most significant gene family expansions (41) and contractions (561). Viewing these results in an ecological context, *P. nagasau* likely transitioned from an ant-garden ant to the highly specialised agricultural lifestyle which involved evolving new functions as well as losing certain traits, the most relevant being the ability to construct a carton nest. When considering the other facultative-generalist mutualists (ant-garden ants, *P. laevigata*, some of *P. sp. 8*, *P. cordata*, and some of *Anonychomyrma sp.*) and the non-mutualists (some of *P. sp. 8*, *P. sp. 5*, *P. sp. 66*, *P. sp. 70*, *Iridomyrmex cf. pallidus* and some of *Anonychomyrma sp.*) there is no clear trend of differences in gene family expansion and contraction rate relating to mutualism strategy. It is not clear, apart from in the case of *Iridomyrmex cf. pallidus*, which is a ground nesting ant, whether the non-mutualists studied here have the capacity to produce ant-gardens under

certain conditions, or even possibly at different stages of the colonies life span – it may be more necessary for ants to expand the size of their carton nests via the stabilising effect of epiphyte roots when the colony size has expanded past a certain point. The genomic similarities between ant-garden ant non-mutualist ant populations suggests that this trait maybe phenotypically plastic. This could also explain how plant cultivation seems to repeatedly evolve (a highly conservative estimate is of 15 origins (see Chapter 2 and Campbell et al. 2023)) the genera *Crematogaster* (Davidson 1988, Dejean et al. 1997, 2000, Marini-Filho 1999, Kaufmann et al. 2001, Kaufmann 2002, Kaufmann and Maschwitz 2006, Menzel and Blüthgen 2010), *Camponotus* (Davidson 1988, Dejean et al. 1997, 2000, Marini-Filho 1999, Kaufmann et al. 2001, Kaufmann 2002, Kaufmann and Maschwitz 2006, Menzel and Blüthgen 2010) and *Pheidole* (Davidson 1988, Kaufmann et al. 2001, Kaufmann 2002) have evolved plant cultivation at least twice, once in the Neotropics and once in Southeast Asia, and yet relatively few species of these speciose genera are known to engage mutualistically with plants.

There appears to be many more orthogroup contractions than expansions, this is especially pronounced in the species in which we have multiple samples such as *P. nagasau* (41 expansions and 561 contractions), *P. laevigata* (8 expansions and 191 contractions), *P. sp. 8* (15 expansions and 264). Although there are other multi-sample species which do not have such large differences, for example the genus *Philidris* as a whole (5 expansions and 12 contractions) and *P. nagasau* (Taveuni clade) (1 expansion and 4 contractions and *Anonychomyrma* had more expansions than contractions (30 expansions and 7 contractions). It is not clear whether these results are due to gene losses or due to the population-level sampling or a bias produced by the method of orthogroup clustering, which is a known issue with performing gene expansion and contraction analysis (Demuth and Hahn 2009). Here, we used OrthoFinder the most widely used approach (Xia et al. 2020, Schrader et al. 2021, Wang et al. 2021, Ewart et al. 2024) and most effective automated approach (Emms and Kelly 2019). OrthoFinder combines reciprocal best-hits with phylogenetic information (Emms and Kelly 2019) which leads to an increase in accuracy of orthologue prediction compared to other software such as Orthologue and OrthoMCL which do not incorporate phylogenetic inference (Li et al. 2003, Fulton et al. 2006, Ludwig and Mrázek 2024).

One of the key results is that orthogroups containing olfactory receptor (OR) genes were either expanding, contracting or both in all species apart from *P. sp. 8*. Additionally, orthogroups containing gustatory receptors (GRs) were expanding in *P. nagasau* (one orthogroup expanding species wide and one orthogroup expanding in the Vanua Levu clade) as well as in *P. sp. 5*, *Anonychomyrma sp.*, *P. cordata*, and contracting in *Anonychomyrma*,

Iridomyrmex cf. pallidus and *P. laevigata*. *Philidris nagasau* is highly specific with the seed it collects, only cultivating seeds from six species of *Squamellaria* (Chomicki and Renner 2016a). In the majority of cases, *Philidris nagasau* farms monocultures of single *Squamellaria* species (Chomicki et al. 2020b). Unlike typical (terrestrial) ant seed dispersal that is mediated by the presence of food rewards called elaiosomes (Handel and Beattie 1990, Lengyel et al. 2010) elaiosomes are almost entirely absent from ant-garden plants (see Chapter 2 Figure 2.7 and Campbell et al., 2023). Work on ant-gardens in Peru demonstrated that ants are attracted to the volatile chemicals methyl 6-methylsalicylate (6-MMS) which is present in seeds of *Peperomia macrostachya*, the most dominant ant-garden plant in South America (Youngsteadt et al. 2008), therefore it seems likely that chemical compounds also mediate ant-garden mutualism in Southeast Asia and the obligate farming mutualism between *P. nagasau* and *Squamellaria*. The finding that some OR genes have intensified while others have relaxed is in line with the eco-evolutionary observation that *P. nagasau* has made behavioural gains, for example increased specificity, optimising seed planting for light exposure of adult plants (Chomicki et al. 2020b) and the ability to recognise and access concealed food rewards (Chomicki et al. 2016) accompanied by the loss of the ability to build carton nests (Chomicki and Renner 2016a).

Another recurring theme was expansions or contractions in gene families related to the neurotransmitter glutamate (Table 4.8) as well as other nervous system related GO terms (Tables 4.3 and 4.5). Changes in the nervous system are related to shifts in behavioural change (Rubin and Moreau 2016). There were more contractions in glutamate-related genes (4 in *P. nagasau* clade, 2 in *P. nagasau* (Vanua Levu clade)) compared to expansions (one expansion on the *P. nagasau* (Vanua Levu clade)). The expansion was annotated as a ionotropic glutamate receptor, which in insects has been found to function as a gustatory receptor (although it is phylogenetically distinct to GR genes) (Croset et al. 2010, Rytz et al. 2013, Wicher and Miazzi 2021). Three of the contracting glutamate gene families were annotated as metabotropic glutamate receptors; metabotropic glutamate receptors are expressed in bee brains as well as at neuromuscular junctions (Mitri et al. 2004).

Genomics alone cannot answer whether olfactory signals mediate plant cultivation in ants. Future work should involve testing the function of the genes here identified to be under diversifying selection in *P. nagasau*, as well as the genes experienced gene family expansions, and perform a genetic knockout study to establish which, if any, of the genes cause the ants to stop being interested in the seeds following a knockout. Since *Philidris* is not a genetically tractable system, cross-species complementation could be an option. Additionally, it would be of use to analyse the chemical components of *Squamellaria* seeds and perform a cafeteria experiment. By introducing each chemical in turn it would be

possible to establish which, are attractive to *P. nagasau*, as was carried out in Neotropical ant-garden ants establishing the role of methyl 6-methylsalicylate (6-MMS) in attracting ant-garden ants to *Peperomia macrostachya* (Youngsteadt et al. 2008).

Conclusions

The shift to highly specialised, obligate farming in *P. nagasau* has been accompanied by a higher amount of gene family expansions and contractions compared to other species in the genus. In contrast, there is no clear pattern when comparing facultative generalist farmers with non-mutualist populations, suggesting that facultative generalist farming may be phenotypically plastic. Key expanding and contracting gene families include olfactory receptors (ORs), gustatory receptors (GRs) and genes relating to the neurotransmitter glutamate, including an ionotropic glutamate receptor which functions similarly to a GR. These results suggest that plant farming mutualisms are mediated by adaptive evolution of sensory receptors.

Supplementary material (Chapter 4)

Table S4.1 Olfactory receptor (OR) orthogroups (CDS) with significant evidence for diversifying selection in *Philidris nagasau*. aBSREL, part of the HyPhy package (version 2.5.60) (Smith et al. 2015, Kosakovsky Pond et al. 2020) was used to test every nagasau branch in each OR containing orthogroup for evidence of diversifying selection. aBSREL optimises the number of rate classes per branch. Results from significant branches (LRT >3.41) are presented in bold.

Orthogroup	Branch name	Corrected P-value	(w)	Non-synonymous subs/site	Synonymous subs/site	LRT	Rate Distributions	Rate classes
CDS OG0000042.fa	Pnag@_80Phi_927_000001.1	7.09E-14	0.410	0.410	0.000	62.668	[[0, 0.995306119982048], [100000, 0.004693880017952012]]	2
	Pnag@_82Phi_445_000001.1	5.58E-03	0.152	0.152	0.001	12.391	[[0, 0.9989271849555653], [100000, 0.001072815044434683]]	2
	Pnag@_75Phi_682_000002.1	1.00E+00	0.002	0.001	0.001	0.000	[[0.2149687909693241, 1]]	1
	Pnag@_79Phi_779_000001.1	1.00E+00	0.001	0.000	0.000	0.000	[[0.4423382241192017, 1]]	1
	Pnag@_83Phi_910_000001.1	1.00E+00	0.000	0.000	0.000	0.000	[[0, 1]]	1
	Pnag@_85Phi_921_000001.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_86Phi_727_000002.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_87Phi_2527_000001.1	1.00E+00	0.005	0.000	0.005	0.000	[[0, 1]]	1
CDS OG0000048.fa	Pnag@_ptg000070l_000009.1	1.00E+00	0.001	0.000	0.000	0.000	[[0.4435957441702558, 1]]	1
	Pnag@_ptg001636l_000013.1	4.71E-05	0.041	0.041	0.000	20.503	[[10000000000, 1]]	1
	Pnag@_83Phi_982_000013.1	4.11E-03	54.766	54.707	0.060	11.054	[[0.3828416354205011, 0.6762663233436331], [1143.506294056057, 0.3237336766563669]]	2
	Pnag@_79Phi_739_000008.1	3.01E-01	0.007	0.006	0.001	0.650	[[2.23957113389991, 1]]	1
CDS OG0000058.fa	Pnag@_ptg001636l_000011.1	5.20E-01	0.012	0.010	0.002	0.899	[[2.018165779528221, 1]]	1
	Pnag@_ptg001194l_000025.1	9.79E-05	0.079	0.079	0.000	19.855	[[1, 0.9692889604157005], [100000, 0.03071103958429955]]	2
	Pnag@_ptg001194l_000024.1	2.82E-02	0.022	0.020	0.002	8.262	[[0.8987925753504837, 0.9921701152379955], [434.988913805767, 0.007829884762004524]]	2
	Pnag@_86Phi_2336_000006.1	7.52E-01	0.002	0.002	0.000	1.478	[[10000000000, 1]]	1
	Pnag@_80Phi_3607_000003.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_80Phi_3607_000005.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
CDS OG0000175.fa	Pnag@_ptg001194l_000026.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_ptg000752l_000052.1	2.90E-02	0.009	0.008	0.002	6.403	[[0.1215501531791432, 0.9761083592607237], [96.30898860702527, 0.0238916407392763]]	2

	Pnag@_ptg000752l_000051.1	2.50E-01	0.002	0.002	0.000	0.970	[[10000000000, 1]]	1
CDS OG0000228.fa	Pnag@_ptg001467l_000011.1	5.36E-11	3.413	3.412	0.001	48.927	[[0.0006600413021006226, 1.56517764622548e-10], [1, 0.9843482235377452], [100000, 0.01565177630573703]]	3
	Pnag@_87Phi_1764_000002.1	1.00E+00	0.011	0.008	0.003	0.017	[[1.16192523828966, 1]]	1
	Pnag@_ptg001199l_000011.1	1.00E+00	0.028	0.019	0.008	0.000	[[0.8663117246661298, 1]]	1
	Pnag@_ptg001199l_000012.1	1.00E+00	0.007	0.005	0.002	0.000	[[0.9159949998167566, 1]]	1
	Pnag@_ptg001199l_000013.1	1.00E+00	0.029	0.000	0.028	0.000	[[0.00597883327665546, 1]]	1
	Pnag@_ptg001199l_000014.1	1.00E+00	0.012	0.009	0.003	0.021	[[1.102391461733385, 1]]	1
	Pnag@_ptg001199l_000015.1	1.00E+00	0.002	0.001	0.001	0.000	[[0.8368556566321104, 1]]	1
CDS OG0000234.fa	Pnag@_ptg000355l_000029.1	2.51E-03	0.095	0.087	0.008	13.046	[[0.2326641771947966, 0.9827156452983679], [264.3372120802139, 0.01728435470163214]]	2
	Pnag@_82Phi_1481_000002.1	1.00E+00	0.008	0.003	0.005	0.000	[[0.2384241143412938, 1]]	1
	Pnag@_83Phi_626_000023.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_85Phi_225_000022.1	1.00E+00	0.001	0.000	0.001	0.000	[[0, 1]]	1
	Pnag@_ptg000355l_000028.1	1.00E+00	0.004	0.002	0.003	0.000	[[0.2582512421763634, 1]]	1
CDS OG0000357.fa	Pnag@_ptg000528l_000046.1	4.82E-02	0.005	0.003	0.003	5.406	[[0, 0.9965233635467089], [116.3997994597693, 0.003476636453291104]]	2
	Pnag@_ptg000528l_000047.1	1.22E-01	0.007	0.007	0.000	2.282	[[10000000000, 1]]	1
CDS OG0000535.fa	Pnag@_80Phi_3500_000003.1	1.07E-14	18.202	18.198	0.004	63.453	[[1, 0.9799610017539782], [100000, 0.02003899824602184]]	2
	Pnag@_ptg001467l_000006.1	1.00E+00	0.011	0.007	0.004	0.000	[[0.6020266684000501, 1]]	1
CDS OG0000552.fa	Pnag@_75Phi_249_000010.1	1.08E-04	5.532	5.528	0.004	19.959	[[0.7419146759411799, 0.9763993392594599], [24242.24058910811, 0.02360066074054012]]	2
	Pnag@_84Phi_527_000008.1	7.23E-01	0.006	0.006	0.000	2.305	[[10000000000, 1]]	1
	Pnag@_79Phi_1988_000010.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_83Phi_630_000007.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_ptg000528l_000009.1	1.00E+00	0.006	0.006	0.000	0.400	[[10000000000, 1]]	1
	Pnag@_ptg000528l_000010.1	1.00E+00	0.036	0.026	0.010	-0.074	[[1.17707559204354, 1]]	1
	Pnag@_ptg000528l_000014.1	1.00E+00	0.013	0.004	0.009	0.000	[[0.1915311484005757, 1]]	1
CDS OG0001104.fa	Pnag@_87Phi_1768_000006.1	1.92E-03	0.104	0.104	0.000	13.572	[[0, 0.9944732425842568], [100000, 0.005526757415743155]]	2
	Pnag@_ptg000070l_000025.1	2.50E-01	0.006	0.006	0.000	3.556	[[10000000000, 1]]	1

	Pnag@_ptg000070l_000026.1	8.41E-01	0.013	0.011	0.002	0.768	[[1.899389089361151, 1]]	1
	Pnag@_81Phi_1481_000007.1	1.00E+00	0.004	0.003	0.001	-0.014	[[1.133331579803108, 1]]	1
	Pnag@_ptg000070l_000028.1	1.00E+00	0.010	0.006	0.004	0.000	[[0.5828850481996769, 1]]	1
CDS OG0001404.fa	Pnag@_ptg001636l_000007.1	2.09E-04	0.028	0.024	0.004	14.781	[[1, 0.9908989667190471], [183.3341451883724, 0.009101033280952908]]	2
CDS OG0002188.fa	Pnag@_ptg000528l_000048.1	4.82E-02	0.015	0.012	0.003	4.058	[[0, 0.9728245349733616], [60.13693411980182, 0.02717546502663837]]	2
CDS OG0002254.fa	Pnag@_ptg001242l_000005.1	8.00E-05	2.568	2.564	0.003	16.693	[[0.3318563109959238, 0.9962208235109152], [100000, 0.003779176489084768]]	2
	Pnag@_87Phi_1281_000007.1	3.32E-05	0.023	0.023	0.000	20.630	[[0, 0.9912414484550197], [0, 0.003585195924573073], [100000, 0.005173355620407214]]	3
CDS OG0002294.fa	Pnag@_75Phi_810_000009.1	5.62E-01	0.001	0.001	0.000	0.765	[[100000000000, 1]]	1
	Pnag@_ptg000355l_000006.1	1.00E+00	0.001	0.000	0.001	0.000	[[0, 1]]	1
	Pnag@_ptg000528l_000006.1	7.16E-05	4.732	4.729	0.003	19.671	[[0, 0.9921243667864799], [100000, 0.007875633213520139]]	2
CDS OG0002769.fa	Pnag@_81Phi_111_000007.1	1.00E+00	0.001	0.000	0.001	0.000	[[0, 1]]	1
	Pnag@_84Phi_527_000006.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_ptg000528l_000007.1	1.00E+00	0.002	0.002	0.000	-1.441	[[100000000000, 1]]	1
	Pnag@_87Phi_3821_000003.1	2.96E-07	2.723	2.722	0.001	30.038	[[1, 0.9830403642849668], [100000, 0.01695963571503323]]	2
CDS OG0005578.fa	Pnag@_79Phi_63_000003.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_ptg000360l_000004.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
CDS OG0007863.fa	Pnag@_ptg000060l_000010.1	1.02E-04	2.541	2.538	0.002	16.218	[[0.2798108590277785, 0.006256606081006062], [0.3135531643141838, 0.9888579607882046], [100000, 0.00488543313078929]]	3
CDS OG0008887.fa	Pnag@_ptg000325l_000017.1	1.00E-05	6.085	6.081	0.004	22.204	[[0, 0.9936703255019782], [100000, 0.006329674498021798]]	2
	Pnag@_ptg000325l_000018.1	1.00E+00	0.005	0.002	0.003	0.000	[[0.3838864328032798, 1]]	1
	Pnag@_82Phi_7865_000002.1	3.21E-09	152.441	152.435	0.006	39.638	[[0.1064462757223849, 0.9182139275264658], [100000, 0.0817860724735342]]	2
CDS OG0009273.fa	Pnag@_79Phi_1988_000009.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_85Phi_3130_000003.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
	Pnag@_ptg000528l_000008.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1
CDS OG0009417.fa	Pnag@_ptg000132l_000018.1	2.64E-04	0.051	0.048	0.003	14.320	[[1, 0.9896880679619302], [581.2918790202139, 0.01031193203806979]]	2

CDS OG0009596.fa	Pnag@_ptg001268l_000005.1	2.03E-06	0.068	0.068	0.000	24.010	[[1, 0.93410887126411], [100000, 0.06589112873588998]]	2
CDS OG0009654.fa	Pnag@_ptg001194l_000010.1	1.22E-05	0.207	0.201	0.007	20.432	[[1, 0.9948603239784864], [2382.082562935151, 0.005139676021513551]]	2
CDS OG0010134.fa	Pnag@_83Phi_580_000008.1	1.15E-06	259.101	259.087	0.014	26.515	[[0.4561386507068011, 0.9074791965108777], [100000, 0.09252080348912228]]	2
	Pnag@_82Phi_559_000003.1	1.86E-01	0.013	0.013	0.000	1.496	[[10000000000, 1]]	1
CDS OG0010275.fa	Pnag@_ptg000325l_000016.1	3.28E-04	0.962	0.960	0.002	13.890	[[0, 0.9975107079227673], [100000, 0.002489292077232697]]	2
CDS OG0010362.fa	Pnag@_ptg001194l_000023.1	4.93E-04	0.016	0.013	0.003	13.080	[[0.342509588538337, 0.9956762182661402], [409.1489856266459, 0.004323781733859833]]	2
CDS OG0010793.fa	Pnag@_ptg000132l_000011.1	3.57E-02	0.012	0.009	0.003	4.643	[[0, 0.9501530814113998], [25.82595364936972, 0.04984691858860024]]	2
CDS OG0011090.fa	Pnag@_82Phi_1783_000007.1	7.25E-03	0.340	0.301	0.039	9.130	[[0, 0.7866301932165666], [13.61222463610737, 0.2133698067834334]]	2
	Pnag@_ptg000077l_000038.1	1.08E-02	106.950	106.940	0.010	9.139	[[1, 0.9594948079521812], [100000, 0.04050519204781877]]	2
	Pnag@_ptg000077l_000036.1	1.51E-01	0.006	0.006	0.000	1.883	[[10000000000, 1]]	1
OG0011111.fa	Pnag@_ptg000086l_000028.1	8.96E-05	0.050	0.048	0.002	16.466	[[0.6024897992897406, 0.9924659983311155], [1312.77231075134, 0.007534001668884516]]	2
CDS OG0011122.fa	Pnag@_ptg000077l_000045.1	3.45E-02	0.056	0.052	0.004	7.859	[[0.2790708479427222, 0.9877170079303274], [468.1706259722945, 0.01228299206967265]]	2
	Pnag@_83Phi_5423_000001.1	1.00E+00	0.002	0.002	0.001	0.000	[[0.9783096114982986, 1]]	1
	Pnag@_ptg000077l_000047.1	1.00E+00	0.002	0.000	0.002	0.000	[[0, 1]]	1
	Pnag@_ptg000077l_000049.1	1.00E+00	0.014	0.009	0.005	0.000	[[0.8094212369619666, 1]]	1
	Pnag@_ptg000277l_000009.1	1.00E+00	0.053	0.035	0.018	0.000	[[0.8664224679875452, 1]]	1
CDS OG0011137.fa	Pnag@_85Phi_3979_000004.1	1.07E-05	0.653	0.629	0.024	22.073	[[0.2546119030506676, 0.6271944724038826], [25.66642804325861, 0.3728055275961174]]	2
	Pnag@_ptg000070l_000031.1	4.69E-01	0.011	0.008	0.003	0.028	[[1.194352059796741, 1]]	1
CDS OG0012530.fa	Pnag@_82Phi_4947_000001.1	4.91E-03	0.149	0.146	0.002	8.531	[[1, 0.9897480148747344], [3074.324489055994, 0.01025198512526559]]	2
CDS OG0012633.fa	Pnag@_ptg000325l_000005.1	4.10E-03	0.037	0.030	0.007	10.257	[[0, 0.8826634805951201], [15.73209057778493, 0.1173365194048799]]	2
	Pnag@_ptg000325l_000004.1	1.00E+00	0.011	0.004	0.007	0.000	[[0.2403577017556631, 1]]	1

CDS OG0012698.fa	Pnag@_ptg000325l_000014.1	4.50E-06	79.980	79.978	0.002	23.801	[[1, 0.8498637329933598], [100000, 0.1501362670066402]]	2
	Pnag@_ptg000325l_000013.1	1.00E+00	0.000	0.000	0.000	0.000	[[0, 1]]	1
CDS OG0013683.fa	Pnag@_ptg000077l_000043.1	0.00E+00	299.356	299.344	0.012	92.991	[[0.4551846938847472, 0.9034372270607827], [100000, 0.09656277293921733]]	2
	Pnag@_ptg000077l_000041.1	1.53E-03	0.016	0.012	0.004	12.214	[[0.1508164841841904, 0.9841901934234751], [64.34461729208016, 0.01580980657652487]]	2
	Pnag@_ptg000077l_000046.1	4.36E-01	0.014	0.011	0.003	0.100	[[1.340298490356181, 1]]	1
CDS OG0016602.fa	Pnag@_86Phi_2264_000010.1	4.00E-03	0.024	0.024	0.000	10.306	[[0, 0.9805092311862857], [98111.25219099695, 0.01949076881371425]]	2
	Pnag@_ptg000077l_000040.1	1.00E+00	0.000	0.000	0.000	0.000	[[1, 1]]	1

Table S4.2 Olfactory receptor (OR) orthogroups which have significant evidence of either intensified or relaxed selection following a RELAX test, performed using HyPhy. RELAX (Wertheim et al. 2015) model fits. RELAX produces a null, alternative, or partitioned descriptive (PD) models. lnL is the log likelihood. AICc is the small sample-corrected Akaike Information and alternative models on 1 degree of freedom. K is the relation/intensification parameter ($K < 1$ suggests relaxation and $K > 1$ suggests intensification). Partition refers to the tree branch partition (Test = *P. nagasau* and Ref = background). ω_0 ω_1 ω_2 give the inferred ratio of nonsynonymous to synonymous mutations (dN/dS) of each site class and p0 p1 and p2 give the site proportions of each site class. The null model is constrained to having a K value of 1.

gene	model	lnL	AICc	LRT	p value	k	partition	ω_0	ω_1	ω_2	p0	p1	p2
Orthogroups with significant evidence of intensified natural selection													
CDS OG0000058	null	-4317.99	8800.883			1	Reference	0	1	135.6005	0.559217	0.437913	0.002871
	alt	-4314.5	8795.913	6.99133	0.008191	1.987258	Reference	0	1	27.03227	0.584302	0.407662	0.008036
	alt						Test	0	1	700.6814	0.584302	0.407662	0.008036
	PD	-4311.74	8798.491				Reference	0	1	125.6302	0.564176	0.433586	0.002238
	PD						Test	1	1	559.0736	0.622531	0.361974	0.015494
CDS OG0000175	null	-4673.6	9459.486			1	Reference	0	0	3.980535	0.824863	0.098581	0.076556
	alt	-4669.21	9452.734	8.762754	0.003074	2.634464	Reference	8.55879E-19	8.55879E-19	3.746809	0.824435	0.103659	0.071906
	alt						Test	2.52108E-48	2.52108E-48	32.4557	0.824435	0.103659	0.071906
	PD	-4668.49	9459.338				Reference	0.056634244	0.117109787	3.107508	0.850696	0.083408	0.065895
	PD						Test	0	3.99847E-08	106.1001	0.968881	0.00296	0.028158
CDS OG0000535	null	-4076.65	8265.991			1	Reference	0	1	697.4561	0.278931	0.719595	0.001474
	alt	-4068.49	8251.697	16.31873	5.35E-05	2.782788	Reference	0	1	21.00683	0.320644	0.668804	0.010552
	alt						Test	0	1	4784.634	0.320644	0.668804	0.010552
	PD	-4070.23	8263.288				Reference	0	1	571.7704	0.284687	0.71471	0.000602
	PD						Test	0.50423607	1	1E+10	0.298957	0.692587	0.008456
CDS OG0001062	null	-2325.12	4732.92			1	Reference	3.14457E-14	1.17874E-10	2.052426	0.000298	0.674844	0.324858
	alt	-2323.13	4730.978	3.975103	0.046178	5.953485	Reference	7.86142E-13	1.17919E-10	2.067657	0	0.692168	0.307832
	alt						Test	8.63066E-73	7.78623E-60	75.54351	0	0.692168	0.307832
	PD	-2323.06	4738.978				Reference	3.94463E-16	1.1738E-10	1.861433	0.002567	0.662709	0.334724
	PD						Test	3.94463E-16	1.1738E-10	26.73924	1.59E-05	0.000112	0.999873
CDS OG0001418	null	-2275.26	4659.119			1	Reference	3.47043E-20	3.47043E-20	5.543693	0.879409	0.073325	0.047266
	alt	-2273.25	4657.118	4.023001	0.044884	4.060946	Reference	8.67608E-19	8.67608E-19	5.006836	0.876802	0.0744	0.048799
	alt						Test	4.49278E-74	4.49278E-74	693.2481	0.876802	0.0744	0.048799
	PD	-2273.19	4665.088				Reference	1.38817E-21	1.38817E-21	5.064816	0.87886	0.072987	0.048154

	PD						Test	1.38817E-21	3.04076E-21	33.86268	0.000719	0	0.999281
CDS OG0002254	null	-1775.55	3631.897			1	Reference	0.562391434	1	1263.468	0.998395	0.001226	0.000379
	alt	-1772.55	3627.932	6.005234	0.014264	2.739004	Reference	0	0.597834808	22.11172	0.010342	0.986318	0.00334
	alt						Test	0	0.244373963	4818.666	0.010342	0.986318	0.00334
	PD	-1772.4	3635.815				Reference	0.572728194	1	19.63489	0.929819	0.070181	0
	PD						Test	0	0.332039867	1E+10	0.001072	0.995122	0.003807
CDS OG0002294	null	-2289.74	4666.075			1	Reference	0.195436514	0.249578276	472.5621	0.996174	0.002738	0.001088
	alt	-2287.55	4663.721	4.382024	0.03632	2.292951	Reference	0.187092813	0.188576159	188.9335	0.995349	0.003023	0.001628
	alt						Test	0.021422128	0.021813568	165754.2	0.995349	0.003023	0.001628
	PD	-2287.35	4671.446				Reference	0.200050326	1	341.0084	0.996887	0.00226	0.000853
	PD						Test	0	0	3851.067	0.995562	0.001776	0.002662
CDS OG0002769	null	-1938.69	3964.073			1	Reference	0.361717157	1	468.8004	0.996353	0.002285	0.001362
	alt	-1936.55	3961.824	4.281919	0.03852	3.569214	Reference	0.348053674	0.553882217	108.3781	0.992665	0.00437	0.002965
	alt						Test	0.023122642	0.121395327	18329083	0.992665	0.00437	0.002965
	PD	-1937.18	3971.219				Reference	0.376038616	1	293.3171	0.997041	0.001587	0.001372
	PD						Test	0.195736922	1	1E+10	0.995646	0.002686	0.001668
CDS OG0003084	null	-2335.12	4750.76			1	Reference	0.381288423	1	1239.41	0.998087	0.001645	0.000268
	alt	-2330.61	4743.766	9.019865	0.002671	50	Reference	0.363909926	0.917360429	26.65593	0.985763	0.012107	0.00213
	alt						Test	1.12123E-22	0.013396771	1.95E+71	0.985763	0.012107	0.00213
	PD	-2330.26	4751.162				Reference	0.228374673	1	8.399129	0.860594	0.126894	0.012511
	PD						Test	0	0	1071980	0.997766	0.000478	0.001756
CDS OG0003627	null	-2959.4	5989.251			1	Reference	0.572399919	0.613740538	179.0732	0.171056	0.827626	0.001318
	alt	-2957.31	5987.089	4.188195	0.040706	4.265088	Reference	0.605919184	0.689442768	91.65917	0.210464	0.7876	0.001936
	alt						Test	0.118026509	0.204729629	2.34E+08	0.210464	0.7876	0.001936
	PD	-2957.25	5995.085				Reference	0.679448534	0.687654717	162.3149	0.808618	0.190333	0.001049
	PD						Test	0.111438942	0.171394688	219.474	0.564994	0.430737	0.00427
CDS OG0007490	null	-2215.91	4506.433			1	Reference	0.431125695	1	1.62124	1	0	0
	alt	-2213.92	4504.477	3.988661	0.045807	7.448556	Reference	0.43494466	0.439748613	2.446115	0.984752	0.012446	0.002802
	alt						Test	0.002027009	0.002199828	782.6942	0.984752	0.012446	0.002802
	PD	-2213.89	4512.547				Reference	0.440983544	0.944558896	1.451417	1	0	0

	PD						Test	0	0	769.4816	0.95329	0.043841	0.002869
CDS OG0007830	null	-2110.4	4291.512			1	Reference	0.018835321	0.436657476	5.654433	0	1	0
	alt	-2108.32	4289.398	4.154449	0.041525	39.01336	Reference	0.019748721	0.457304967	8.168487	0	1	0
	alt						Test	3.18597E-67	5.53975E-14	3.85E+35	0	1	0
	PD	-2108.32	4297.575				Reference	0.017956046	0.457438004	4.264743	0	1	0
	PD						Test	0	0.017956046	4.264743	1	0	0
CDS OG0008031	null	-2442.24	4961.101			1	Reference	0.177152517	0.856917728	1.260233	0.584677	0.415323	0
	alt	-2439.81	4958.261	4.872608	0.027286	14.80748	Reference	0.217804864	0.911845098	1.416018	0.579301	0.420699	0
	alt						Test	1.57925E-10	0.254995248	172.5629	0.579301	0.420699	0
	PD	-2439.79	4966.362				Reference	0.176779446	0.880738318	1.50672	0.567392	0.381764	0.050844
	PD						Test	0.101622056	0.225211844	1.136061	0.963347	0.036653	0
CDS OG0009273	null	-557.529	1192.795			1	Reference	0	1	11.82379	0.947222	0.002164	0.050614
	alt	-544.767	1169.363	25.52371	4.37E-07	50	Reference	0.007313423	1	1.172217	0.864121	0.0533	0.082579
	alt						Test	1.6071E-107	1	2820.926	0.864121	0.0533	0.082579
	PD	-544.578	1177.38				Reference	0.137542281	0.998060429	2.222053	1	0	0
	PD						Test	0.106241545	0.117465956	1E+10	0.913299	0.004986	0.081716
CDS OG0009556	null	-2040.18	4152.983			1	Reference	0.257168796	0.268080127	285.0412	0.995889	0.003706	0.000405
	alt	-2038.12	4150.903	4.115516	0.042492	2.524467	Reference	0.304959355	0.370993843	289.0582	0.996476	0.003153	0.00037
	alt						Test	0.049886804	0.081823925	1631838	0.996476	0.003153	0.00037
	PD	-2037.98	4158.774				Reference	0.305914425	0.309827711	276.5969	0.995582	0.003973	0.000445
	PD						Test	0.050526299	0.123619068	2.410121	1	0	0
CDS OG0010134	null	-2362.99	4828.634			1	Reference	0.467189227	1	132.9918	0.958587	0.036926	0.004488
	alt	-2355.32	4815.321	15.33967	8.98E-05	4.417236	Reference	0	1	6.072138	0.668096	0.254753	0.077151
	alt						Test	0	1	2885.395	0.668096	0.254753	0.077151
	PD	-2351.6	4815.984				Reference	0.568043796	0.580050871	542.9873	0.989219	0.009619	0.001162
	PD						Test	0.402553756	0.407655905	2749.858	0.029458	0.887628	0.082914
CDS OG0010275	null	-2363.9	4794.089			1	Reference	0.619936097	1	null	0.869125	0.045391	null
	alt	-2356.9	4782.118	13.98933	0.000184	50	Reference	0.658668887	0.669911311	null	0.234015	0.763479	null
	alt						Test	0	1.99924E-09	null	0.234015	0.763479	null
	PD	-2356.9	4788.172				Reference	0.65439788	0.679294915	null	0.423423	0.576577	null

	PD						Test	0	0	null	0.97495	0.022538	null
CDS OG0011111	null	-2862.99	5804.479			1	Reference	0.803485879	1	780.8031	0.537313	0.461125	0.001562
	alt	-2859.41	5799.347	7.158622	0.00746	1.903316	Reference	0.380811939	1	46.04564	0.321097	0.669419	0.009483
	alt						Test	0.15920615	1	1464.106	0.321097	0.669419	0.009483
	PD	-2859.19	5807.021				Reference	0	1	39.16048	0.200614	0.786762	0.012624
	PD						Test	0	0.633219965	1480.481	0	0.992773	0.007227
CDS OG0011429	null	-2220.92	4514.426			1	Reference	0.309465332	0.334834318	134.2655	0.647398	0.351552	0.00105
	alt	-2218.82	4512.257	4.202273	0.04037	36.72545	Reference	0.323342368	0.394723875	177.4774	0.664742	0.334455	0.000802
	alt						Test	9.82065E-19	1.49174E-15	3.99E+82	0.664742	0.334455	0.000802
	PD	-2218.42	4519.595				Reference	0.338904985	0.340395928	119.0775	0.574851	0.423749	0.0014
	PD						Test	0	0	20.23336	0.010528	0.989472	0
CDS OG0012439	null	-1885.78	3818.421			1	Reference	0.332861399	1	58.91035	0.88501	0.06609	0.0489
	alt	-1880.52	3809.988	10.50905	0.001188	5.490139	Reference	0	1	3.151423	0.577703	0.285237	0.13706
	alt						Test	0	1	545.5958	0.577703	0.285237	0.13706
	PD	-1883.51	3824.29				Reference	0.605357446	1	65.73885	0.865932	0.121083	0.012984
	PD						Test	1	1	1E+10	0.885051	0.060019	0.05493
CDS OG0013683	null	-4621.2	9302.697			1	Reference	0.044335428	0.32890451	28.23516	0.539072	0.384323	0.076605
	alt	-4613.62	9289.545	15.17084	9.82E-05	2.112087	Reference	0	1	8.95427	0.665614	0.253274	0.081112
	alt						Test	0	1	102.511	0.665614	0.253274	0.081112
	PD	-4613.18	9296.749				Reference	0	0.154585338	7.708115	0.878494	0	0.121506
	PD						Test	0	1	106.5821	0.63847	0.279234	0.082296

Orthogroups with significant evidence of relaxed natural selection

CDS OG0000406	null	-4154.55	8437.682			1	Reference	0.290338524	1	135.0315	0.785872	0.209977	0.004151
	alt	-4150.59	8431.777	7.922905	0.004881	0	Reference	0.259736488	0.979156256	156.504	0.77861	0.216842	0.004548
	alt						Test	1	1	1	0.77861	0.216842	0.004548
	PD	-4150.58	8439.848				Reference	0.258267044	0.997292694	168.1193	0.776859	0.21878	0.004361
	PD						Test	1	1	19.46558	0.351504	0.648496	0
CDS OG0001404	null	-2667.16	5412.74			1	Reference	0.33505446	0.335448149	1E+10	0.997232	0.001488	0.00128
	alt	-2664.61	5409.655	5.106919	0.023831	0.34559	Reference	0.277460012	0.28180055	1E+10	0.001484	0.997232	0.001284
	alt						Test	0.64206007	0.645513655	2856.928	0.001484	0.997232	0.001284

	PD	-2662.32	5413.177				Reference	0.274317172	0.274412102	1E+10	0.000746	0.998284	0.00097
	PD						Test	1	1	31999.9	0.99319	0.000794	0.006015
CDS OG0004376	null	-2390.98	4854.528		1		Reference	0.226408244	0.28800629	88.01127	0.992598	0.00553	0.001871
	alt	-2388.89	4852.398	4.162313	0.041333	0	Reference	0.20275109	0.207121968	95.60273	0.004841	0.993376	0.001783
	alt						Test	1	1	1	0.993376	0.004841	0.001783
	PD	-2388.74	4860.228				Reference	0.205794361	0.208956373	102.175	0.00346	0.994827	0.001714
	PD						Test	1	1	4.170099	0.685527	0.046774	0.267699
CDS OG0005999	null	-2646.62	5369.862		1		Reference	0.07195554	0.89577654	9.864348	0.453536	0.452829	0.093635
	alt	-2644.54	5367.721	4.17327	0.041066	0.000451	Reference	0	1	10.94651	0.497775	0.396682	0.105543
	alt						Test	0	1	1.001079	0.497775	0.396682	0.105543
	PD	-2644.41	5375.599				Reference	0.11359666	0.886109444	11.11368	0.468791	0.432694	0.098515
	PD						Test	0.068421639	0.413889002	6.123904	0	1	0
CDS OG0011122	null	-4095.74	8283.964		1		Reference	0.506122512	0.6429453	57.19318	0.913533	0.075805	0.010662
	alt	-4093.73	8281.969	4.015187	0.045092	0.666896	Reference	0.44006521	0.442702127	52.28887	0.883086	0.101987	0.014927
	alt						Test	0.578446186	0.580755421	13.99588	0.883086	0.101987	0.014927
	PD	-4092.27	8287.136				Reference	0.273329457	0.456709235	53.03796	0.116613	0.868166	0.01522
	PD						Test	0.707911029	0.726433806	575.1804	0.965679	0.032601	0.001721

Chapter 5: Genome-wide signatures of mutualistic dependence



Abstract

Cooperation in mutualisms is widely understood as mutually beneficial exploitation. This poses the question: does evolution of mutualism have similar dynamics to that seen in coevolution between host and parasite and predator and prey? There are two main hypotheses of how rates of molecular evolution should evolve in mutualists (i) the Red Queen hypothesis: rates should increase in mutualistic lineages, mirroring a classic arms race as seen between host and parasite and predator and prey and (ii) the Red King effect which predicts that mutualists benefit from evolving more slowly, thus positing that molecular evolutionary rates should be slower in mutualists. Here, I investigate the mutualistic ant-genus *Philidris* (Dolichoderinae). *Philidris nagasau* experienced a shift towards obligate specialist mutualism, wherein it has lost the ability to build its own carton nests and instead relies on farming six species of *Squamellaria* which provide nesting space in the form of domatia. I find that rates of nucleotide substitution have increased in *P. nagasau* compared background branches, indicating Red Queen dynamics. I also find evidence of genome-wide relaxed selection accompanied by some positive selection.

1. Introduction

Mutualisms have historically caused confusion for evolutionary biologists – why would a species evolve traits seemingly for the benefit of other species? Some early evolutionary biologist even dismissed the existence of mutualisms as anthropomorphising, viewing interspecies interactions as inherently antagonistic (Bronstein 2015). Our understanding of mutualisms has now advanced, and we appreciate that while a species may evolve highly complex traits for the benefit of another species, this is in return for services or rewards at least as valuable as the cost of the said trait. The evolution of mutualistic cooperation between species prompts two questions, firstly, how do mutualisms persist in spite of threats from cheating and exploitation and second, how are the benefits of mutualism allocated between partners (Bergstrom and Lachmann 2003a). The first question has received much more attention (Bull and Rice 1991, Sachs et al. 2004, 2010, Frederickson 2013, 2017, Chomicki et al. 2016) compared to the latter (Bergstrom and Lachmann 2003a, b, Rubin and Moreau 2016, Ametrano et al. 2022).

With the aim to understand how the benefits of mutualism are allocated between mutualists –sometimes termed the surplus produced by mutualism, borrowing from the language of economics (Bergstrom and Lachmann 2003a)– there are two main schools of thought of how mutualists are expected to evolve. First, the Red Queen Hypothesis first applied to mutualisms by Herre et al. (1999) predicts that the evolution of mutualism would lead to increased rates of molecular evolution, similar to a classic arms race seen between predator and prey or host and parasite as originally described by Van Valen (1976). In contrast, the Red King Effect, identified by Bergstrom and Lachmann (2003a, b) using game theory modelling, predicts that successful mutualists will evolve more slowly, characterising evolution of mutualists as a bargaining process over the surplus produced by mutualism: *“In this bargaining process, fast evolution does not allow a species to outrun a partner — it simply causes this species to yield to whatever threats are made.”* Here, the mutualist gets to retain “selfish” behaviour for longer by delaying coevolutionary changes although this is highly dependent on the circumstances of the interaction (Rubin and Moreau 2016). A mutualist may also evolve more slowly due to a “lock and key” effect, wherein mutualists are well adapted to each other, and therefore are incentivised to evolve more slowly. Modelling by Yoder and Nuismer (2010) showed how coevolutionary interactions (including mutualisms, antagonisms and competition) affect species diversification (Yoder and Nuismer 2010). Their results indicate that mutualisms mediated by phenotype differences, when a species maximises its coevolutionary fitness by having a greater phenotype value, regardless of the phenotype value of the other interacting species, as seen in classic arms race dynamics are not expected to affect species diversification whereas mutualisms that

are mediated by phenotype matching, when the fitness outcome of the interaction depends on how closely the trait in one species matches the trait in the other, regardless of the relative values of the traits, restrict phenotype diversification (Yoder and Nuismer 2010). Empirical studies have provided support for the 'lock and key' effect at the phenotypic level. This effect appears to be especially strong in highly specialised and obligate mutualisms involving coadapted traits, and has been found to characterise morphological evolution of both specialised oil-bee pollination (Davis et al. 2014) and ant-plant mutualisms (Chomicki and Renner 2017b). At present, our knowledge of genome evolution and mutualists is mostly limited to the evolution of bacterial endosymbionts. Obligate bacterial endosymbionts often have greatly reduced genome sizes, as low as 1 Mb (McCutcheon and Moran 2012). Along with small genome size obligate endosymbiont genomes are characterised by genes loss, often with reciprocal gene gains in the host, and of interest here, rapid genome evolution (McCutcheon and Moran 2012). These changes are attributed to endosymbionts having small effective population sizes, asexual reproduction and a deletion bias in bacteria (McCutcheon and Moran 2012). This prompts the question, are these genome changes a result of a mutualistic lifestyle or are they inherent to the bacterial endosymbiont lifestyle?

There have been very few studies on the effects of mutualism on genome evolution outside of endosymbionts. One study of the classic plant-ant genus *Pseudomyrmex* which includes the classic mutualists the acacia-ants, ants that nest in the hollow thorns of Central American acacia trees in return for providing defence from herbivory (Ward and Downie 2005). There are an additional two clades of mutualistic clades within *Pseudomyrmex* that instead of nesting in hollow thorns, they nest in twigs and leaf petioles domatia in the plant genera *Triplaris* and *Tachigali*, respectively (Chomicki et al., 2015). When comparing mutualist clades with closely related non-mutualists, the mutualists have a higher rate of molecular evolution compared to the non-mutualist relatives, suggesting that the evolution of mutualism conforms to Red Queen dynamics (Rubin and Moreau 2016). Similarly, early work on lichenised fungi found that lichenised fungi had higher rates of nucleotide substitutions compared to their non-lichenised relatives, although the authors hypothesise that this difference is as a result of increased exposure to UV radiation (Lutzoni and Pagel 1997). A more recent and comprehensive comparison between lichenised and non-lichenised fungi found consistently lower rates of nucleotide substitution rates in mutualistic lichenised fungi, (Ametrano et al. 2022). Ametrano et al. (2022) caveat this finding with the fact that it is not clear whether this is as a result of the fungi remaining "selfish" for longer as predicted by the Red King Hypothesis, or whether this is an effect of the slower life histories of lichenised fungi compared to their non-lichenised relatives (Ametrano et al. 2022). While the work on

shifts to mutualism are limited and inconclusive, work investigating the effect on genome evolution in response to shifts in mutualistic dependence are non-existent.

Here, I investigate another ant-plant mutualism within the ant genus, *Philidris* (Dolichoderinae). *Philidris nagasau* is a highly specialised obligate mutualist of six species of the genus *Squamellaria* (Rubiaceae) (Chomicki and Renner 2016a). Within the same genus, there are several species that engage in ant-garden mutualisms, wherein the ants first create a nutrient rich carton nest and then collect epiphyte seeds from many plant families, as the seeds grow roots this provides structural stability to the nest, allowing the nests to be larger, although the presence of plants provides a fitness benefit, the ants appear to be able to nest without plants (Kaufmann 2002, Peeters and Wiwatwitaya 2014, Campbell et al. 2023). Additionally, there appear to be populations that do not engage in any plant mutualism. Using *Philidris* we ask whether a shift to specialised, obligate mutualism as seen in *P. nagasau* leads to differing levels of molecular evolution, and whether these differences reflect Red Queen dynamics or a Red King effect. Additionally, I investigate how this change in mutualism strategy effects signatures of natural selection.

2. Methods

See Chapter 3 for details on genome assembly (section 2.2) and annotation (section 2.3) and details on phylogenetic constructions (section 2.4).

2.1 Codon alignments

In Chapter 3 I assembled and annotated two genomes using PacBio HiFi reads and a further 38 using Illumina resequencing data, this sampling represents a combination of species-level and population-level sampling, with some species represented by many samples and some species with just one. While it was possible to utilise this population-level sampling in the gene family evolution analysis in Chapter 4, it was not sensible for signatures of selection analysis. There is no utility to comparing identical samples. Using sequences with identical or near-identical sequences provides the model with little usable information, particularly due to a lack of non-synonymous rate substitutions (Yang 2014) and at worst dN/dS analysis on intraspecific samples can be actively misleading (Kryazhimskiy and Plotkin 2008). In light of this, I created a subset from my dataset so that each species was represented by just one genome, a phylogeny of this subset dataset can be seen in Figure 5.1. Genomes for each species were chosen at random, with the exception of *P. nagasau* and *P. laevigata* wherein the reference genomes, assembled from HiFi reads, were utilised.

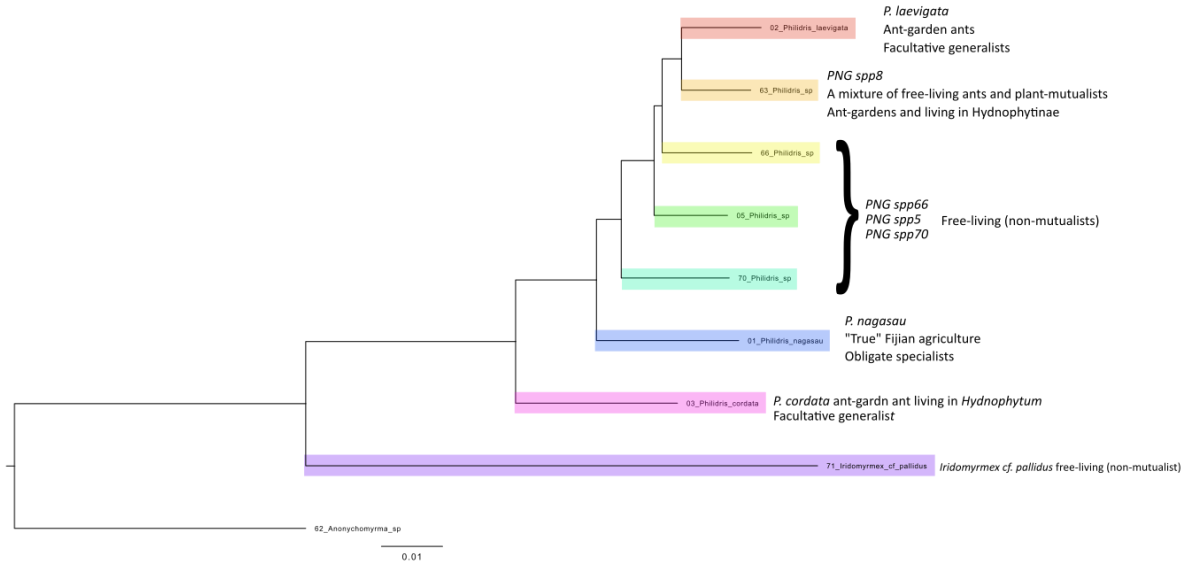


Figure 5.1 Maximum likelihood phylogeny calculated from a concatenated super matrix of 3,736 BUSCO genes²³. This dataset represented a subset of the full *Philidris* genome dataset used in the rest of this thesis, pruned so that each species is represented by only one genome.

OrthoFinder version 2.5.5 (Emms and Kelly 2019) was used to cluster coding sequences (CD) into orthogroups. OrthoFinder identified 2377 single copy orthologues. Each single copy orthologue was aligned using MAFFT version 7.505 (Katoh and Standley 2013) and gene trees were inferred using IQTree version 2.2.3 (Minh et al. 2020). Codon aware alignments were calculated using PRANK version 170427 (Löytynoja 2014), and using the gene trees as guide trees. The alignment was successful for 1985 of the single copy orthologues, which were used to detect genome-wide signatures of selection.

2.2 Rates of molecular evolution

Nucleotide substitution rates were calculated using *baseML*, part of the PAML package (version 4.10.1) (Yang 1997, 2007). Using the codon alignments and gene trees as inputs. The parameters *model* = 7, *Mgene* = 0, *clock* = 0, *fix_alpha* = 0, *Malpha* = 0, *ncatG* = 10, and *cleandata* = 0 were used, as described by Ametrano et al (2022). Substitution rates were extracted per species, per gene, and then an average of the background sister species of *P. nagasau* (*P. laevigata*, *P. sp. 8*, *P. sp. 66*, *P. sp. 5* and *P. sp. 70*) were used as a comparison to *P. nagasau*, mirroring the sister comparisons seen in other work on genome evolution in mutualisms (Rubin and Moreau 2016, Ametrano et al. 2022).

²³ The phylogenies in this thesis are based on the number of BUSCO genes present and complete in all genomes included in the tree. As expected, more complete BUSCO genes were identified in the subset tree (9 genomes, 3,736 BUSCO genes) compared to the full tree (40 genomes, 2,100 genes).

2.3 Signatures of selection analysis

2.3.1 Tests for directional selection

codeML, part of the PAML package (version 4.10.1) (Yang 1997, 2007) was used to perform a branch test, comparing dN/dS values (ω) between the *Philidris nagasau* and background branches as described in Álvarez-Carretero et al. (2023). For the null model the following parameters were used: *model* = 0, *Mgene* = 0, *fix_alpha* = 1, *Malpha* = 0, *ncatG* = 8, and *cleandata* = 1 and for the branch model *model* = 2. By specifying *model* = 2 we are specifying that the foreground branches may evolve under a different ω value than the background branches (Álvarez-Carretero et al. 2023).

When conducting a branch test with *codeML* the result is the average dN/dS over all sites in a gene, this can sometimes mask sites within a gene that are under positive selection, because the rest of the sites are highly conserved and under strong purifying selection. A branch-site test allows for heterogeneity in omega not only on different branches of the tree, but also between sites on the gene. *codeML* (part of PAML version 4.10.1) was used to conduct a branch-site test as described in Álvarez-Carretero et al. (2023).

2.3.2 Intensity of selection

To investigate potential shifts in selection intensity, I used the software RELAX (part of the HyPhy package, version 2.5.6) (Kosakovsky Pond et al. 2020). The RELAX model compares the ratio of non-synonymous to synonymous substitution rates (dN/dS, ω) in three site classes representing purifying ($\omega_0 < 1$), purifying-to-neutral ($\omega_1 \leq 1$) and positively selected ($\omega_2 > 1$) sites between foreground and reference branch partitions. RELAX relies on the differential effects relaxed natural selection has on omega values below one, wherein relaxed selection causes omega to increase towards one whereas omega values above one, indicating positive selection, relaxed selection causes omega to decrease (Wertheim et al. 2015). The RELAX model introduces an additional parameter, *k* (the selection intensity parameter), if the presence of *k* leads to a statistically significant improvement of in the model fit, then we can conclude that selection on the test branch has been intensified if *k* is above 1 or relaxed if *k* is below 1 (Wertheim et al. 2015).

2.4 GO annotation and enrichment

All significant orthogroups from the branch test, branch site test and RELAX test were annotated with InterProScan version 5.47-82.0 (Jones et al. 2014) using the option *-goterms*. GO terms were extracted and summarised for each test (i.e. genes with $\omega > 1$ for *P. nagasau*, ii. genes with $\omega < 1$ but the ω of *P. nagasau* is higher than the background and iii. genes with $\omega < 1$ and is decreasing on the *P. nagasau* branch.), for the branch test, branch

site test and the RELAX test (relaxed genes and intensified genes) using the R package *rvvgo* (Sayols 2023) using *Drosophila melanogaster* as the comparison set.

2.5 Statistical analysis

To compare overall rates of molecular evolution a Wilcoxon-Ranked test was performed in R version 4.3.1 (R Core Team 2023) to compare nucleotide substitution rates between *P. nagasau* and a sister background clade (*P. laevigata*, *P. sp. 8*, *P. sp. 66*, *P. sp. 5* and *P. sp. 70*). Omega values over 10 were filtered out to exclude potentially saturated markers in line with other studies (Rubin and Moreau 2016, Ametrano et al. 2022). Additionally, to test for genome-wide genome relaxation a Wilcoxon-Ranked test was performed to compare the ω_2 produced by RELAX between the *nagasau* and background as well as combining ω_1 and ω_0 as described by Schrader et al. (2021). In line with Schrader et al. (2021) we did not include genes with an omega over 10 in these genome-wide comparisons.

3. Results

3.1 Rates of molecular evolution

Philidris nagasau had higher average rates of nucleotide substitution compared to the sister background species (0.0046 and 0.0019 respectively). A paired Wilcoxon signed-rank test was performed and was statistically significant ($Z = -34.318$, $p < 2.2e-16$).

3.2 Branch test results

Of the 1,985 gene tested we found 133 wherein the codeML branch test had a better fit than the null test, indicating that natural selection was acting differently on the *P. nagasau* branch compared to the rest of the phylogeny for these genes (the background branches), see Table S5.1.

Some 53 genes had an omega of >1 on the *P. nagasau* branch, indicating positive selection (see Figure 5.2 A). Enriched GO terms include the sensory perception of smell, olfactory receptor activity, nervous system development, axon guidance (see Figures 5.3-5 for TreeMaps of the biological, cellular and molecular GO terms enriched in these genes).

Some 80 genes had an omega of <1 indicating purifying selection. Of these 80 genes, 37 had a greater omega value of the *P. nagasau* branch compared to the background branches indicating that there could either be positive selection acting on these genes, or relaxed purifying selection. GO Terms enriched for these genes include RNA and mRNA processing, translation, protein phosphorylation, ATP hydrolysis, protein kinase activity and magnesium ion binding, see Figures 5.6-8 for TreeMaps of the biological, cellular and molecular GO terms enriched in these genes. The remaining 43 genes had a lower omega value on the *P.*

nagasau branch, indicating that purifying selection has intensified on these genes. GO terms enriched for these genes included several genes related to metal ion binding (zinc, magnesium, and calcium) as well as ATP, DNA, rRNA and nucleic acid binding. See Figures 5.9-11 for TreeMaps of the biological, cellular and molecular GO terms enriched in these genes.

When considering the whole dataset, regardless of significance of the model fit, 106 had an omega value of 999 and 581 had an omega value of 0.0001 (either, in the background branches or the *P. nagasau* foreground branch), these values are the maximum and minimum possible values produced by codeML, a value of 999 is caused by a lack of synonymous variation and is essentially infinity (Álvarez-Carretero et al. 2023), therefore we do not include them when comparing average values. However, we do consider these genes when looking at shifts, as the LRT value is still valid and they are still informative of a trend if not the precise value (Álvarez-Carretero et al. 2023). The average omega value of the *P. nagasau* branch was higher (2.18) compared to the background branches (0.26), see Figure 5.2 (A). Most of the genes (1,170) of the genes had an omega value below 1 for both the *P. nagasau* branch and the background branches, 709 of these genes had a higher (but still below 1) omega on the *P. nagasau* branch compared to the background, see Figure 5.2 (B). There are two possible explanations of this trend, the first is that these genes are experience relaxed purifying selection, symptomatic of large demographic change, specifically a reduced effective population size or a removal of a functional constraint in the form of a new ecological niche (Lahti et al. 2009, Hunt et al. 2011, Wertheim et al. 2015), alternatively, it may be the increase in omega is due to positive selection. To investigate this further we conducted a branch-site test within PAML codeML, which has a higher power to detected positive selection than a standard branch test (Álvarez-Carretero et al. 2023) and tested for selection intensity using RELAX, part of the HyPhy package (Wertheim et al. 2015).

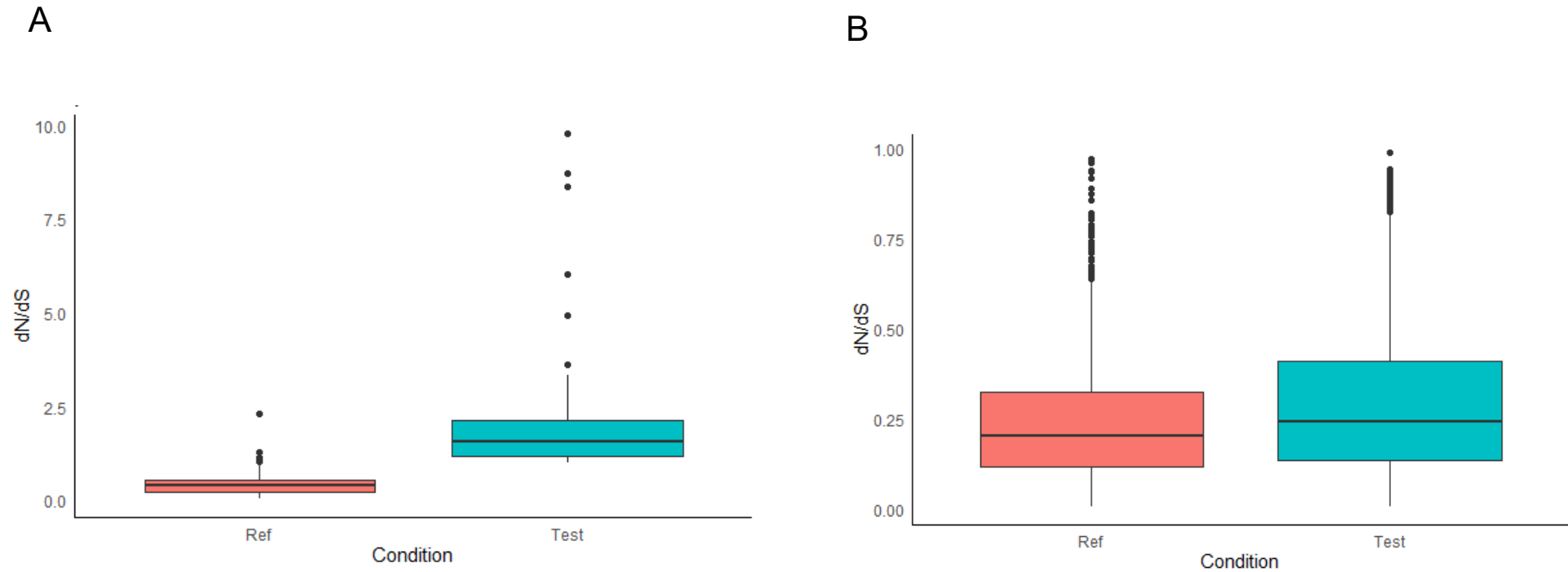


Figure 5.2 Distribution of evolutionary rates for regions in 1359 single-copy orthologs across all species (filtered for $dN/dS < 10$ and $dN/dS = 0.0001$) **(A)** under positive selection ($dN/dS < 1$) (Paired Wilcoxon test $Z = 8.48$ $p < 1e-04$) and **(B)** purifying selection ($dN/dS \geq 1$, (Paired Wilcoxon test $Z = 6.77$ $p < 1e-04$) calculated using a branch-test, using codeML part of PAML, this data includes both significant and non-significant model fits. Boxplot centres show the median, hinges show the first and third quantiles, and whiskers show the $1.5 \times$ inter-quantile range. The red box represents the “reference” background branches and blue represents the “test” *Pholidris nagasau* branch. Evolutionary rates have increased in both genes under positive selection and genes under purifying selection in *P. nagasau*.

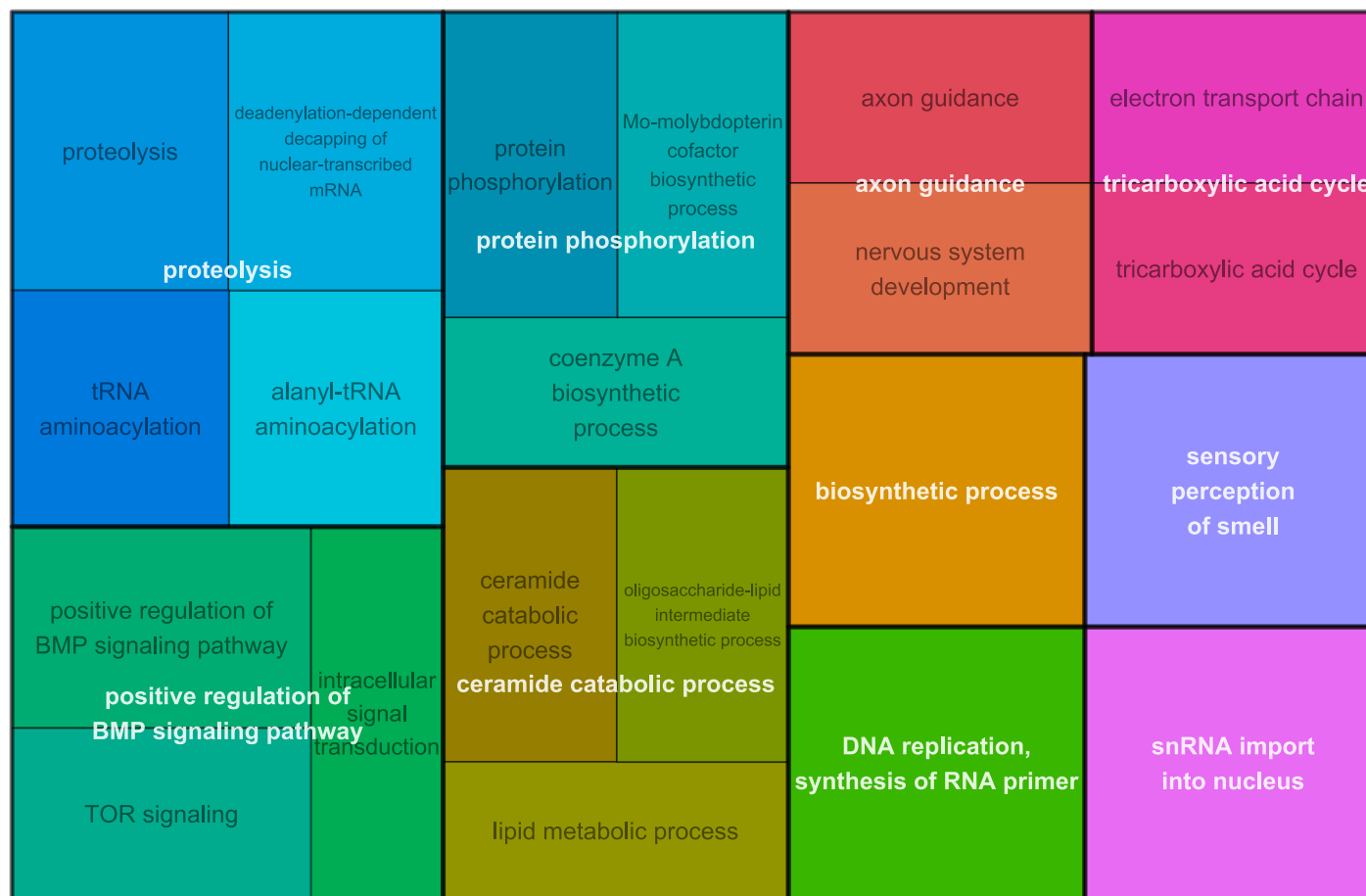


Figure 5.3 Treemap summarising biological process GO terms of genes under positive selection ($\omega > 1$) in *Philidris nagasau* following a branch test performed with codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

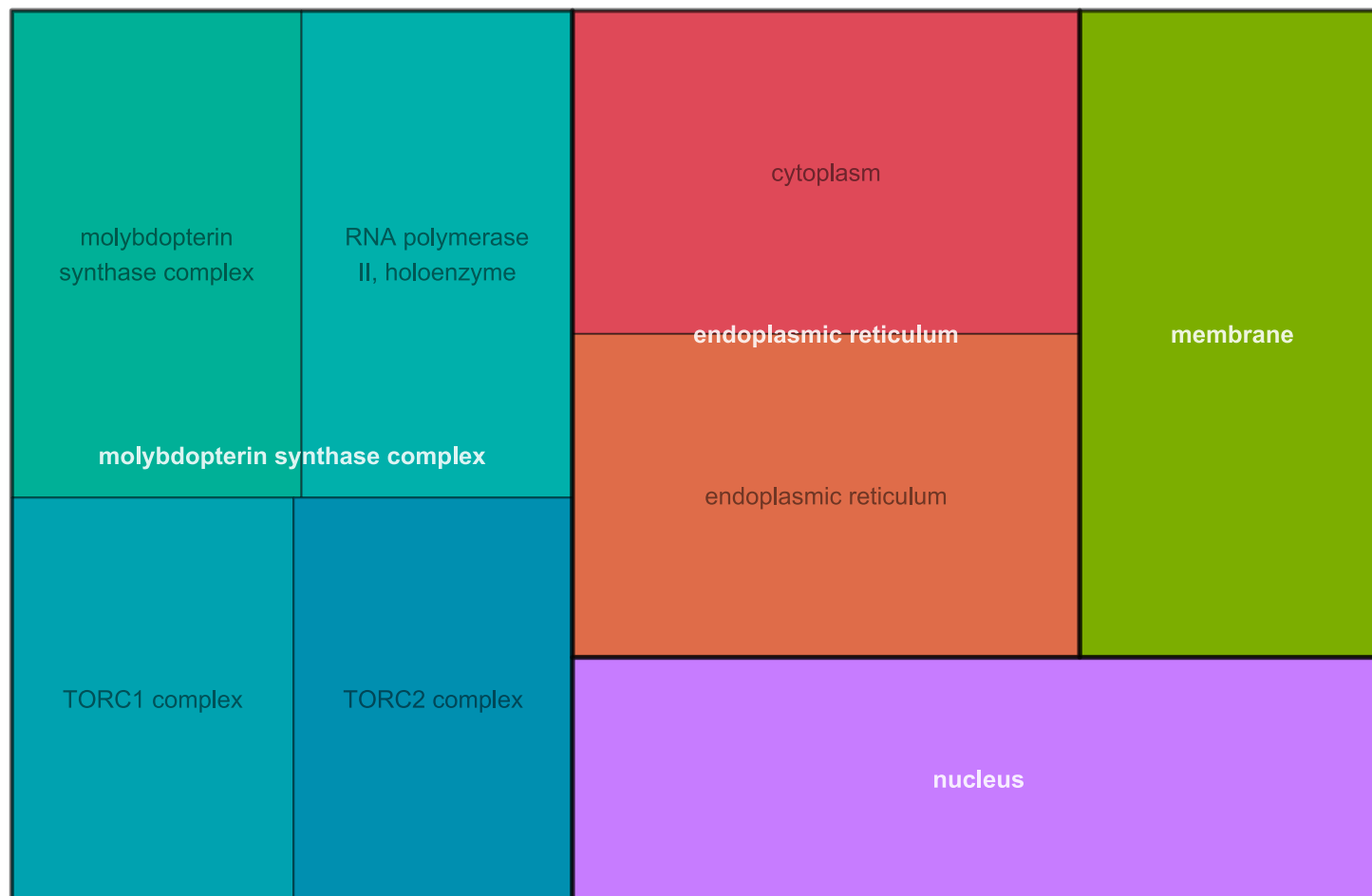


Figure 5.4 TreeMap summarising cellular component GO terms of genes under positive selection ($\omega > 1$) in *Philidris nagasau* following a branch test performed with codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.



Figure 5.5 TreeMap summarising molecular process GO terms of genes under positive selection ($\omega > 1$) in *Philidris nagasau* following a branch test performed with codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

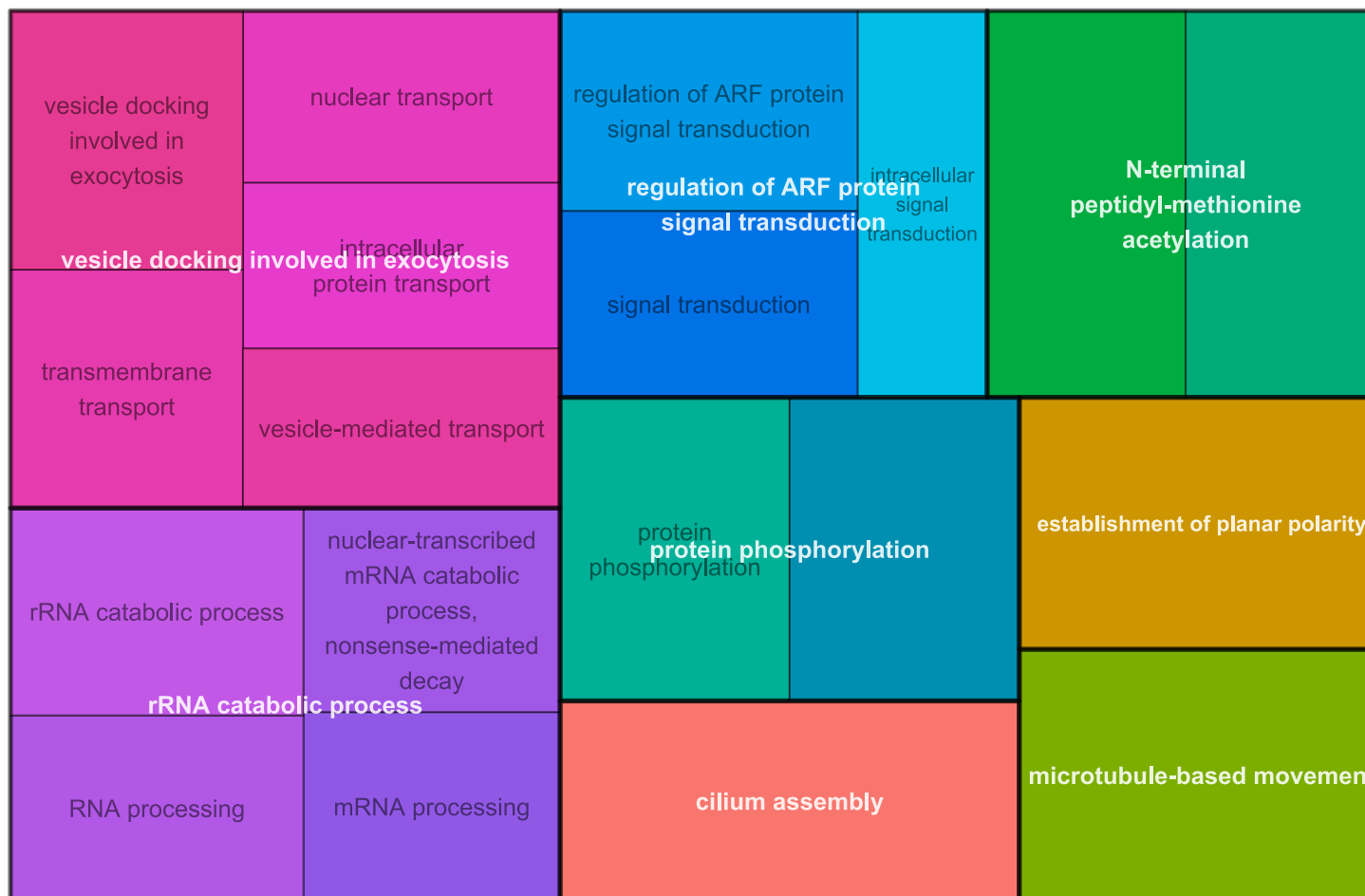


Figure 5.6 TreeMap summarising biological function GO terms of genes under purifying $\omega < 1$ in both the background branches and *Pholidris nagasau*, but the ω is greater in *P. nagasau* following a branch test performed with codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

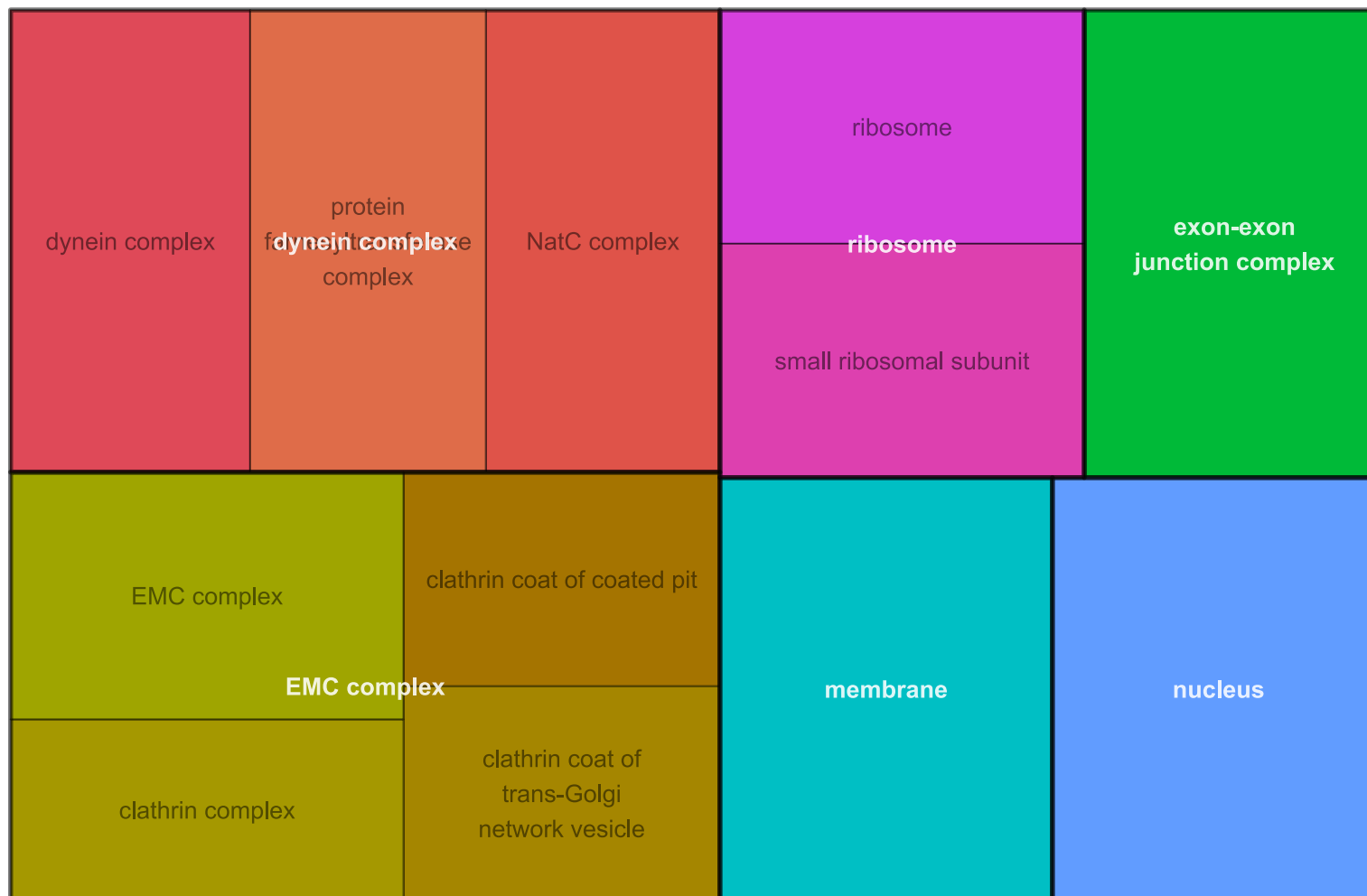


Figure 5.7 TreeMap summarising cellular component GO terms of genes under purifying $\omega < 1$ in both the background branches and *Philidris nagasau*, but the ω is greater in *P. nagasau* following a branch test performed with codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

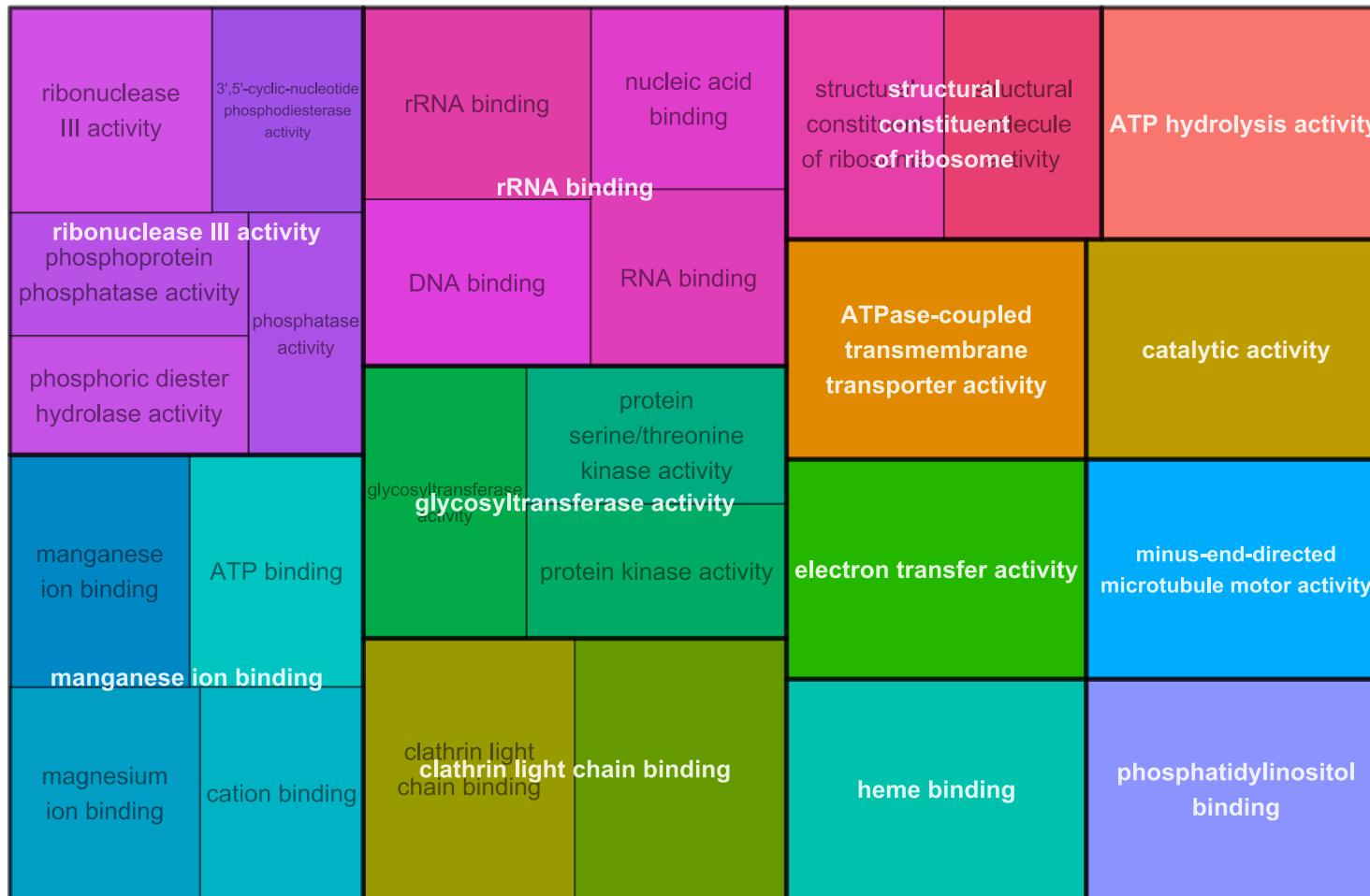


Figure 5.8 TreeMap summarising molecular function GO terms of genes under purifying $\omega > 1$ in both the background branches and *Philidris nagasau*, but the ω is greater in *P. nagasau* following a branch test performed with codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.



Figure 5.9 TreeMap summarising biological function GO terms of genes under purifying $\omega < 1$ in both the background branches and *Phildris nagasau*, but the ω is decreasing in *P. nagasau*. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

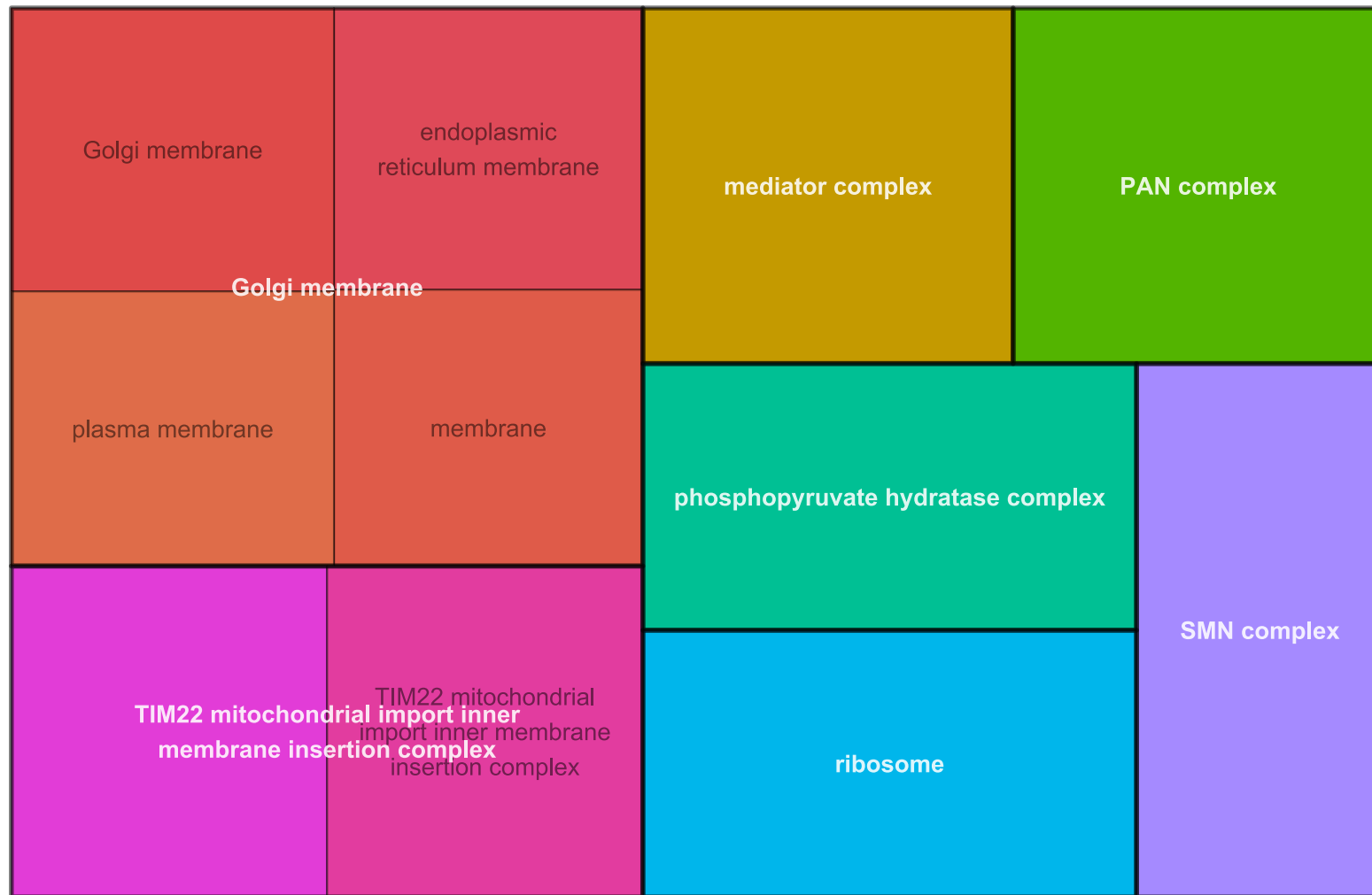


Figure 5.10 TreeMap summarising cellular GO terms of genes under purifying $\omega < 1$ in both the background branches and *Philidris nagasau*, but the ω is decreasing in *P. nagasau*. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.



Figure 5.11 TreeMap summarising molecular process GO terms of genes under purifying $\omega < 1$ in both the background branches and *Pholidris nagasau*, but the ω is decreasing in *P. nagasau*. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

3.3 Branch-site results

Of the 1,985 genes tested, there were 38 genes wherein the branch-site model had the best fit, see Table S5.2. Enriched GO terms for these 38 genes include nervous system development, axon guidance, DNA repair, DNA replication and DNA metabolic processes. See Figures 5.12-14 for TreeMaps of the biological, cellular and molecular GO terms enriched in these genes.

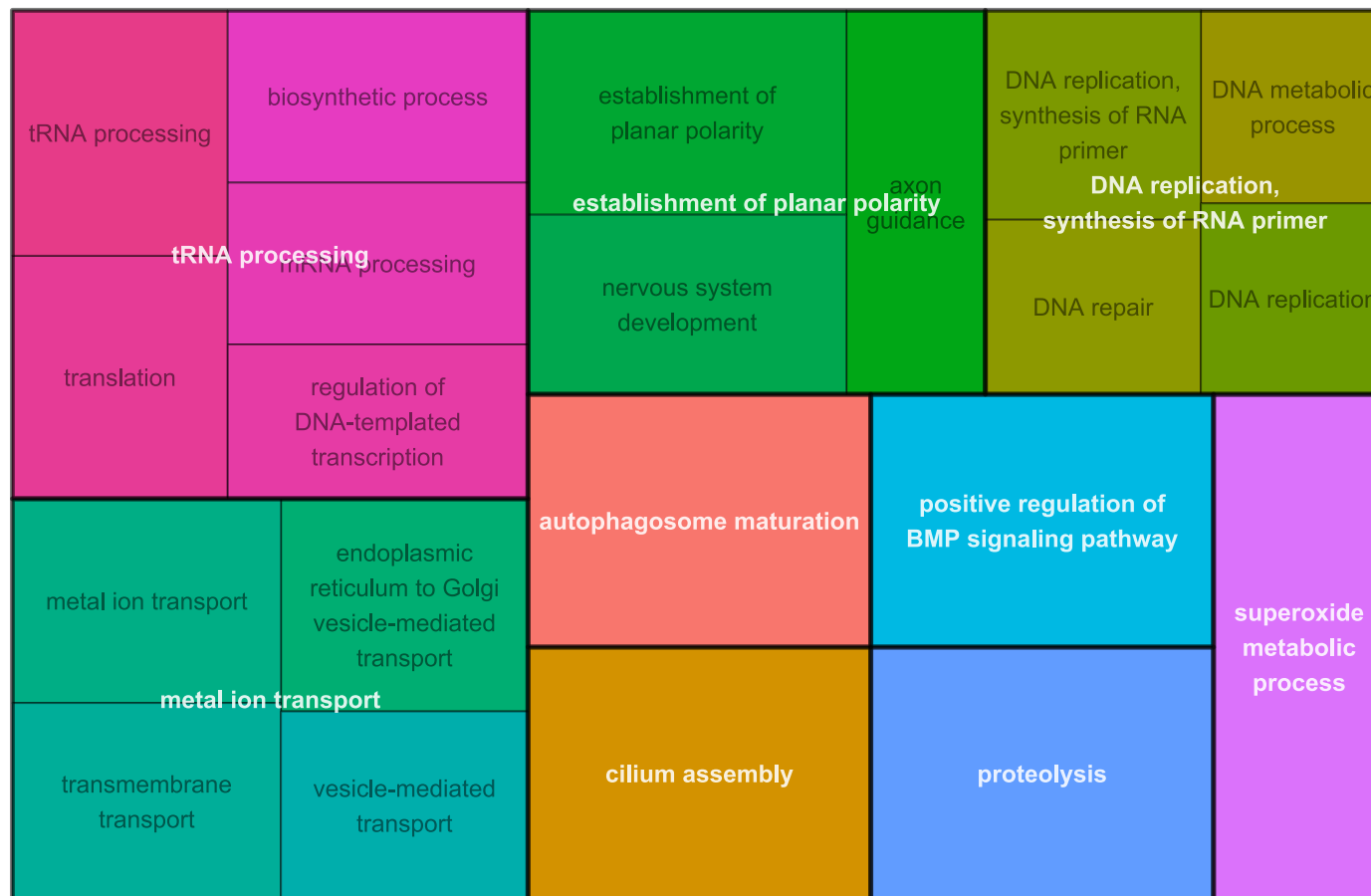


Figure 5.12 TreeMap summarising biological function GO terms of genes with significant evidence for positive selection on the *Philidris nagasau* branch, following a branch-site test performed using codeML, part of PAML. GO terms were summarised using the R package rvvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

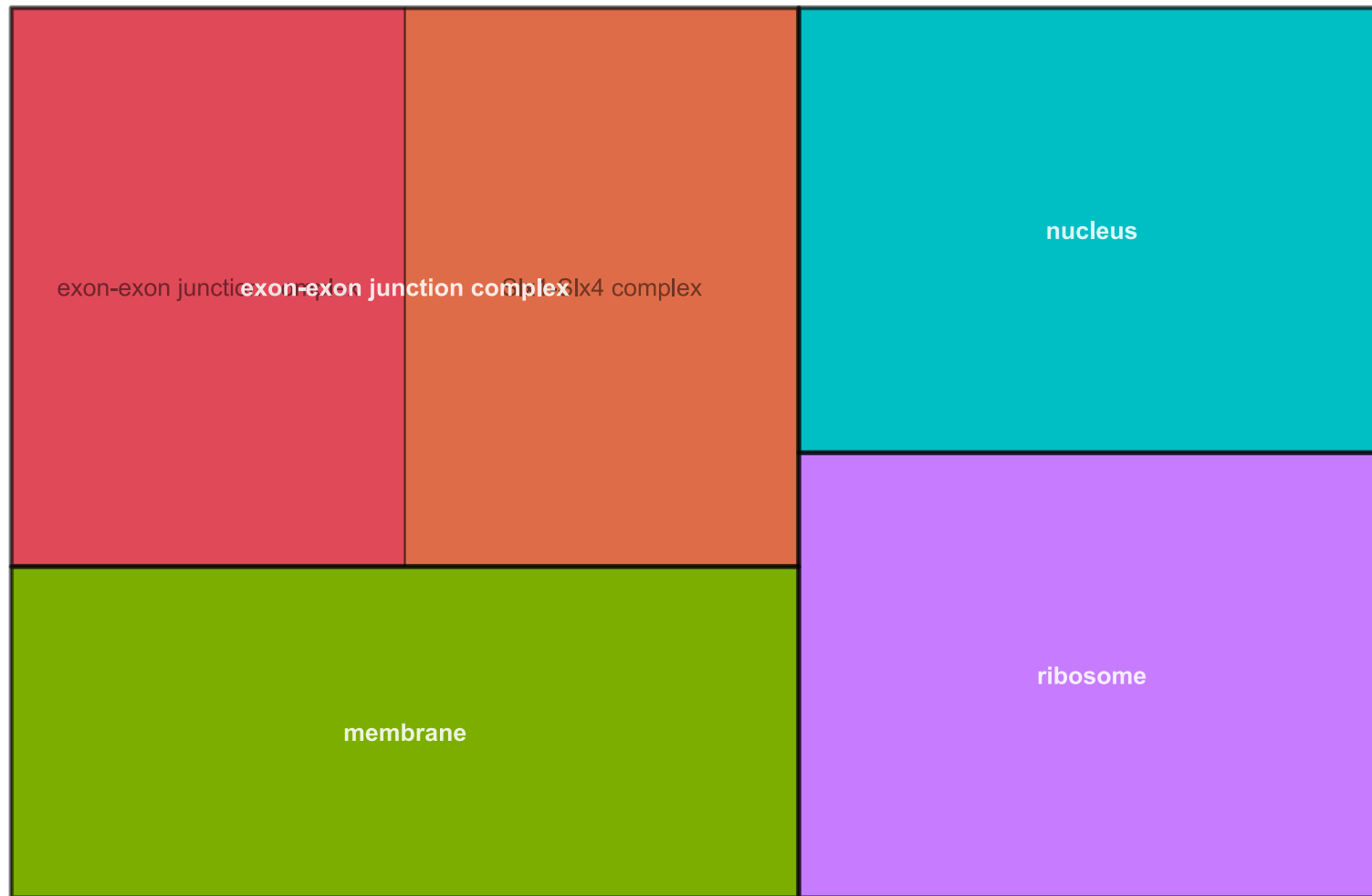


Figure 5.13 TreeMap summarising cellular component GO terms of genes with significant evidence for positive selection on the *Philidris nagasau* branch, following a branch-site test performed using codeML, part of PAML. GO terms were summarised using the R package rrvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

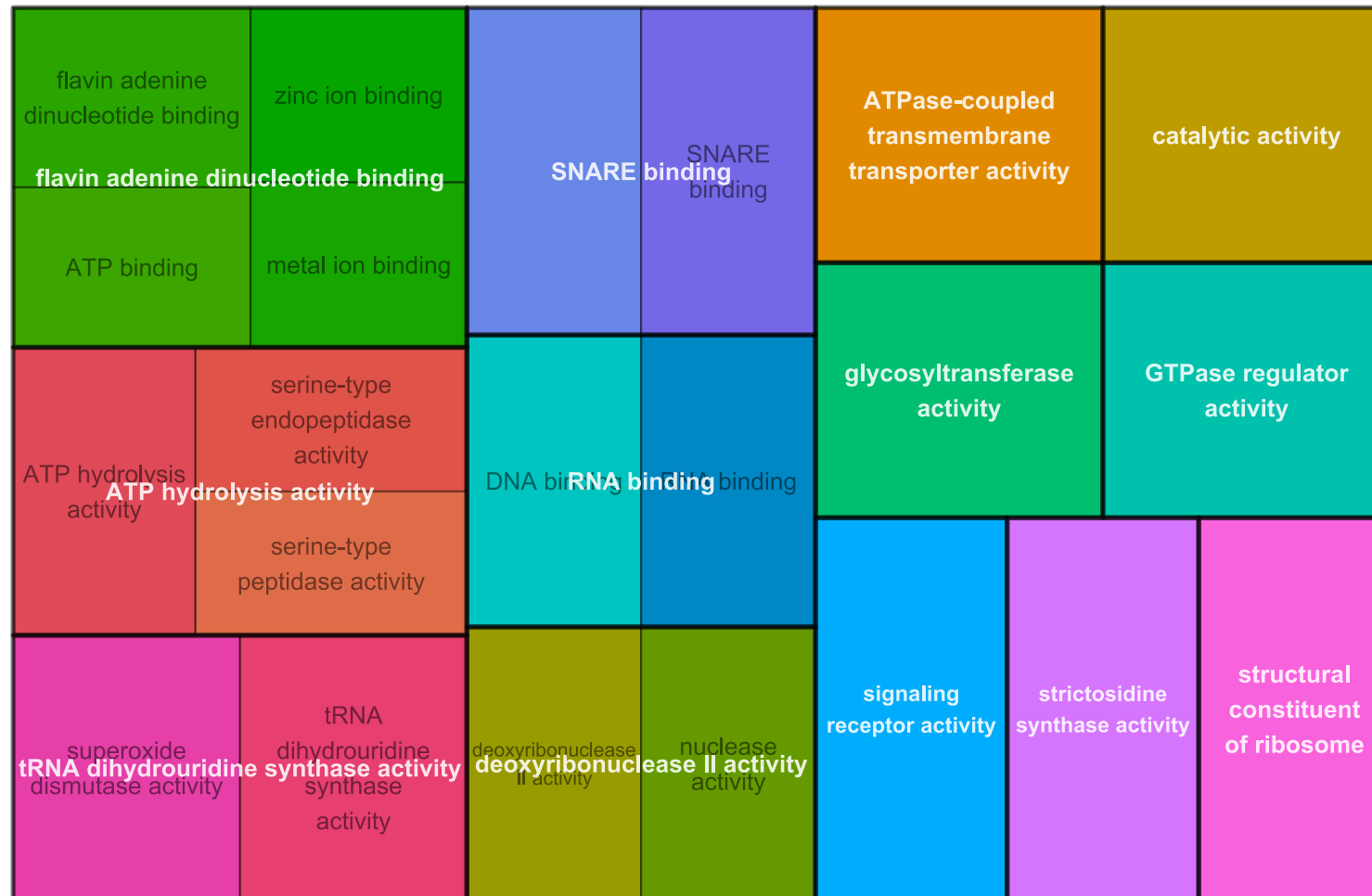


Figure 5.4 TreeMap summarising molecular function GO terms of genes with significant evidence for positive selection on the *Philidris nagasau* branch, following a branch-site test performed using codeML, part of PAML. GO terms were summarised using the R package rvvgo. The size of each box is proportional to the frequency of each GO term in the dataset.

3.4 Intensity of selection

RELAX, part of the Hyphy package, was used to investigate whether natural selection had either intensified or relaxed. Most of the genes had the best model fit to a selection of parameter of 1, indicating that there is no significant evidence of shifts in selection intensity (Figure 5.15), the next largest peak was at 0 indicating relaxed selection followed by several small positive peaks, indicating intensified selection. Of the 1985 genes tested 45 showed significant evidence for relaxed selection and 60 showed significant evidence for intensified selection, see Table S5.3 and Figures 5.16 and 5.20. Of the 60 genes that experienced intensified selection, the GO Terms metal ion transport, RNA methylation, DNA metabolic process and signalling receptor binding were enriched, see Figures 5.17-19 for TreeMaps of the biological, cellular and molecular GO terms enriched in these genes. Of the 45 genes that showed signs of relaxed selection the GO terms sensory perception of smell, DNA replication, RNA processing, ATP binding and protein binding were enriched) see Figures 5.21-23 for TreeMaps of the biological, cellular and molecular GO terms enriched in these genes. A test for genome-wide relaxation was significant ($Z = 4.9271$ $p = 8.35E-07$), see Table 5.1 for full results.

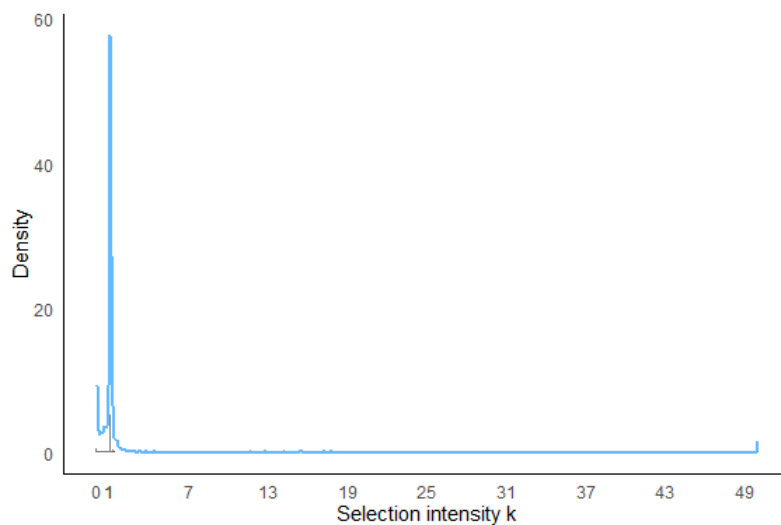


Figure 5.15 Distribution of selection intensity parameter (k) of single copy orthologues as calculated by RELAX. Most of the single copy orthologues has a selection parameter of 1, indicating that there was no significant shift in selection intensity. The next highest peak is at 0, indicating that a substantial proportion of genes were under relaxed selection.

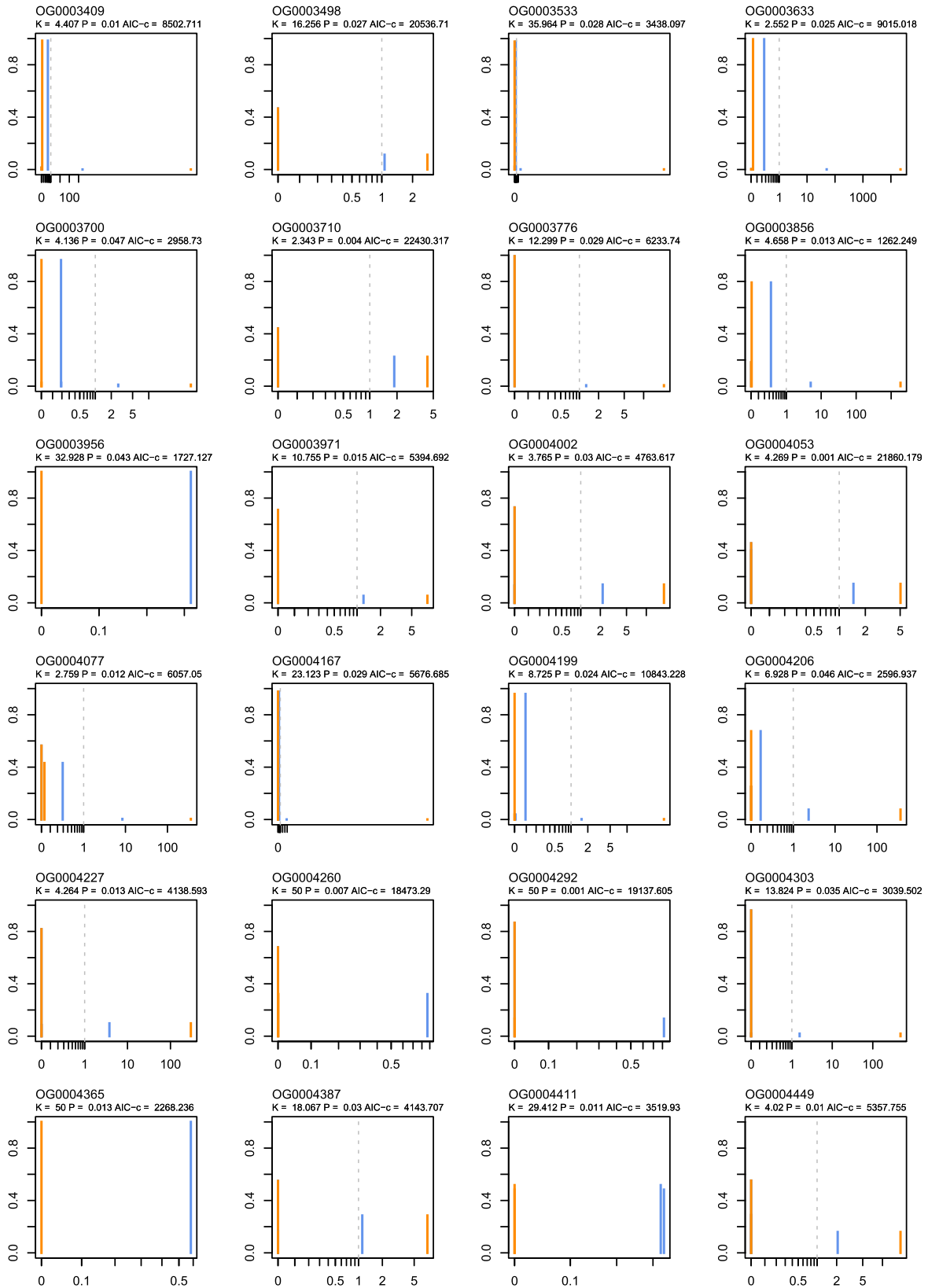


Figure 5.16 Single copy orthologue orthogroups with significant evidence for intensified selection in *Philidris nagasau*. Figure continues below.

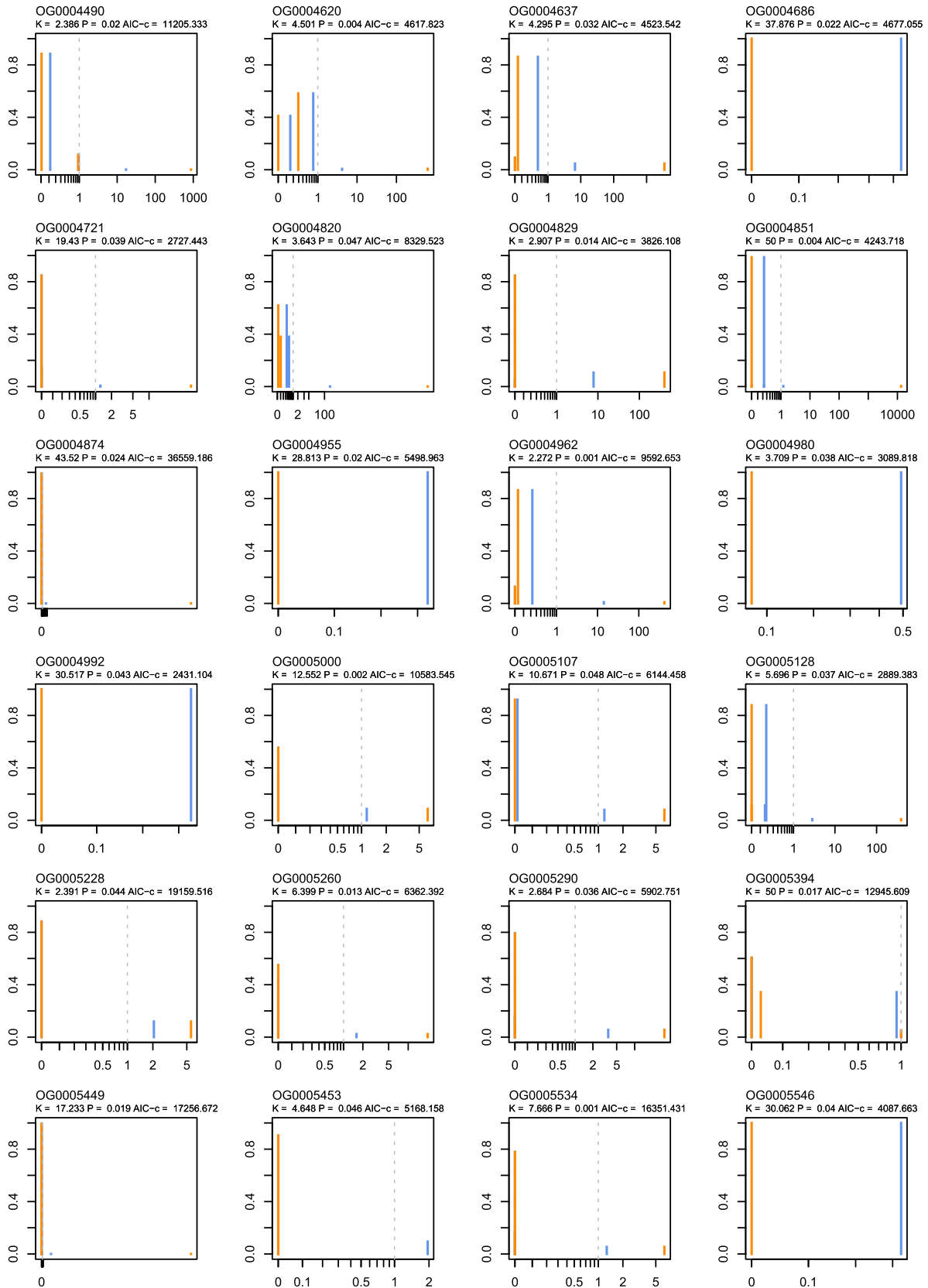


Figure 5.16 (continued) Single copy orthologue orthogroups with significant evidence for intensified selection in *Philidris nagasau*. Figure continues below.

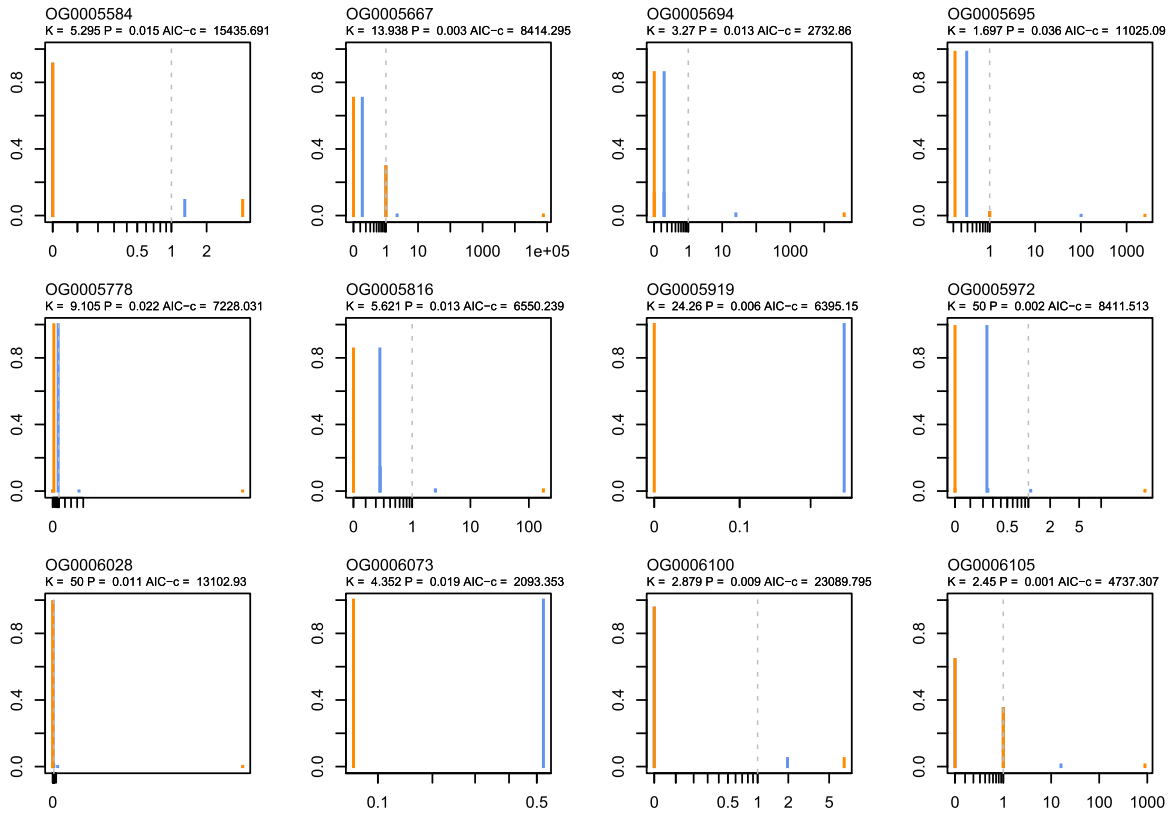


Figure 5.16 Single copy orthologue orthogroups with significant evidence for intensified selection in *Philidris nagasau*. Plots of model fits from the RELAX alternative model (Wertheim et al. 2015). Each pair of bars represents the site proportion assigned to that site class for reference (orange) and test (blue) branch partitions, while the x-axis position gives the inferred dN/dS (omega) of that site partition. The grey dotted line represents a neutral omega value (1). Note that site proportions are constrained to be equal between reference and test groups. In each case, K gives the inferred intensification/relaxation parameter, which corresponds to relaxed selection if $K < 1$ and intensified selection if $K > 1$. The P-value of the likelihood ratio test on one degree of freedom of the RELAX alternative against the RELAX null which constrains $K = 1$ is shown. AIC-c gives the small sampled-corrected Akaike Information Criterion for the RELAX alternative model.



Figure 5.17 TreeMap summarising biological process GO terms of genes with significant evidence for intensified selection (selection parameter $k > 1$), following a RELAX test, performed using HyPhy. GO terms were summarised using the R package rvgo. The size of each box is proportion to frequency of each GO term in the dataset.

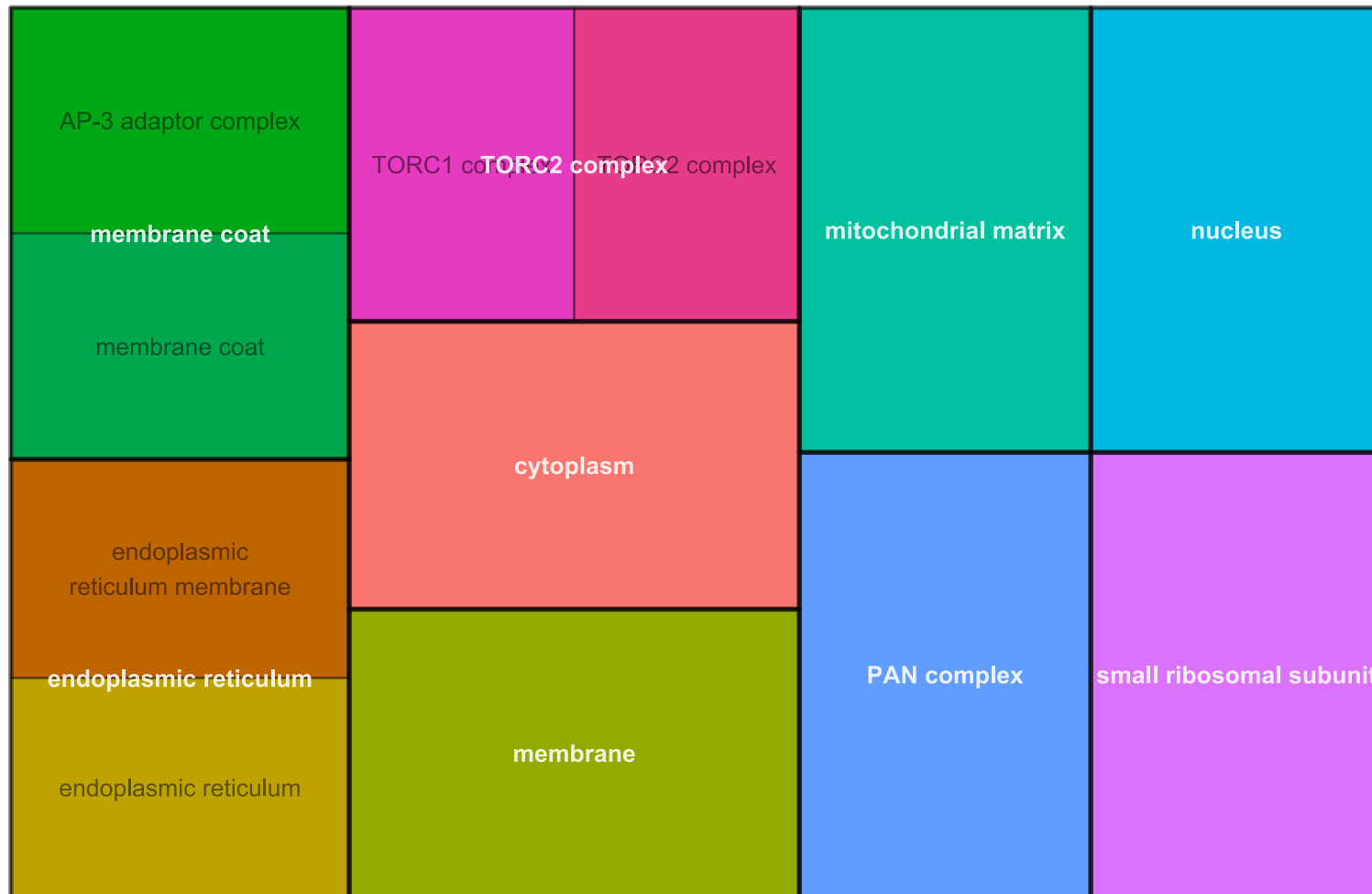


Figure 5.18 TreeMap summarising cellular component GO terms of genes with significant evidence for intensified selection (selection parameter $k > 1$), following a RELAX test, performed using HyPhy. GO terms were summarised using the R package rvggo. The size of each box is proportion to frequency of each GO term in the dataset.

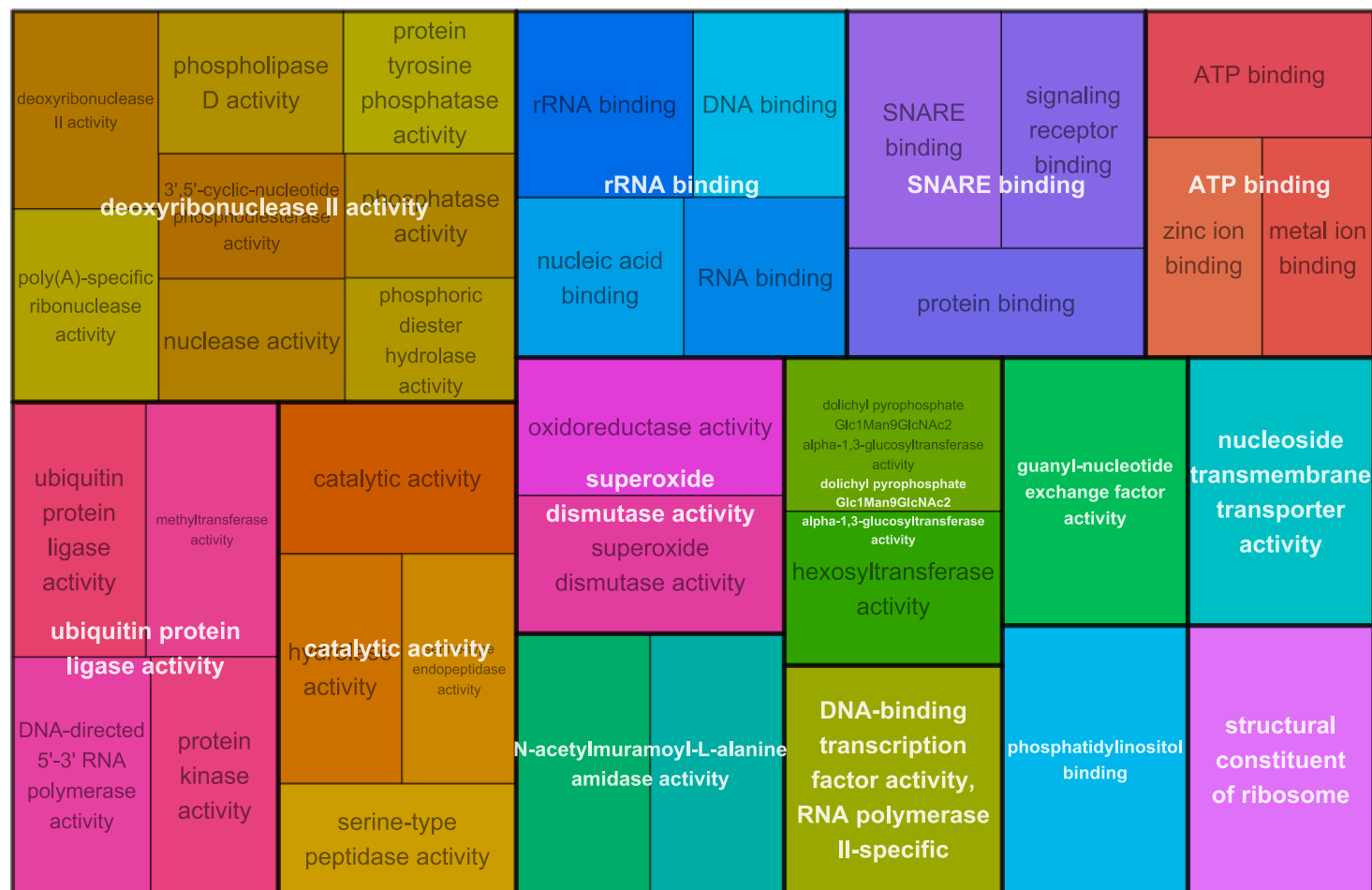


Figure 5.19 TreeMap summarising molecular process GO terms of genes with significant evidence for intensified selection (selection parameter $k > 1$), following a RELAX test, performed using HyPhy. GO terms were summarised using the R package rvvgo. The size of each box is proportion to frequency of each GO term in the dataset.

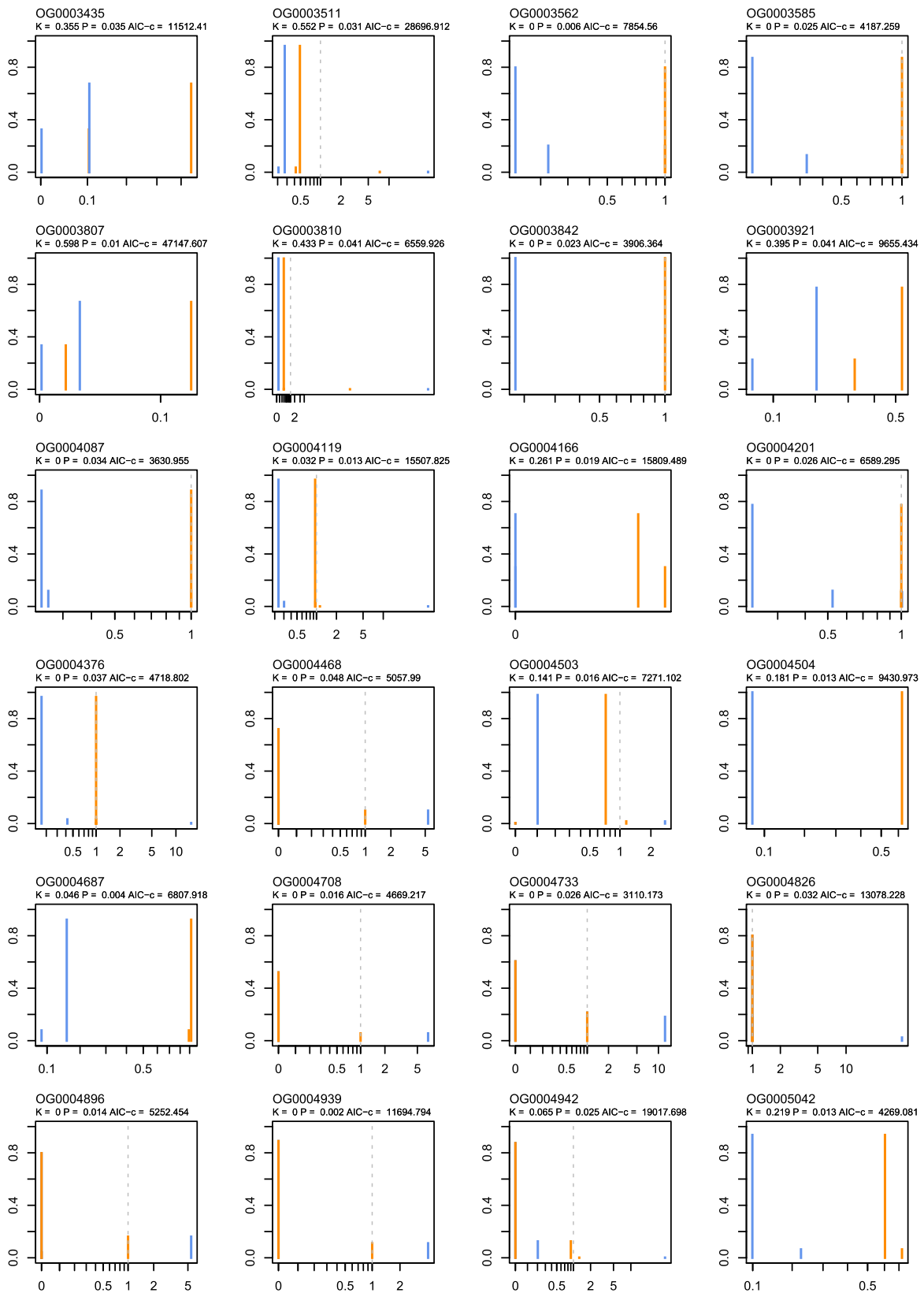


Figure 5.20 Single copy orthologue orthogroups with significant evidence for relaxed selection. Figure continues below.

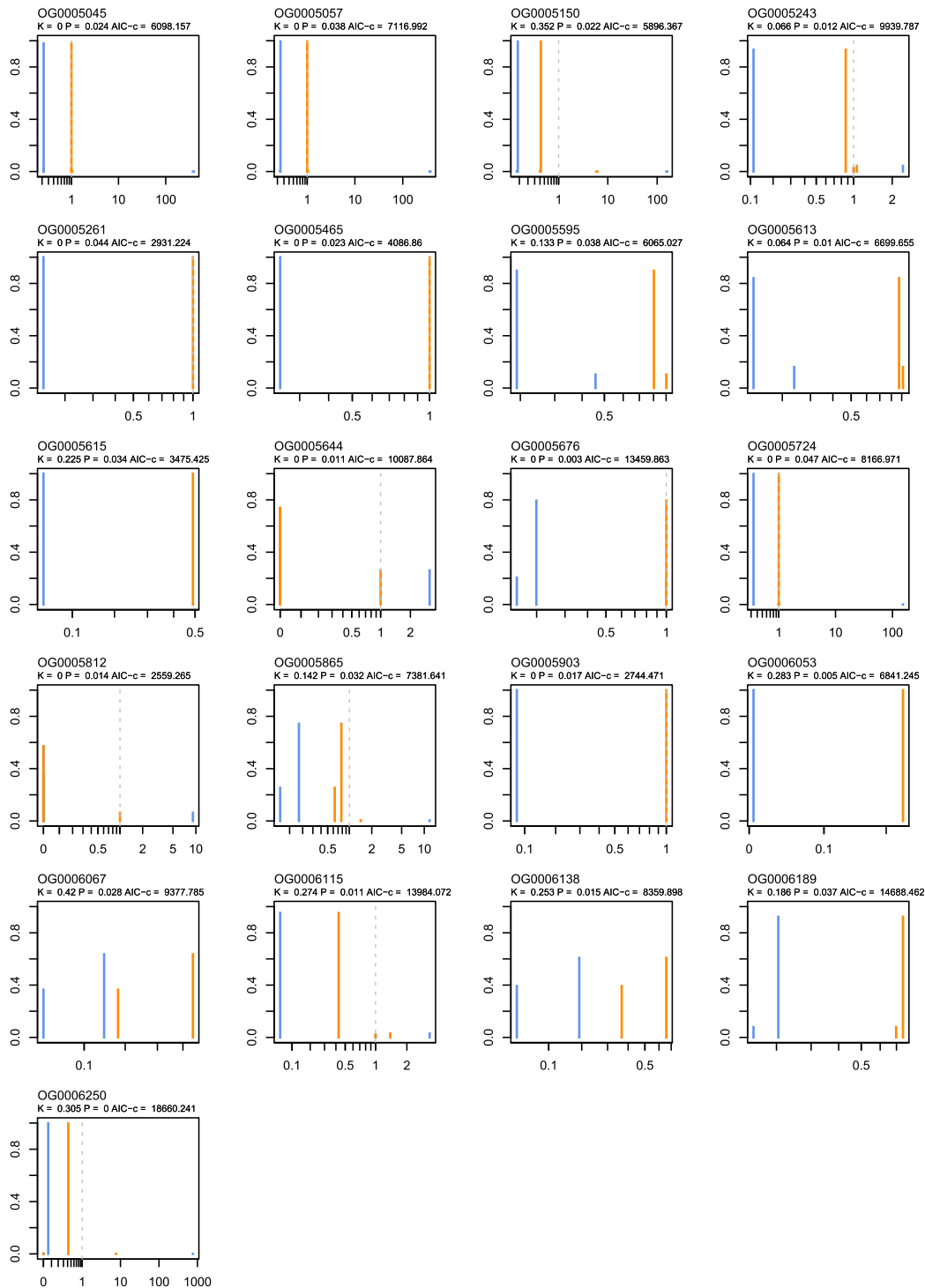


Figure 5.20 Single copy orthologue orthogroups with significant evidence for relaxed selection. Plots of model fits from the RELAX alternative model (Wertheim et al. 2015). Each pair of bars represents the site proportion assigned to that site class for reference (orange) and test (blue) branch partitions, while the x-axis position gives the inferred dN/dS (omega) of that site partition. The grey dotted line represents a neutral omega value (1). Note that site proportions are constrained to be equal between reference and test groups. In each case, K gives the inferred intensification/relaxation parameter, which corresponds to relaxed selection if $K < 1$ and intensified selection if $K > 1$. The P-value of the likelihood ratio test on one degree of freedom of the RELAX alternative against the RELAX null which constrains $K = 1$ is shown. AIC-c gives the small sampled-corrected Akaike Information Criterion for the RELAX alternative model.

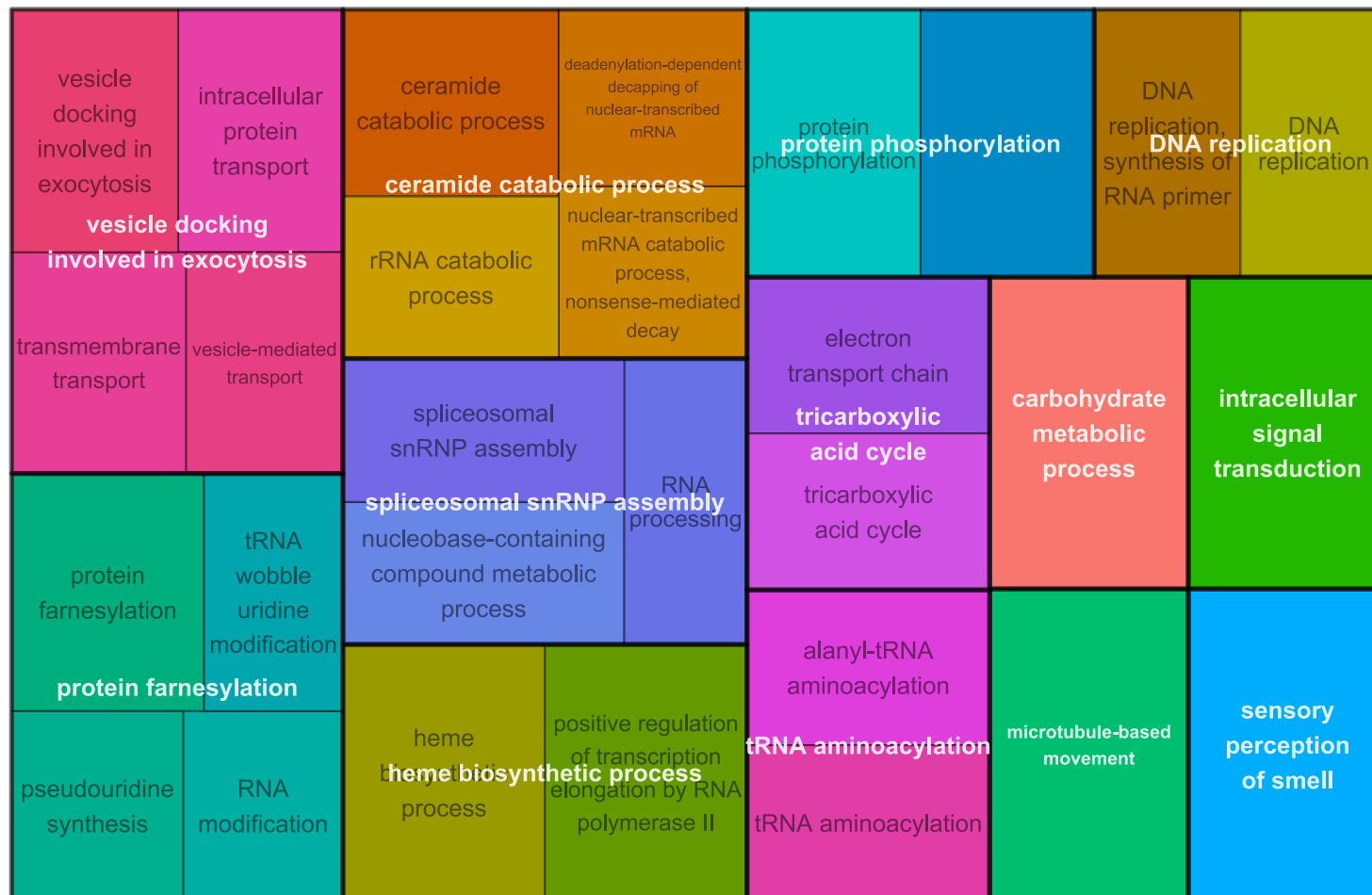


Figure 5.21 Treemap summarising biological function GO terms of genes with significant evidence for relaxed selection (selection parameter $k < 1$), following a RELAX test, performed using HyPhy. GO terms were summarised using the R package rrvgo. The size of each box is proportion to frequency of each GO term in the dataset.

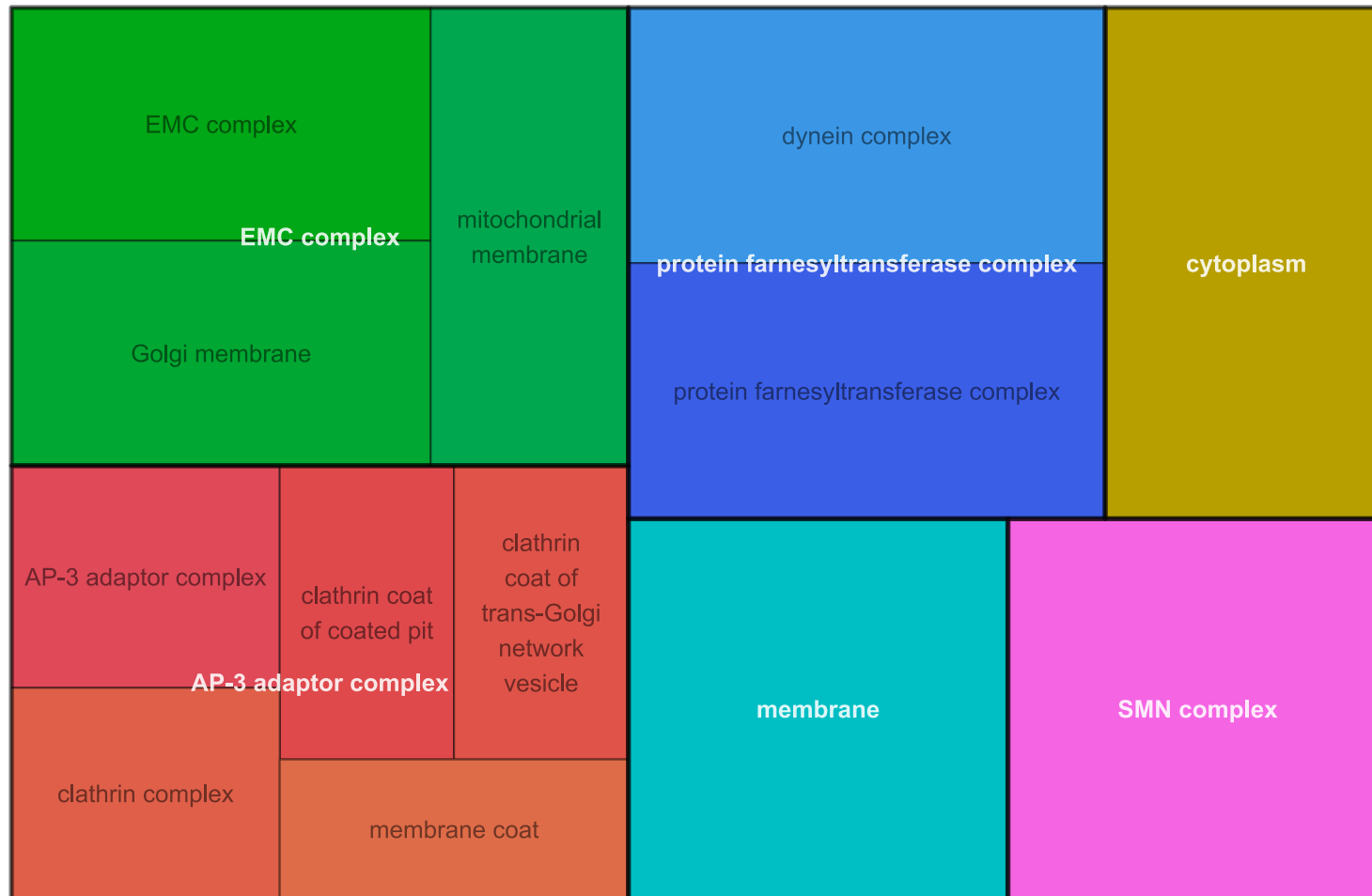


Figure 5.22 TreeMap summarising cellular component GO terms of genes with significant evidence for relaxed selection (selection parameter $k < 1$), following a RELAX test, performed using HyPhy. GO terms were summarised using the R package rvvgo. The size of each box is proportion to frequency of each GO term in the dataset.



Figure 5.23 Treemap summarising molecular process GO terms of genes with significant evidence for relaxed selection (selection parameter $k < 1$), following a RELAX test, performed using HyPhy. GO terms were summarised using the R package rvgo. The size of each box is proportion to frequency of each GO term in the dataset.

Table 5.1 Results of Wilcox test comparing the *Philidris nagasau* branch omega value to the background branches produced by HyPhy RELAX. ω_2 was tested with values filtered to no omega values higher than 10 as in Schrader et al (2021). ω_1 and ω_0 were combined to produce the background omega.

	Z	p
ω_2	4.9271	8.35E-07
background ω	-6.4442	1.16E-10

4. Discussion

Overall, there were significantly higher rates of molecular evolution, as measured by nucleotide substitution rate for the *Philidris nagasau* branch compared to the background branches (0.0046 and 0.0019 respectively), suggesting that the evolution of obligate mutualism follows Red Queen rather than Red King dynamics. This result mirrors work comparing mutualistic and non-mutualist *Pseudomyrmex* (Rubin and Moreau 2016) as well as lichens (Lutzoni and Pagel 1997)²⁴ and many bacterial endosymbionts (Moran 1996, McCutcheon and Moran 2012, McCutcheon et al. 2024) and even obligate parasites such as parasitic plants (Bromham et al. 2013) and lice (Yoshizawa and Johnson 2003). This suggests that Red Queen dynamics may not necessarily a result of mutualistic or antagonistic evolution, and perhaps rather a consequence of symbiosis.

The ratio of non-synonymous to synonymous substitutions (dN/dS) was consistently higher on the *P. nagasau* branch compared to the background (2.18 and 0.26 respectively), see Figure 5.2. Considering the entire dN/dS dataset the most common category of gene were genes that had an omega below one for both the *nagasau* and background branches, indicating purifying selection (1,170 genes (excluding 581 genes with $\omega = 0.00010$)), but where the *nagasau* omega was higher than the background (709 genes) indicating that either relaxed purifying selection was occurring, or that positive selection was occurring. A genome wide test comparing ω_2 (produced by RELAX) of *P. nagasau* and the background branches indicates genome-wide relaxed selection ($Z = -4.9271$, p-value 8.35E-07). RELAX identified 60 genes that were had significant evidence for intensified selection and 45 genes where selection had been relaxed ($p < 0.05$). Enriched GO terms for the genes experienced intensified natural selection included DNA metabolic process, metal ion transport and deoxyribonuclease II activity and ATP binding (Figures 5.17-19). Genes that experienced

²⁴ The authors hypothesised that this result was due to increased exposure of UV radiation and more recent work on lichen symbiosis suggests that molecular evolution slows down for mutualist fungi compared to free-living fungi (Ametrano et al., 2022).

relaxed selection were enriched for the terms Vesicle docking involved with exocytosis, protein farnesylation and ceramide catabolic process as well as sensory perception of smell.

While most genes were either under purifying selection, there were 53 genes showed signs of significant positive selection following the branch test (Table S5.1), GO enrichment of these include functions predicted to relate to the farming mutualism including sensory perception of smell, axon guidance and nervous system development. It is hypothesised that dramatic behavioural changes, such as change in mutualistic strategy, would be accompanied by changes in genes related to the nervous system (Rubin and Moreau 2016). Fewer (38) genes were found to have significant evidence of positive selection according to the branch site test (see Table S5.2). The branch site test is a more robust test which takes into account the that each site in a given gene does not experience selection uniformly, with some sites being highly conserved and others potentially under positive selection, some researchers characterise these patterns as evolutionary fingerprints of genes (Kosakovsky Pond et al. 2010). The branch site test in some cases lead to a higher power to detect positive selection, for example in genes where just a few sites have a high ω compared to most site being highly conserved, under purifying selection $\omega < 1$ (Wertheim et al. 2015, Álvarez-Carretero et al. 2023). However, in this study fewer genes had significant evidence for positive selection compared to the branch test (38 genes vs 53). These genes were enriched for the terms tRNA processing, metal ion transport, nervous system development, axon guidance and signalling receptor activity (see Figures 5.12-14).

Some genes had contradictory results when comparing the results from the branch test (performed in codeML, part of PAML) and the RELAX test (part of the HyPhy package), for example gene OG0004376 had significant evidence that it was under positive selection ($\omega > 1$) according to the branch test (LRT = 4.66) whereas according to the RELAX test there was significant evidence for relaxed selection. Examining the RELAX results closer we can see that for the background branches most of the sites are under purifying selection (97.19% of sites $\omega = 0.16$) whereas a small proportion of the sites are under positive selection (0.48% of sites $\omega = 13.2$) with the remaining sites under neutral selection. In comparison, *Philidris nagasau* appear to be experiencing neutral selection throughout the sites, which by comparison makes the average ω higher when considering the sites on average (e.g. the ω results from the branch test are 0.21416 for the background and 1.84073 for *P. nagasau*). This discrepancy highlights the benefits of using models such as RELAX that model different site classes. Gene OG0004376 did not have significant evidence for positive selection when using the PAML branch-site test, which also take site class into account, suggesting that we should be cautious when interpreting branch test results.

It has been suggested genome-wide relaxed selection, along with positive selection on mutualism-related genes, is symptomatic of mutualism (Rubin and Moreau 2016) with these changes expected to be more dramatic in endosymbionts than in autonomous animals such as ants. There are many evolutionary scenarios that can lead to relaxed selection: reduced effective population size and bottlenecks, changes in genetic architecture such as polyploid or gene duplication, wherein extra gene copies can evolve freely due to having a redundant copy, phenotypic plasticity and environmental change (Lahti et al. 2009). Regarding reduced population size, ants as a group have a reduced population size due to their eusocial lifestyle/division of reproductive labour (Romiguier et al. 2014) and given that *P. nagasau* is endemic to two Fijian islands, Vanua Levu and Taveuni, it seemed possible that *P. nagasau* may have a reduced effective population size compared to other species of *Philidris*. However, this appears to not be the case. Between 1 million and 100,000 years ago *P. nagasau* appears to experience an initial decrease in effective population size, followed by an increase between 100,000 and 10,000 years ago (unpublished analysis by collaborator Lin Cheng, Agricultural Genomics Institute, Shenzhen, China). Additionally, it appears that obligate farming of *Squamellaria* allows *P. nagasau* to produce larger colonies compared to ant-garden and carton nesting species of *Philidris*, while this has not been quantified yet, it would be congruent with the effect of *P. nagasau* initially experiencing an effective population size reduction, and then increase once obligate agriculture had evolved. These results suggest that some genes have become more important following the evolution of the Fijian agriculture system and some genes have become obsolete, this mirrors patterns seen in the fungus farming mutualism (Nygaard et al. 2016).

Conclusions

The evolution of obligate specialist mutualism appears to follow Red Queen dynamics rather than Red King as predicted from modelling. While some genes (53 or 38) did experience positive selection, most genes were under purifying selection and appear to be under relaxed selection on the *P. nagasau* branch. This work mirrors findings in other mutualistic symbiosis, including endosymbiosis but also obligate parasitism, suggesting that these dynamics are as a result of closely living with other species, rather than being unique to either mutualism or antagonism.

Table S5.1 Genes which have significant evidence of positive selection on the *Philidris nagasau* branch following a branch test using codeML (Yang 2007). Branch model analysis was performed on 1,985 single copy orthologs between *Philidris nagasau* and the remaining background branches. There are two fitted models (null M0 and alternative branch), lnL gives the log-likelihood of the fitted model given the codon alignment data and the gene tree. ω null gives the overall ratio of nonsynonymous to synonymous mutations (dN/dS), ω background gives the dN/dS ratio of the background branches for the branch model and ω *nagasau* gives the dN/dS for the foreground (*P. nagasau*) branch for the branch model. Genes are organised based on the signature of selection result, with (i) genes wherein the *P. nagasau* branch with a $\omega > 1$ indicating positive selection; (ii) second genes which have a $\omega < 1$ for both the background and foreground *P. nagasau* branch but where the *P. nagasau* branch has a greater ω value indicating that either relaxed purifying selection is occurring or positive selection; and (iii) lastly, genes which have a $\omega < 1$ for both the background and foreground *P. nagasau* branch and the *P. nagasau* branch has a lower ω value, indicating purifying selection.

Gene	ω null	ω background	ω <i>nagasau</i>	lnL null	lnL branch	x-2	LRT	GO Terms
Genes wherein the nagasau branch has a $w > 1$, indicating positive selection								
OG0003419.fa	0.02004	0.0001	999	-1242.95	-1238.57	4.382257	8.764514	GO:0016021
OG0003562.fa	0.1355	0.10895	1.32634	-3265.23	-3260.26	4.966679	9.933358	GO:0016627 GO:0022900 GO:0050660 GO:0055114 GO:0006099 GO:0016491
OG0003710.fa	0.43335	0.39513	1.06736	-9421.66	-9415.92	5.743747	11.487494	GO:0005085
OG0003750.fa	0.59253	0.5462	1.42466	-6076.53	-6074.47	2.059244	4.118488	GO:0005515 GO:0016021 GO:0005887 GO:0007399 GO:0007411 GO:0030513 GO:0038023 GO:0055072
OG0003842.fa	0.1904	0.16665	999	-1801.67	-1797.91	3.754358	7.508716	GO:0000290 GO:0016787
OG0003859.fa	0.28078	0.25955	999	-3462.11	-3458.75	3.357012	6.714024	N/A
OG0003900.fa	0.2718	0.256	999	-2062.33	-2060.26	2.076982	4.153964	GO:0004672 GO:0006468 GO:0005524

OG0003985.fa	0.45982	0.39574	999	-1473.76	-1471.65	2.110573	4.221146	GO:0009055 GO:0020037
OG0004002.fa	0.30977	0.28146	2.93372	-2309.41	-2306.78	2.6335	5.267	GO:0005515
OG0004087.fa	0.23849	0.20837	1.74624	-1796.47	-1794.00	2.476634	4.953268	GO:0003824 GO:0016833 GO:0004419
OG0004120.fa	0.02667	0.0001	3.85872	-318.22	-315.90	2.315779	4.631558	GO:0005515 GO:0030695
OG0004182.fa	0.47063	0.44275	2.90321	-3936.45	-3934.23	2.227666	4.455332	N/A
OG0004201.fa	0.36716	0.32575	2.40633	-3198.38	-3195.68	2.698429	5.396858	GO:0004423 GO:0008484 GO:0003824
OG0004206.fa	0.22013	0.18564	999	-1274.37	-1272.14	2.232453	4.464906	N/A
OG0004207.fa	0.44945	0.3949	3.31797	-2228.53	-2225.66	2.873239	5.746478	N/A
OG0004227.fa	0.34078	0.30598	999	-2041.46	-2038.60	2.86106	5.72212	N/A
OG0004248.fa	0.22987	0.20712	1.60918	-2528.06	-2525.96	2.10052	4.20104	GO:0006777 GO:0046872 GO:0003824 GO:0051536 GO:0019008 GO:0051539
OG0004303.fa	0.04097	0.03066	999	-1501.78	-1499.30	2.48061	4.96122	GO:0005515 GO:0031929 GO:0031931 GO:0031932
OG0004376.fa	0.23704	0.21416	1.84073	-2263.74	-2261.41	2.329575	4.65915	GO:0004984 GO:0005549 GO:0007608 GO:0016020

OG0004387.fa	0.23025	0.20478	999	-1380.72	-1378.30	2.412559	4.825118	GO:0005515 GO:0005634 GO:0005737 GO:0061015
OG0004414.fa	0.12853	0.10445	999	-816.53	-813.88	2.650237	5.300474	GO:0016972 GO:0055114 GO:0016971
OG0004430.fa	0.47787	0.4311	999	-1881.10	-1877.90	3.207551	6.415102	GO:0006629 GO:0016788
OG0004449.fa	0.33516	0.29353	2.99361	-2615.75	-2612.20	3.554753	7.109506	N/A
OG0004693.fa	0.54118	0.48335	999	-1671.27	-1668.84	2.426052	4.852104	N/A
OG0004763.fa	0.05809	0.0001	999	-195.29	-192.47	2.810909	5.621818	N/A
OG0004785.fa	0.13911	0.12755	999	-1935.98	-1933.40	2.580212	5.160424	N/A
OG0004809.fa	0.03047	0.0001	999	-643.01	-639.85	3.158502	6.317004	GO:0003676 GO:0008270
OG0004829.fa	0.78438	0.70412	999	-1876.38	-1872.80	3.579561	7.159122	GO:0004252 GO:0006508
OG0004900.fa	0.19375	0.17486	999	-1540.47	-1538.00	2.469414	4.938828	GO:0004672 GO:0005524 GO:0006468
OG0005019.fa	0.44992	0.4318	1.16226	-8836.22	-8834.04	2.182199	4.364398	GO:0005515
OG0005045.fa	0.25547	0.21671	2.034	-2539.49	-2536.74	2.74924	5.49848	GO:0016757 GO:0000030
OG0005057.fa	0.23521	0.22021	1.809	-3367.21	-3364.96	2.249129	4.498258	GO:0045162
OG0005068.fa	0.28542	0.26092	999	-1846.70	-1844.09	2.613985	5.22797	GO:0005524 GO:0016887
OG0005076.fa	0.28886	0.23686	999	-1122.77	-1119.91	2.863501	5.727002	GO:0005515
OG0005163.fa	0.15592	0.1271	999	-1115.36	-1112.94	2.416796	4.833592	GO:0004140 GO:0005524 GO:0015937

OG0005243.fa	0.2325	0.21253	1.02032	-4776.27	-4772.49	3.779675	7.55935	N/A
OG0005261.fa	0.15962	0.14051	999	-1337.39	-1334.65	2.748664	5.497328	GO:0003725
OG0005290.fa	0.17178	0.14516	1.70231	-2389.21	-2386.21	3.002868	6.005736	GO:0016758 GO:0005783 GO:0006490 GO:0042283
OG0005297.fa	0.28406	0.19589	999	-428.39	-425.18	3.207099	6.414198	N/A
OG0005341.fa	0.05757	0.0479	999	-1630.66	-1628.48	2.179117	4.358234	N/A
OG0005430.fa	0.02079	0.01037	999	-1340.20	-1336.79	3.408403	6.816806	GO:0035556
OG0005446.fa	0.09526	0.06328	999	-827.78	-825.54	2.24323	4.48646	N/A
OG0005465.fa	0.28395	0.21137	1.48741	-2035.71	-2031.91	3.790612	7.581224	GO:0006269
OG0005596.fa	0.06487	0.04853	999	-1284.68	-1282.60	2.088587	4.177174	GO:0005515
OG0005674.fa	0.34221	0.2849	2.53838	-1681.20	-1678.66	2.537126	5.074252	N/A
OG0005676.fa	0.18286	0.16815	1.58435	-6287.79	-6282.65	5.138975	10.27795	GO:0004813 GO:0000166 GO:0005524 GO:0005737 GO:0006419 GO:0003676 GO:0004812 GO:0043039
OG0005724.fa	0.3294	0.28051	3.10185	-2080.74	-2076.98	3.763844	7.527688	GO:0017040 GO:0046514
OG0005730.fa	0.21821	0.20026	999	-1944.48	-1941.49	2.985951	5.971902	N/A
OG0005903.fa	0.08618	0.06432	999	-1352.07	-1348.43	3.639807	7.279614	GO:0051536 GO:0009055 GO:0006099 GO:0016491 GO:0055114 GO:0051537
OG0006059.fa	0.39579	0.34577	2.08435	-1768.40	-1766.46	1.941936	3.883872	GO:0016591
OG0006085.fa	0.71333	0.65348	999	-3326.13	-3321.16	4.966166	9.932332	N/A

OG0006126.fa	0.64129	0.55635	999	-2736.74	-2730.75	5.982851	11.965702	GO:0009058 GO:0016844
OG0006223.fa	0.16763	0.10228	1.14182	-602.82	-600.75	2.068682	4.137364	N/A
Genes were w < 1 but nagasau w is greater than background indication either relaxed purifying selection or positive selection								
OG0003435.fa	0.07783	0.06868	0.32238	-5750.01	-5747.80	2.209684	4.419368	GO:0000184 GO:0003723 GO:0005515
OG0003498.fa	0.12279	0.11231	0.32229	-9693.05	-9690.05	2.995147	5.990294	GO:0005086 GO:0032012 GO:0051169
OG0003611.fa	0.0246	0.01865	0.55094	-3155.20	-3153.27	1.92626	3.85252	GO:0006886 GO:0008536
OG0003615.fa	0.20891	0.18214	0.80791	-4094.44	-4091.05	3.393174	6.786348	GO:0003723 GO:0006397 GO:0035145
OG0003776.fa	0.02292	0.00813	0.24981	-2394.51	-2391.24	3.263523	6.527046	GO:0005515
OG0003807.fa	0.02356	0.02077	0.08996	-23467.15	-23463.68	3.471391	6.942782	GO:0005524 GO:0007018 GO:0008569 GO:0030286
OG0003921.fa	0.16727	0.14822	0.41848	-4811.04	-4808.99	2.050851	4.101702	N/A
OG0003971.fa	0.07441	0.05341	0.34201	-2572.62	-2569.47	3.154945	6.30989	GO:0016791
OG0004053.fa	0.20855	0.19144	0.69422	-10844.23	-10837.60	6.631772	13.263544	GO:0003677
OG0004119.fa	0.26004	0.24364	0.87148	-6475.88	-6473.26	2.626544	5.253088	GO:0005524 GO:0016021 GO:0042626 GO:0055085 GO:0016887
OG0004166.fa	0.00243	0.0001	0.04475	-7550.08	-7547.25	2.829821	5.659642	GO:0005198 GO:0006886 GO:0016192 GO:0030130 GO:0030132 GO:0005515 GO:0032051 GO:0071439
OG0004503.fa	0.15542	0.14125	0.5985	-3545.18	-3543.10	2.088202	4.176404	GO:0016021
OG0004504.fa	0.08848	0.07736	0.59052	-3421.56	-3419.29	2.269406	4.538812	GO:0006464 GO:0005524
OG0004721.fa	0.01708	0.0001	0.53975	-951.88	-948.81	3.065053	6.130106	GO:0019843 GO:0003723 GO:0003735

								GO:0006412 GO:0015935
OG0004881.fa	0.21929	0.19122	0.85487	-2503.15	-2500.97	2.181771	4.363542	N/A
OG0004962.fa	0.29571	0.26526	0.76823	-4596.93	-4594.94	1.983378	3.966756	GO:0005515 GO:0001736 GO:0060271 GO:0016192
OG0005000.fa	0.1033	0.08999	0.39551	-4129.13	-4126.59	2.542064	5.084128	GO:0004114 GO:0007165 GO:0008081
OG0005042.fa	0.13784	0.09424	0.57867	-2038.31	-2034.98	3.323574	6.647148	GO:0003824 GO:0005965 GO:0018343
OG0005143.fa	0.01361	0.0001	0.18224	-1399.29	-1396.90	2.388124	4.776248	GO:0000287 GO:0004721 GO:0030145 GO:0016791 GO:0043169
OG0005150.fa	0.10647	0.09002	0.37228	-2945.29	-2943.23	2.054117	4.108234	GO:0006904 GO:0016192
OG0005215.fa	0.02329	0.0001	0.34178	-638.77	-636.40	2.365799	4.731598	GO:0003735 GO:0005840 GO:0006412
OG0005260.fa	0.04901	0.03549	0.41403	-2702.68	-2699.31	3.372074	6.744148	N/A
OG0005366.fa	0.1527	0.13209	0.76019	-2569.63	-2566.94	2.681496	5.362992	GO:0005524
OG0005534.fa	0.07154	0.06051	0.2533	-7669.20	-7664.85	4.345674	8.691348	GO:0005515
OG0005584.fa	0.11016	0.09605	0.37778	-7165.06	-7160.77	4.292022	8.584044	GO:0005515 GO:0007165
OG0005613.fa	0.15856	0.13053	0.84483	-3204.16	-3200.82	3.341662	6.683324	GO:0035091
OG0005615.fa	0.05192	0.03989	0.47113	-1714.12	-1711.87	2.253826	4.507652	GO:0004672 GO:0006468 GO:0005524
OG0005865.fa	0.17558	0.1608	0.72146	-3665.93	-3663.48	2.457722	4.915444	GO:0005524 GO:0042626 GO:0055085 GO:0016021 GO:0016887 GO:0009055 GO:0020037
OG0005937.fa	0.06996	0.06066	0.3662	-4183.39	-4181.40	1.990482	3.980964	GO:0003676
OG0006053.fa	0.01513	0.00529	0.21628	-3406.41	-3402.53	3.878176	7.756352	GO:0017196 GO:0031417
OG0006067.fa	0.12127	0.09869	0.34637	-3665.68	-3663.34	2.341076	4.682152	GO:0005515 GO:0072546

OG0006100.fa	0.10539	0.08796	0.37195	-6659.40	-6655.82	3.575813	7.151626	N/A
OG0006115.fa	0.13471	0.12199	0.34798	-6527.96	-6525.70	2.267419	4.534838	GO:0004525 GO:0006396 GO:0003723 GO:0016075
OG0006138.fa	0.13187	0.11102	0.47413	-4125.41	-4122.57	2.836887	5.673774	GO:0004674 GO:0005524 GO:0006468 GO:0035556 GO:0004672
OG0006210.fa	0.28687	0.25936	0.93087	-2640.38	-2638.42	1.960425	3.92085	GO:0016020 GO:0016757 GO:0003676
OG0006246.fa	0.4099	0.38305	0.93001	-7204.83	-7202.62	2.211276	4.422552	GO:0005515 GO:0006396 GO:0005634 GO:0006397
OG0006250.fa	0.06087	0.0535	0.27234	-4795.97	-4793.49	2.483745	4.96749	GO:0005515
Genes where in both background and nagasau w < 1 and nagasau w is lower, indicating purifying selection.								
OG0003409.fa	0.42424	0.46475	0.05385	-3254.442713	-3251.256399	3.186314	6.372628	GO:0005337 GO:0016020 GO:1901642
OG0003425.fa	0.13047	0.14454	0.00715	-4190.57201	-4188.306731	2.265279	4.530558	GO:0005524 GO:0006457 GO:0051082 GO:0004672
OG0003430.fa	0.18457	0.19573	0.04028	-7616.382361	-7613.403588	2.978773	5.957546	GO:0006468 GO:0005524 GO:0005515 GO:0004713
OG0003461.fa	0.09464	0.10213	0.0001	-7234.157365	-7231.687598	2.469767	4.939534	GO:0003712 GO:0006357 GO:0016592
OG0003633.fa	0.24471	0.26908	0.03157	-4463.162685	-4459.515488	3.647197	7.294394	N/A
OG0003762.fa	0.0845	0.09613	0.0001	-3348.948404	-3346.111945	2.836459	5.672918	GO:0005515 GO:0007165 GO:0003677
OG0003806.fa	0.3079	0.33727	0.0001	-2912.832477	-2909.658492	3.173985	6.34797	GO:0006260 GO:0005524 GO:0016887 GO:0000015
OG0003876.fa	1.42321	1.53213	0.0001	-2824.875858	-2821.462126	3.413732	6.827464	GO:0000287 GO:0004634 GO:0006096
OG0003896.fa	0.14114	0.17033	0.0001	-1709.541601	-1707.005421	2.53618	5.07236	GO:0042302

OG0003956.fa	0.2593	0.30631	0.0001	-835.913024	-833.828119	2.084905	4.16981	GO:0004126 GO:0008270 GO:0009972 GO:0016787 GO:0003824
OG0004167.fa	0.20309	0.22249	0.0001	-2263.487405	-2261.537823	1.949582	3.899164	GO:0050825 GO:0005102 GO:0003899
OG0004260.fa	0.24811	0.25875	0.0001	-9079.048088	-9075.773975	3.274113	6.548226	GO:0006351 GO:0008270 GO:0003677
OG0004292.fa	0.09326	0.10011	0.0001	-9496.722741	-9491.532622	5.190119	10.380238	GO:0008270 GO:0061630 GO:0071596 GO:0030163
OG0004340.fa	0.18329	0.19796	0.0001	-2747.800564	-2745.823951	1.976613	3.953226	GO:0005515
OG0004365.fa	0.4338	0.54403	0.0001	-1082.398668	-1079.318555	3.080113	6.160226	N/A
OG0004388.fa	0.43311	0.53171	0.0001	-678.944888	-676.818644	2.126244	4.252488	GO:0003735 GO:0005840 GO:0006412 GO:0019843
OG0004411.fa	0.29059	0.33043	0.0001	-1711.403745	-1708.11264	3.291105	6.58221	GO:0005759
OG0004641.fa	0.15301	0.17123	0.0001	-2899.372699	-2897.079909	2.29279	4.58558	GO:0046872 GO:0006508
OG0004686.fa	0.43509	0.48704	0.0001	-1251.718652	-1249.565254	2.153398	4.306796	GO:0008745 GO:0009253 GO:0008270
OG0004708.fa	0.26607	0.29732	0.0001	-2280.58818	-2277.132394	3.455786	6.911572	N/A
OG0004795.fa	0.20075	0.23417	0.0001	-1169.858247	-1167.332436	2.525811	5.051622	GO:0042721
OG0004820.fa	0.28693	0.31704	0.0001	-2734.706574	-2731.409706	3.296868	6.593736	N/A
OG0004864.fa	0.13861	0.14871	0.02644	-6327.216426	-6324.987881	2.228545	4.45709	GO:0005509 GO:0007156 GO:0016020 GO:0005886 GO:0007155 GO:0003677 GO:0003676 GO:0008270 GO:0000981 GO:0006355
OG0004874.fa	0.07999	0.08503	0.0001	-8808.162778	-8805.655224	2.507554	5.015108	GO:0000139
OG0004896.fa	0.57834	0.63743	0.07642	-2414.563311	-2411.938818	2.624493	5.248986	N/A
OG0004939.fa	0.43331	0.48281	0.0001	-4816.495187	-4809.653876	6.841311	13.682622	N/A
OG0004955.fa	0.25201	0.27781	0.0001	-2561.724703	-2559.105342	2.619361	5.238722	N/A

OG0004992.fa	0.29305	0.34424	0.0001	-1188.689349	-1186.631955	2.057394	4.114788	GO:0005789 GO:0006506
OG0005005.fa	0.33567	0.42177	0.05942	-1148.621621	-1146.68233	1.939291	3.878582	N/A
OG0005306.fa	0.07281	0.07927	0.0001	-6714.675931	-6711.958191	2.71774	5.43548	GO:0003677
OG0005374.fa	0.32243	0.35991	0.0001	-1288.480223	-1286.537257	1.942966	3.885932	N/A
OG0005619.fa	0.32672	0.34524	0.0001	-3173.892062	-3171.656842	2.23522	4.47044	N/A
OG0005644.fa	0.63864	0.69011	0.1983	-4459.457754	-4456.584354	2.8734	5.7468	GO:0006260 GO:0055114 GO:0004748 GO:0005524
OG0005778.fa	0.65466	0.71785	0.07761	-3455.423468	-3452.635338	2.78813	5.57626	N/A
OG0005809.fa	0.33651	0.36045	0.0001	-2862.04173	-2859.239039	2.802691	5.605382	N/A
OG0005812.fa	0.31889	0.39496	0.0001	-1255.596196	-1251.73224	3.863956	7.727912	GO:0000387 GO:0032797 GO:0016021 GO:0000166 GO:0000287 GO:0005524 GO:0015914 GO:0140326
OG0005914.fa	0.1244	0.13223	0.0001	-4301.932978	-4299.556813	2.376165	4.75233	GO:0000387 GO:0032797 GO:0016021 GO:0000166 GO:0000287 GO:0005524 GO:0015914 GO:0140326
OG0005919.fa	0.228	0.25989	0.0001	-3153.618672	-3149.87503	3.743642	7.487284	N/A
OG0005964.fa	0.28348	0.34192	0.0001	-1987.141028	-1984.306024	2.835004	5.670008	GO:0006812 GO:0008324 GO:0016021 GO:0055085
OG0005972.fa	0.1892	0.20852	0.02759	-3819.40929	-3816.342986	3.066304	6.132608	N/A
OG0005995.fa	0.51713	0.59124	0.0001	-1085.147158	-1083.135202	2.011956	4.023912	N/A
OG0006028.fa	0.13828	0.15413	0.0001	-3961.490151	-3958.897857	2.592294	5.184588	GO:0003676 GO:0000289 GO:0004535 GO:0006397 GO:0031251 GO:0005515
OG0006073.fa	0.36053	0.44967	0.05304	-1014.249318	-1011.55215	2.697168	5.394336	N/A

Table S5.2 Genes which have significant evidence of positive selection on the *Philidris nagasau* branch following a branch-site test using codeML (Yang 2007). Branch model analysis was performed on 1985 single copy orthologs between *Philidris nagasau* and the remaining background branches. The branch-site model models four site classes ω_0 , ω_1 , ω_{2a} and ω_{2b} and their proportions. ω_0 are sites under purifying selection ($\omega < 1$), ω_1 are under neutral selection $\omega = 1$, ω_{2a} are sites that are under positive selection ($\omega > 1$) on the test branch but under purifying selection in the background and ω_{2b} are under positive selection in the foreground and neutral in the background. $\ln L$ gives the log-likelihood of the fitted model given the codon alignment data and the gene tree. The chi-squared statistic (X^2) is calculated as twice the difference between the log-likelihood of the test model and the null model ($2 \times (\ln L_{\text{test}} - \ln L_{\text{null}})$), which is then used to calculate the likelihood ratio test (LRT). GO Terms were annotated using InterProScan using -*goterms*.

Gene	ω_0			ω_1			ω_{2a}			ω_{2b}			$\ln L$		x-2	LRT	GO Terms
	Back.	Fore.	Prop.	Back.	Fore.	Prop.	Back.	Fore.	Prop.	Back.	Fore.	Prop.	(test)	(null)			
OG0003615.fa	0	0	0.78813	1	1	0.19839	0	95.7984	0.01077	1	95.7984	0.00271	-4083.174449	-4086.45	3.272574	6.545148	GO:0003723 GO:0006397 GO:0035145
OG0003668.fa	0.03446	0.03446	0.97326	1	1	0.0239	0.03446	48.47636	0.00278	1	48.47636	0.00007	-3300.675074	-3303.2	2.521893	5.043786	N/A
OG0003700.fa	0.18649	0.18649	0.99584	1	1	0	0.18649	461.93111	0.00416	1	461.93111	0	-1448.5734	-1453.13	4.552961	9.105922	GO:0016020 GO:0016192 GO:0000149 GO:0006888 GO:0097111 GO:0097352
OG0003750.fa	0	0	0.48048	1	1	0.49875	0	35.52558	0.01019	1	35.52558	0.01058	-6065.689172	-6067.77	2.076094	4.152188	GO:0005515 GO:0016021 GO:0005887 GO:0007399 GO:0007411 GO:0030513 GO:0038023 GO:0055072
OG0003761.fa	0.01849	0.01849	0.879	1	1	0.11944	0.01849	264.19689	0.00137	1	264.19689	0.00019	-7866.869571	-7870.43	3.558821	7.117642	N/A
OG0003856.fa	0	0	0.74925	1	1	0.23015	0	652.01724	0.01576	1	652.01724	0.00484	-593.716185	-597.476	3.760129	7.520258	GO:0003677 GO:0006355
OG0004053.fa	0.00857	0.00857	0.78021	1	1	0.20879	0.00857	103.11792	0.00868	1	103.11792	0.00232	-10810.81537	-10817.6	6.759573	13.51915	GO:0003677
OG0004077.fa	0.14999	0.14999	0.9978	1	1	0	0.14999	735.9837	0.0022	1	735.9837	0	-2987.837053	-2991.26	3.426324	6.852648	N/A
OG0004110.fa	0	0	0.43263	1	1	0.5273	0	40.85849	0.01806	1	40.85849	0.02201	-1896.074778	-1898.9	2.822589	5.645178	N/A
OG0004120.fa	0	0	0.98367	1	1	0	0	999	0.01633	1	999	0	-311.040022	-315.354	4.313887	8.627774	GO:0005515 GO:0030695
OG0004182.fa	0.09614	0.09614	0.59272	1	1	0.39529	0.09614	248.18084	0.0072	1	248.18084	0.0048	-3928.413174	-3932.83	4.417231	8.834462	N/A

OG0004282.fa	0.00434	0.00434	0.80438	1	1	0.18965	0.00434	123.8722	0.00484	1	123.8722	0.00114	-3346.847876	-3349.36	2.507413	5.014826	GO:0008033 GO:0017150 GO:0050660 GO:0055114 GO:0003824
OG0004482.fa	0	0	0.73737	1	1	0.26096	0	165.8404	0.00123	1	165.8404	0.00044	-5354.874086	-5357	2.130678	4.261356	GO:0005515
OG0004620.fa	0.45172	0.45172	0.99468	1	1	0	0.45172	999	0.00532	1	999	0	-2265.314351	-2270.68	5.366797	10.73359	GO:0003677 GO:0004518
OG0004660.fa	0	0	0.86982	1	1	0.12818	0	91.45645	0.00174	1	91.45645	0.00026	-6426.450331	-6428.6	2.151632	4.303264	GO:0005515
OG0004767.fa	0	0	0.77755	1	1	0.21999	0	140.10436	0.00191	1	140.10436	0.00054	-4329.33299	-4331.96	2.629303	5.258606	GO:0005524 GO:0016887 GO:0016021 GO:0042626 GO:0055085
OG0004829.fa	0	0	0.40944	1	1	0.51164	0	999	0.03508	1	999	0.04384	-1865.672606	-1870.16	4.48969	8.97938	GO:0004252 GO:0006508
OG0004889.fa	0.15233	0.15233	0.73744	1	1	0.25611	0.15233	999	0.00479	1	999	0.00166	-1002.439555	-1007.63	5.189316	10.37863	N/A
OG0004947.fa	0	0	0.80836	1	1	0.19034	0	800.67473	0.00105	1	800.67473	0.00025	-6039.682618	-6044.84	5.157242	10.31448	GO:0005634 GO:0008270
OG0004961.fa	0.02166	0.02166	0.73128	1	1	0.26056	0.02166	999	0.00602	1	999	0.00214	-1572.524368	-1581.58	9.060429	18.12086	GO:0005515
OG0004962.fa	0.14135	0.14135	0.83262	1	1	0.16151	0.14135	693.11098	0.00492	1	693.11098	0.00095	-4571.678873	-4592.9	21.21979	42.43959	GO:0005515 GO:0001736 GO:0060271 GO:0016192
OG0005128.fa	0.10105	0.10105	0.93719	1	1	0.05167	0.10105	999	0.01055	1	999	0.00058	-1347.24913	-1350.81	3.564296	7.128592	GO:0006801 GO:0046872 GO:0004784 GO:0055114 GO:0030001
OG0005215.fa	0	0	0.99041	1	1	0	0	999	0.00959	1	999	0	-631.026105	-635.962	4.935598	9.871196	GO:0003735 GO:0005840 GO:0006412
OG0005453.fa	0	0	0.83767	1	1	0.16012	0	613.05493	0.00186	1	613.05493	0.00036	-2553.301876	-2556.6	3.300699	6.601398	GO:0006508 GO:0008236
OG0005465.fa	0.03808	0.03808	0.77248	1	1	0.17924	0.03808	47.90624	0.03919	1	47.90624	0.00909	-2027.667792	-2030.1	2.42792	4.85584	GO:0006269
OG0005529.fa	0	0	0.24478	1	1	0.75109	0	237.38468	0.00101	1	237.38468	0.00311	-2198.059804	-2200.6	2.543373	5.086746	N/A
OG0005667.fa	0	0	0.624	1	1	0.37195	0	999	0.00254	1	999	0.00151	-3581.575528	-3595.76	14.18354	28.36708	N/A

OG0005792.fa	0.15455	0.15455	0.61171	1	1	0.38706	0.15455	858.45669	0.00075	1	858.45669	0.00048	-8228.434722	-8232.28	3.844751	7.689502	GO:0005515 GO:0005634 GO:0006260 GO:0006281 GO:0033557
OG0005816.fa	0.24352	0.24352	0.99319	1	1	0	0.24352	128.681	0.00681	1	128.681	0	-3263.918679	-3267.18	3.265706	6.531412	GO:0005515
OG0005826.fa	0	0	0.34517	1	1	0.65319	0	228.04171	0.00057	1	228.04171	0.00107	-7800.753573	-7802.76	2.003988	4.007976	N/A
OG0005933.fa	0	0	0.59013	1	1	0.39938	0	62.28764	0.00626	1	62.28764	0.00424	-2307.517918	-2309.5	1.983362	3.966724	N/A
OG0006085.fa	0	0	0	1	1	0	0	548.55268	0.41923	1	548.55268	0.58077	-3314.71177	-3318.32	3.61145	7.2229	N/A
OG0006105.fa	0	0	0.62637	1	1	0.36311	0	442.00675	0.00666	1	442.00675	0.00386	-2295.814386	-2311.02	15.20406	30.40811	GO:0004531 GO:0006259
OG0006126.fa	0	0	0	1	1	0	0	343.84368	0.52989	1	343.84368	0.47011	-2722.615917	-2724.78	2.16878	4.33756	GO:0009058 GO:0016844
OG0006210.fa	0	0	0.72945	1	1	0.25779	0	53.52461	0.00943	1	53.52461	0.00333	-2625.461636	-2628.2	2.73754	5.47508	GO:0016020 GO:0016757
OG0006223.fa	0.1109	0.1109	0.98525	1	1	0	0.1109	616.30674	0.01475	1	616.30674	0	-595.459598	-600.434	4.974346	9.948692	N/A

Table S5.3 RELAX (Wertheim et al. 2015) model fits. RELAX produces a null, alternative, or partitioned descriptive (PD) models. lnL is the log likelihood. AICc is the small sample-corrected Akaike Information and alternative models on 1 degree of freedom. K is the relation/intensification parameter ($K < 1$ suggests relaxation and $K > 1$ suggests intensification). Partition refers to the tree branch partition (Test = *P. nagasau* and Ref = background). ω_0 ω_1 ω_2 give the inferred ratio of nonsynonymous to synonymous mutations (dN/dS) of each site class and p0 p1 and p2 give the site proportions of each site class. The null model is constrained to having a K value of 1. Each gene was annotation was annotated using InterProScan using the option -goterms.

gene	model	lnL	AICc	LRT	p value	k	partition	ω_0	ω_1	ω_2	p0	p1	p2	GO terms
Genes which have significant evidence for intensification of natural selection														
OG0003409.fa	null	-4219.46	8507.25			1.000	Ref	0.000	0.436	5.0E+03	0.014	0.985	0.001	GO:0005337 GO:0016020 GO:1901642
	alt	-4216.17	8502.71	6.563	0.010	4.407	Ref	0.000	0.470	2.7E+03	0.015	0.984	0.001	
	alt						Test	0.000	0.036	1.3E+15	0.015	0.984	0.001	
	PD	-4215.67	8509.80				Ref	0.460	1.000	5.4E+03	0.984	0.015	0.001	
	PD						Test	0.000	0.182	1.9E+01	0.705	0.295	0.000	
OG0003498.fa	null	-10237.74	20539.59			1.000	Ref	0.000	0.000	1.0E+00	0.343	0.528	0.129	GO:0005086 GO:0032012
	alt	-10235.29	20536.71	4.892	0.027	16.256	Ref	0.000	0.000	1.1E+00	0.466	0.421	0.113	
	alt						Test	0.000	0.000	2.8E+00	0.466	0.421	0.113	
	PD	-10234.47	20543.10				Ref	0.066	0.104	1.0E+00	0.743	0.213	0.044	
	PD						Test	0.116	0.253	1.0E+00	0.092	0.812	0.096	
OG0003533.fa	null	-1687.08	3440.89			1.000	Ref	0.403	0.611	6.7E+02	0.980	0.020	0.001	N/A
	alt	-1684.66	3438.10	4.841	0.028	35.964	Ref	0.373	0.392	1.5E+02	0.021	0.977	0.002	
	alt						Test	0.000	0.000	1.5E+78	0.021	0.977	0.002	
	PD	-1684.09	3445.15				Ref	0.414	0.465	4.0E+02	0.980	0.019	0.001	
	PD						Test	0.000	0.000	1.0E+10	0.981	0.015	0.004	
OG0003633.fa	null	-4475.87	9018.04			1.000	Ref	0.000	0.224	3.1E+01	0.009	0.988	0.003	N/A
	alt	-4473.34	9015.02	5.044	0.025	2.552	Ref	0.004	0.258	5.0E+01	0.003	0.995	0.001	
	alt						Test	0.000	0.032	2.2E+04	0.003	0.995	0.001	
	PD	-4471.78	9019.98				Ref	0.240	0.246	3.2E+01	0.985	0.011	0.003	
	PD						Test	0.016	0.130	1.2E+01	0.844	0.156	0.000	
OG0003700.fa	null	-1448.94	2960.63			1.000	Ref	0.000	0.052	7.4E+00	0.012	0.956	0.032	

	alt	-1446.97	2958.73	3.947	0.047	4.136	Ref	0.189	0.192	2.7E+00	0.963	0.026	0.011	GO:0016020
	alt						Test	0.001	0.001	6.1E+01	0.963	0.026	0.011	GO:0016192
	PD	-1446.93	2966.86				Ref	0.213	0.509	1.0E+00	1.000	0.000	0.000	GO:0000149
	PD						Test	0.000	0.289	6.3E+01	0.950	0.039	0.011	GO:0006888
OG0003710.fa														GO:0097111
														GO:0097352
	null	-11185.23	22436.59			1.000	Ref	0.000	0.000	2.0E+00	0.331	0.441	0.228	
	alt	-11181.09	22430.32	8.278	0.004	2.343	Ref	0.000	0.000	1.9E+00	0.335	0.441	0.224	
	alt						Test	0.000	0.000	4.3E+00	0.335	0.441	0.224	GO:0005085
OG0003776.fa	PD	-11180.27	22436.71				Ref	0.000	0.000	2.0E+00	0.409	0.381	0.210	
	PD						Test	0.830	0.838	1.0E+00	0.038	0.397	0.565	
	null	-3087.08	6236.50			1.000	Ref	0.000	0.000	1.3E+00	0.985	0.001	0.014	
	alt	-3084.69	6233.74	4.780	0.029	12.299	Ref	0.000	0.000	1.3E+00	0.993	0.000	0.007	
	alt						Test	0.000	0.000	2.1E+01	0.993	0.000	0.007	GO:0005515
OG0003856.fa	PD	-3083.10	6238.63				Ref	0.000	0.000	1.5E+03	0.996	0.004	0.000	
	PD						Test	0.036	0.179	1.0E+00	0.876	0.033	0.091	
	null	-602.26	1266.33			1.000	Ref	0.000	0.271	6.7E+00	0.319	0.630	0.051	
	alt	-599.16	1262.25	6.201	0.013	4.658	Ref	0.000	0.341	5.0E+00	0.182	0.792	0.026	
	alt						Test	0.000	0.007	1.9E+03	0.182	0.792	0.026	GO:0003677
OG0003956.fa	PD	-598.96	1270.37				Ref	0.000	1.000	2.3E+00	0.730	0.158	0.112	GO:0006355
	PD						Test	1.000	1.000	7.6E+02	0.274	0.713	0.012	
	null	-829.65	1729.13			1.000	Ref	0.272	0.931	9.6E+00	1.000	0.000	0.000	
	alt	-827.60	1727.13	4.107	0.043	32.928	Ref	0.320	0.979	2.0E+01	1.000	0.000	0.000	GO:0004126
	alt						Test	0.000	0.490	3.6E+42	1.000	0.000	0.000	GO:0008270
OG0003971.fa	PD	-827.46	1735.32				Ref	0.263	1.000	7.1E+00	0.987	0.000	0.012	GO:0009972
	PD						Test	0.000	0.888	6.3E+00	1.000	0.000	0.000	GO:0016787
	null	-2665.03	5398.54			1.000	Ref	0.000	0.000	1.0E+00	0.001	0.911	0.088	GO:0003824
	alt	-2662.09	5394.69	5.877	0.015	10.755	Ref	0.000	0.000	1.2E+00	0.236	0.710	0.054	
	alt						Test	0.000	0.000	7.9E+00	0.236	0.710	0.054	GO:0016791
OG0004002.fa	PD	-2661.92	5402.48				Ref	0.000	0.000	1.0E+00	0.038	0.899	0.063	
	PD						Test	0.000	0.000	1.5E+00	0.000	0.738	0.262	
	null	-2348.80	4766.26			1.000	Ref	0.000	0.004	2.3E+00	0.734	0.124	0.142	GO:0005515

	alt	-2346.46	4763.62	4.686	0.030	3.765	Ref	0.000	0.000	2.2E+00	0.729	0.132	0.140	
	alt						Test	0.000	0.000	1.9E+01	0.729	0.132	0.140	
	PD	-2346.24	4771.35				Ref	0.008	0.020	2.3E+00	0.098	0.778	0.124	
	PD						Test	1.000	1.000	3.4E+00	0.153	0.120	0.727	
OG0004053.fa	null	-10901.06	21870.26			1.000	Ref	0.000	0.000	1.6E+00	0.455	0.399	0.146	GO:0003677
	alt	-10895.02	21860.18	12.090	0.001	4.269	Ref	0.000	0.000	1.5E+00	0.455	0.400	0.145	
	alt						Test	0.000	0.000	5.0E+00	0.455	0.400	0.145	
	PD	-10892.88	21863.93				Ref	0.000	0.000	1.0E+00	0.285	0.510	0.205	
	PD						Test	0.000	0.000	2.7E+01	0.936	0.032	0.032	
OG0004077.fa	null	-2996.47	6061.40			1.000	Ref	0.000	0.223	1.0E+01	0.558	0.434	0.008	N/A
	alt	-2993.28	6057.05	6.374	0.012	2.759	Ref	0.000	0.289	8.4E+00	0.564	0.431	0.005	
	alt						Test	0.000	0.033	3.6E+02	0.564	0.431	0.005	
	PD	-2993.08	6064.77				Ref	0.000	0.105	3.2E+00	0.370	0.599	0.032	
	PD						Test	0.072	0.169	5.3E+02	0.948	0.049	0.003	
OG0004167.fa	null	-2807.51	5679.42			1.000	Ref	0.201	0.202	6.4E+02	0.023	0.976	0.001	GO:0050825 GO:0005102
	alt	-2805.13	5676.68	4.757	0.029	23.123	Ref	0.226	0.237	6.0E+02	0.977	0.022	0.001	
	alt						Test	0.000	0.000	2.1E+04	0.977	0.022	0.001	
	PD	-2804.64	5683.81				Ref	0.225	0.226	6.0E+02	0.973	0.026	0.001	
	PD						Test	0.000	0.000	2.0E+01	0.110	0.890	0.000	
OG0004199.fa	null	-5389.04	10846.33			1.000	Ref	0.000	0.082	2.3E+00	0.109	0.882	0.009	GO:0016192 GO:0030123 GO:0006886 GO:0030117
	alt	-5386.48	10843.23	5.118	0.024	8.725	Ref	0.007	0.092	1.5E+00	0.038	0.959	0.003	
	alt						Test	0.000	0.000	4.5E+01	0.038	0.959	0.003	
	PD	-5386.44	10851.20				Ref	0.001	0.093	2.0E+00	0.000	1.000	0.000	
	PD						Test	0.000	0.000	4.5E+01	0.882	0.115	0.003	
OG0004206.fa	null	-1266.99	2598.85			1.000	Ref	0.079	0.305	1.0E+00	0.251	0.712	0.037	N/A
	alt	-1265.01	2596.94	3.969	0.046	6.928	Ref	0.000	0.109	2.3E+00	0.250	0.674	0.076	
	alt						Test	0.000	0.000	3.6E+02	0.250	0.674	0.076	
	PD	-1264.77	2604.70				Ref	0.111	0.261	1.1E+00	0.192	0.808	0.000	
	PD						Test	0.956	1.000	3.9E+01	0.000	0.002	0.998	
OG0004227.fa	null	-2039.09	4142.76			1.000	Ref	0.000	0.000	4.4E+00	0.835	0.069	0.096	N/A
	alt	-2035.99	4138.59	6.204	0.013	4.264	Ref	0.000	0.000	3.8E+00	0.817	0.085	0.098	

	alt						Test	0.000	0.000	3.0E+02	0.817	0.085	0.098	
	PD	-2035.13	4145.04				Ref	0.217	0.235	3.1E+01	0.984	0.008	0.008	
	PD						Test	0.935	0.945	6.3E+01	0.000	0.000	1.000	
OG0004260.fa	null	-9205.25	18478.66			1.000	Ref	0.000	0.867	1.1E+00	0.692	0.308	0.000	
	alt	-9201.56	18473.29	7.379	0.007	50.000	Ref	0.000	0.868	1.0E+00	0.679	0.321	0.000	GO:0003899
	alt						Test	0.000	0.001	1.1E+00	0.679	0.321	0.000	GO:0006351
	PD	-9201.55	18481.30				Ref	0.000	0.865	3.7E+00	0.685	0.312	0.003	GO:0008270
	PD						Test	0.000	0.022	1.1E+00	1.000	0.000	0.000	GO:0003677
OG0004292.fa	null	-9538.88	19145.90			1.000	Ref	0.000	0.807	1.1E+00	0.874	0.126	0.000	
	alt	-9533.73	19137.61	10.306	0.001	50.000	Ref	0.000	0.817	1.0E+00	0.866	0.134	0.000	GO:0008270
	alt						Test	0.000	0.000	1.1E+00	0.866	0.134	0.000	GO:0061630
	PD	-9533.72	19145.64				Ref	0.000	0.814	1.6E+00	0.868	0.130	0.002	GO:0071596
	PD						Test	0.000	0.008	1.1E+00	1.000	0.000	0.000	GO:0030163
OG0004303.fa	null	-1490.62	3041.90			1.000	Ref	0.000	0.000	1.0E+00	0.004	0.958	0.037	
	alt	-1488.40	3039.50	4.444	0.035	13.824	Ref	0.000	0.000	1.6E+00	0.021	0.960	0.019	GO:0005515
	alt						Test	0.000	0.000	4.8E+02	0.021	0.960	0.019	GO:0031929
	PD	-1488.19	3047.27				Ref	0.000	0.000	1.0E+00	0.003	0.969	0.028	GO:0031931
	PD						Test	0.000	0.000	6.3E+01	0.105	0.507	0.388	GO:0031932
OG0004365.fa	null	-1101.56	2272.30			1.000	Ref	0.466	1.107	null	1.000	0.000	null	
	alt	-1098.49	2268.24	6.133	0.013	50.000	Ref	0.582	1.222	null	1.000	0.000	null	N/A
	alt						Test	0.000	0.029	null	1.000	0.000	null	
	PD	-1098.49	2274.46				Ref	0.581	1.000	null	1.000	0.000	null	
	PD						Test	0.000	0.027	null	1.000	0.000	null	
OG0004387.fa	null	-2040.91	4146.40			1.000	Ref	0.033	0.423	1.1E+00	0.591	0.148	0.261	
	alt	-2038.55	4143.71	4.730	0.030	18.067	Ref	0.000	0.000	1.1E+00	0.549	0.165	0.285	GO:0005515
	alt						Test	0.000	0.000	7.3E+00	0.549	0.165	0.285	GO:0005634
	PD	-2038.34	4151.45				Ref	0.085	0.251	1.0E+00	0.626	0.165	0.209	GO:0005737
	PD						Test	1.000	1.000	2.4E+00	0.000	0.001	0.999	GO:0061015
OG0004411.fa	null	-1728.77	3524.35			1.000	Ref	0.276	0.297	1.1E+00	0.227	0.773	0.000	GO:0005759
	alt	-1725.53	3519.93	6.473	0.011	29.412	Ref	0.325	0.336	1.0E+00	0.517	0.483	0.000	
	alt						Test	0.000	0.000	2.6E+00	0.517	0.483	0.000	

	PD	-1725.53	3528.14				Ref	0.329	0.345	1.0E+00	0.837	0.163	0.000	
	PD						Test	0.000	0.000	1.2E+00	0.018	0.982	0.000	
OG0004449.fa	null	-2646.88	5362.33			1.000	Ref	0.000	0.000	2.1E+00	0.303	0.520	0.177	N/A
	alt	-2643.58	5357.75	6.604	0.010	4.020	Ref	0.000	0.000	2.1E+00	0.289	0.552	0.159	
	alt						Test	0.000	0.000	1.9E+01	0.289	0.552	0.159	
	PD	-2643.37	5365.48				Ref	0.018	0.024	2.0E+00	0.495	0.349	0.156	
	PD						Test	0.972	1.000	4.9E+00	0.000	0.430	0.570	
OG0004490.fa	null	-5570.25	11208.75			1.000	Ref	0.000	1.000	6.9E+02	0.777	0.223	0.000	
	alt	-5567.54	11205.33	5.426	0.020	2.386	Ref	0.117	0.970	1.7E+01	0.885	0.113	0.001	GO:0004630
	alt						Test	0.006	0.930	8.7E+02	0.885	0.113	0.001	GO:0006654
	PD	-5567.18	11212.67				Ref	0.000	0.938	4.7E+00	0.791	0.197	0.012	GO:0048017
	PD						Test	0.037	0.448	8.5E+02	0.863	0.136	0.001	GO:0003824 GO:0035091
OG0004620.fa	null	-2278.73	4624.02			1.000	Ref	0.456	0.523	7.6E+02	0.401	0.598	0.000	
	alt	-2274.61	4617.82	8.237	0.004	4.501	Ref	0.153	0.761	4.2E+00	0.411	0.582	0.007	
	alt						Test	0.000	0.292	6.2E+02	0.411	0.582	0.007	GO:0003677
	PD	-2274.38	4625.51				Ref	0.488	0.518	2.0E+01	0.136	0.864	0.000	GO:0004518
	PD						Test	0.062	0.270	9.4E+02	0.145	0.849	0.007	
OG0004637.fa	null	-2229.76	4526.10			1.000	Ref	0.000	0.451	9.6E+00	0.068	0.889	0.043	
	alt	-2227.46	4523.54	4.590	0.032	4.295	Ref	0.000	0.477	6.7E+00	0.091	0.862	0.047	GO:0006412
	alt						Test	0.000	0.042	3.5E+03	0.091	0.862	0.047	
	PD	-2227.63	4532.04				Ref	0.000	0.490	7.9E+00	0.054	0.911	0.035	
	PD						Test	0.000	0.000	2.2E+02	0.933	0.009	0.058	
OG0004686.fa	null	-2305.83	4680.29			1.000	Ref	0.344	0.406	1.1E+00	0.000	1.000	0.000	
	alt	-2303.19	4677.06	5.275	0.022	37.876	Ref	0.361	0.436	1.0E+00	0.000	1.000	0.000	
	alt						Test	0.000	0.000	5.6E+00	0.000	1.000	0.000	GO:0008745
	PD	-2303.19	4685.22				Ref	0.437	1.000	1.4E+00	1.000	0.000	0.000	GO:0009253
	PD						Test	0.000	0.334	1.2E+00	1.000	0.000	0.000	GO:0008270
OG0004721.fa	null	-1332.41	2729.66			1.000	Ref	0.000	0.000	1.3E+00	0.330	0.657	0.013	GO:0019843
	alt	-1330.28	2727.44	4.266	0.039	19.430	Ref	0.000	0.000	1.2E+00	0.146	0.847	0.007	GO:0003723
	alt						Test	0.000	0.000	6.0E+01	0.146	0.847	0.007	GO:0003735
	PD	-1329.35	2733.82				Ref	0.000	0.000	1.5E+00	0.351	0.649	0.000	GO:0006412 GO:0015935

	PD						Test	0.000	0.000	2.2E+00	0.254	0.497	0.249	
OG0004820.fa	null	-4131.57	8331.47			1.000	Ref	0.367	0.471	1.9E+02	0.732	0.267	0.001	
	alt	-4129.59	8329.52	3.961	0.047	3.643	Ref	0.370	0.535	2.2E+02	0.619	0.381	0.001	
	alt						Test	0.027	0.102	3.7E+08	0.619	0.381	0.001	N/A
	PD	-4128.95	8336.33				Ref	0.392	0.498	1.8E+02	0.688	0.311	0.001	
	PD						Test	0.065	0.077	1.8E+01	0.991	0.009	0.000	
OG0004829.fa	null	-1881.64	3830.16			1.000	Ref	0.000	0.000	8.8E+00	0.853	0.040	0.107	
	alt	-1878.59	3826.11	6.101	0.014	2.907	Ref	0.000	0.000	7.9E+00	0.046	0.847	0.107	
	alt						Test	0.000	0.000	4.0E+02	0.046	0.847	0.107	GO:0004252 GO:0006508
	PD	-1878.66	3834.47				Ref	0.020	0.285	7.9E+00	0.858	0.040	0.102	
	PD						Test	0.000	0.000	1.7E+02	0.914	0.004	0.082	
OG0004851.fa	null	-2091.63	4249.88			1.000	Ref	0.215	0.231	1.3E+00	0.000	1.000	0.000	
	alt	-2087.53	4243.72	8.205	0.004	50.000	Ref	0.225	0.229	1.2E+00	0.009	0.987	0.004	
	alt						Test	0.000	0.000	1.3E+04	0.009	0.987	0.004	GO:0016491 GO:0055114
	PD	-2091.63	4260.07				Ref	0.206	0.230	1.2E+00	0.000	1.000	0.000	
	PD						Test	0.213	0.261	1.1E+00	0.000	1.000	0.000	
OG0004874.fa	null	-18247.10	36562.26			1.000	Ref	0.000	0.061	7.9E+03	0.008	0.992	0.000	
	alt	-18244.56	36559.19	5.074	0.024	43.520	Ref	0.000	0.064	3.8E+03	0.009	0.991	0.001	GO:0003677 GO:0000981 GO:0006355 GO:0003676 GO:0008270
	alt						Test	0.000	0.000	7.1E+155	0.009	0.991	0.001	
	PD	-18242.48	36563.05				Ref	0.057	1.000	1.0E+10	0.991	0.008	0.001	
	PD						Test	0.000	0.005	1.8E+01	1.000	0.000	0.000	
OG0004955.fa	null	-2717.93	5502.33			1.000	Ref	0.303	0.906	1.1E+00	1.000	0.000	0.000	
	alt	-2715.23	5498.96	5.393	0.020	28.813	Ref	0.333	0.952	1.0E+00	1.000	0.000	0.000	
	alt						Test	0.000	0.243	1.2E+00	1.000	0.000	0.000	N/A
	PD	-2715.23	5507.09				Ref	0.175	0.333	1.0E+00	0.000	1.000	0.000	
	PD						Test	0.000	0.906	1.0E+00	1.000	0.000	0.000	
OG0004962.fa	null	-4767.95	9602.17			1.000	Ref	0.000	0.246	1.4E+02	0.025	0.973	0.002	
	alt	-4762.19	9592.65	11.529	0.001	2.272	Ref	0.000	0.226	1.4E+01	0.126	0.864	0.010	GO:0005515 GO:0001736 GO:0060271 GO:0016192
	alt						Test	0.000	0.034	4.2E+02	0.126	0.864	0.010	
	PD	-4760.51	9597.37				Ref	0.000	0.239	2.7E+01	0.054	0.942	0.004	
	PD						Test	0.000	0.526	2.8E+03	0.030	0.966	0.004	

OG0004980.fa	null	-1514.62	3092.08			1.000	Ref	0.337	0.433	1.1E+00	0.000	1.000	0.000	N/A
	alt	-1512.46	3089.82	4.320	0.038	3.709	Ref	0.354	0.491	1.0E+00	0.000	1.000	0.000	
	alt						Test	0.021	0.072	1.0E+00	0.000	1.000	0.000	
	PD	-1512.46	3098.06				Ref	0.493	1.000	1.0E+00	1.000	0.000	0.000	
	PD						Test	0.071	0.322	1.1E+00	1.000	0.000	0.000	
OG0004992.fa	null	-1185.10	2433.16			1.000	Ref	0.290	0.919	1.1E+00	1.000	0.000	0.000	GO:0005789 GO:0006506
	alt	-1183.04	2431.10	4.114	0.043	30.517	Ref	0.340	0.963	1.0E+00	1.000	0.000	0.000	
	alt						Test	0.000	0.320	2.3E+00	1.000	0.000	0.000	
	PD	-1183.04	2439.37				Ref	0.341	0.496	1.2E+00	1.000	0.000	0.000	
	PD						Test	0.000	0.878	1.2E+00	1.000	0.000	0.000	
OG0005000.fa	null	-5262.22	10590.68			1.000	Ref	0.000	0.000	1.0E+00	0.469	0.410	0.121	GO:0004114 GO:0007165 GO:0008081
	alt	-5257.65	10583.54	9.152	0.002	12.552	Ref	0.000	0.000	1.2E+00	0.553	0.360	0.087	
	alt						Test	0.000	0.000	6.3E+00	0.553	0.360	0.087	
	PD	-5257.37	10591.05				Ref	0.000	0.000	1.0E+00	0.422	0.479	0.099	
	PD						Test	0.000	0.000	2.0E+00	0.453	0.265	0.282	
OG0005107.fa	null	-3038.96	6146.36			1.000	Ref	0.118	0.298	1.0E+00	0.980	0.020	0.000	GO:0004672 GO:0005524 GO:0006468
	alt	-3037.00	6144.46	3.923	0.048	10.671	Ref	0.014	0.856	1.2E+00	0.920	0.000	0.080	
	alt						Test	0.000	0.191	6.3E+00	0.920	0.000	0.080	
	PD	-3036.57	6151.71				Ref	0.099	0.176	1.0E+00	0.979	0.021	0.000	
	PD						Test	0.348	0.653	1.0E+00	0.679	0.172	0.149	
OG0005128.fa	null	-1412.40	2891.66			1.000	Ref	0.189	1.000	5.1E+02	0.994	0.006	0.001	GO:0006801 GO:0046872 GO:0004784 GO:0055114 GO:0030001
	alt	-1410.23	2889.38	4.334	0.037	5.696	Ref	0.161	0.180	2.8E+00	0.113	0.878	0.008	
	alt						Test	0.000	0.000	3.9E+02	0.113	0.878	0.008	
	PD	-1409.88	2896.92				Ref	0.193	0.267	2.0E+01	0.976	0.024	0.000	
	PD						Test	0.283	1.000	1.0E+10	0.992	0.004	0.004	
OG0005228.fa	null	-9546.72	19161.58			1.000	Ref	0.000	0.000	2.1E+00	0.876	0.002	0.122	GO:0004725 GO:0006470 GO:0005515
	alt	-9544.68	19159.52	4.070	0.044	2.391	Ref	0.000	0.000	2.1E+00	0.000	0.881	0.119	
	alt						Test	0.000	0.000	5.6E+00	0.000	0.881	0.119	
	PD	-9544.57	19167.33				Ref	0.000	0.003	2.1E+00	0.000	0.883	0.117	
	PD						Test	0.293	0.918	1.4E+00	0.517	0.361	0.122	
OG0005260.fa	null	-3150.09	6366.57			1.000	Ref	0.000	0.000	4.5E+00	0.245	0.743	0.013	N/A

	alt	-3146.98	6362.39	6.206	0.013	6.399	Ref	0.000	0.000	1.6E+00	0.549	0.427	0.024	
	alt						Test	0.000	0.000	2.0E+01	0.549	0.427	0.024	
	PD	-3146.88	6370.30				Ref	0.000	0.000	1.2E+00	0.876	0.095	0.030	
	PD						Test	0.000	0.000	3.1E+00	0.437	0.429	0.134	
OG0005290.fa	null	-2918.36	5905.15			1.000	Ref	0.000	0.000	4.6E+00	0.809	0.140	0.051	GO:0016758 GO:0005783 GO:0006490 GO:0042283
	alt	-2916.15	5902.75	4.421	0.036	2.684	Ref	0.000	0.000	3.6E+00	0.792	0.153	0.056	
	alt						Test	0.000	0.000	3.1E+01	0.792	0.153	0.056	
	PD	-2915.87	5910.30				Ref	0.000	0.000	4.3E+00	0.818	0.135	0.047	
	PD						Test	0.000	0.000	3.5E+00	0.380	0.110	0.510	
OG0005394.fa	null	-6440.53	12949.29			1.000	Ref	0.000	0.919	1.0E+00	0.616	0.340	0.043	N/A
	alt	-6437.68	12945.61	5.698	0.017	50.000	Ref	0.000	0.931	1.0E+00	0.606	0.343	0.051	
	alt						Test	0.000	0.028	1.0E+00	0.606	0.343	0.051	
	PD	-6437.66	12953.63				Ref	0.000	0.905	1.5E+00	0.620	0.329	0.051	
	PD						Test	0.013	0.751	1.1E+00	0.938	0.062	0.000	
OG0005449.fa	null	-8596.00	17260.16			1.000	Ref	0.150	0.151	1.0E+10	0.006	0.994	0.000	GO:0005515 GO:0007165
	alt	-8593.25	17256.67	5.497	0.019	17.233	Ref	0.157	0.161	1.0E+10	0.994	0.006	0.000	
	alt						Test	0.000	0.000	2.2E+172	0.994	0.006	0.000	
	PD	-8593.22	17264.64				Ref	0.155	0.157	1.0E+10	0.006	0.994	0.000	
	PD						Test	0.000	0.012	1.7E+01	1.000	0.000	0.000	
OG0005453.fa	null	-2549.77	5170.12			1.000	Ref	0.000	1.554	null	0.884	0.115	null	GO:0006508 GO:0008236
	alt	-2547.78	5168.16	3.993	0.046	4.648	Ref	0.000	1.949	null	0.903	0.095	null	
	alt						Test	0.000	0.000	null	0.903	0.095	null	
	PD	-2547.79	5174.29				Ref	0.000	1.649	null	0.884	0.116	null	
	PD						Test	0.000	0.000	null	0.862	0.136	null	
OG0005534.fa	null	-8146.53	16361.23			1.000	Ref	0.000	0.000	1.2E+00	0.782	0.151	0.066	GO:0005515
	alt	-8140.63	16351.43	11.811	0.001	7.666	Ref	0.000	0.000	1.3E+00	0.779	0.167	0.054	
	alt						Test	0.000	0.000	6.4E+00	0.779	0.167	0.054	
	PD	-8140.15	16358.51				Ref	0.000	0.050	1.0E+00	0.020	0.966	0.015	
	PD						Test	0.000	0.326	1.0E+00	0.000	1.000	0.000	
OG0005546.fa	null	-2013.65	4089.85			1.000	Ref	0.292	0.874	6.0E+00	1.000	0.000	0.000	N/A
	alt	-2011.54	4087.66	4.222	0.040	30.062	Ref	0.327	0.917	8.8E+00	1.000	0.000	0.000	

	alt						Test	0.000	0.074	2.4E+28	1.000	0.000	0.000	
	PD	-2011.54	4095.81				Ref	0.327	0.834	4.5E+00	1.000	0.000	0.000	
	PD						Test	0.000	0.834	4.5E+00	1.000	0.000	0.000	
OG0005584.fa	null	-7685.71	15439.61			1.000	Ref	0.001	0.003	1.3E+00	0.000	0.904	0.096	
	alt	-7682.75	15435.69	5.930	0.015	5.295	Ref	0.000	0.000	1.3E+00	0.909	0.000	0.090	
	alt						Test	0.000	0.000	4.0E+00	0.909	0.000	0.090	GO:0005515 GO:0007165
	PD	-7682.50	15443.25				Ref	0.004	0.295	1.3E+00	0.909	0.002	0.089	
	PD						Test	0.191	0.239	1.0E+00	0.000	0.847	0.153	
	null	-4177.49	8421.33			1.000	Ref	0.245	1.000	2.6E+03	0.823	0.177	0.000	
OG0005667.fa	alt	-4172.96	8414.30	9.057	0.003	13.938	Ref	0.134	1.000	2.2E+00	0.704	0.293	0.003	
	alt						Test	0.000	1.000	7.7E+04	0.704	0.293	0.003	N/A
	PD	-4172.93	8422.31				Ref	0.217	1.000	3.5E+00	0.796	0.196	0.007	
	PD						Test	0.289	0.359	1.0E+10	0.926	0.071	0.003	
	null	-1335.99	2737.03			1.000	Ref	0.174	0.219	1.1E+02	0.930	0.065	0.005	
OG0005694.fa	alt	-1332.88	2732.86	6.233	0.013	3.270	Ref	0.144	0.146	2.5E+01	0.132	0.858	0.010	
	alt						Test	0.002	0.002	3.8E+04	0.132	0.858	0.010	N/A
	PD	-1331.84	2739.08				Ref	0.174	0.192	4.5E+01	0.930	0.065	0.005	
	PD						Test	1.000	1.000	1.0E+10	0.932	0.062	0.006	
	null	-5480.64	11027.50			1.000	Ref	0.291	0.346	6.1E+02	0.988	0.012	0.001	
OG0005695.fa	alt	-5478.43	11025.09	4.419	0.036	1.697	Ref	0.286	1.000	1.0E+02	0.980	0.018	0.001	
	alt						Test	0.119	1.000	2.5E+03	0.980	0.018	0.001	GO:0001510 GO:0008168 GO:0009452
	PD	-5477.66	11031.60				Ref	0.273	0.329	6.1E+01	0.956	0.042	0.002	
	PD						Test	0.308	0.310	1.0E+10	0.987	0.011	0.002	
	null	-3581.43	7231.25			1.000	Ref	0.000	0.712	2.6E+03	0.001	0.999	0.001	
OG0005778.fa	alt	-3578.81	7228.03	5.241	0.022	9.105	Ref	0.000	0.767	2.0E+03	0.001	0.999	0.001	
	alt						Test	0.000	0.089	1.3E+30	0.001	0.999	0.001	N/A
	PD	-3578.39	7235.29				Ref	0.767	1.000	1.9E+03	0.999	0.001	0.001	
	PD						Test	0.070	0.239	1.9E+01	0.885	0.115	0.000	
	null	-3244.03	6554.45			1.000	Ref	0.259	0.260	1.7E+02	0.997	0.003	0.000	
OG0005816.fa	alt	-3240.91	6550.24	6.236	0.013	5.621	Ref	0.248	0.253	2.5E+00	0.853	0.139	0.007	GO:0005515
	alt						Test	0.000	0.000	1.8E+02	0.853	0.139	0.007	

	PD	-3240.82	6558.16				Ref	0.249	0.262	2.0E+01	0.000	1.000	0.000	
	PD						Test	0.000	0.000	1.9E+02	0.992	0.000	0.008	
OG0005919.fa	null	-3168.12	6400.62			1.000	Ref	0.226	0.920	7.1E+00	1.000	0.000	0.000	
	alt	-3164.38	6395.15	7.492	0.006	24.260	Ref	0.257	0.967	1.2E+01	1.000	0.000	0.000	
	alt						Test	0.000	0.442	9.2E+25	1.000	0.000	0.000	N/A
	PD	-3164.32	6403.14				Ref	0.206	1.000	2.8E+00	0.975	0.005	0.021	
	PD						Test	0.000	0.867	4.8E+00	1.000	0.000	0.000	
OG0005972.fa	null	-4175.47	8419.26			1.000	Ref	0.216	0.224	1.3E+02	0.999	0.000	0.000	
	alt	-4170.58	8411.51	9.771	0.002	50.000	Ref	0.236	0.246	1.1E+00	0.988	0.008	0.004	
	alt						Test	0.000	0.000	4.0E+01	0.988	0.008	0.004	N/A
	PD	-4170.09	8418.62				Ref	0.214	0.239	2.2E+01	0.000	1.000	0.000	
	PD						Test	0.027	0.105	1.5E+02	0.998	0.000	0.002	
OG0006028.fa	null	-6520.60	13107.39			1.000	Ref	0.154	1.000	2.0E+03	0.991	0.009	0.000	GO:0003676
	alt	-6517.37	13102.93	6.467	0.011	50.000	Ref	0.173	0.892	2.0E+03	0.990	0.009	0.001	GO:0000289
	alt						Test	0.000	0.003	2.8E+165	0.990	0.009	0.001	GO:0004535
	PD	-6517.36	13110.96				Ref	0.172	1.000	2.0E+03	0.990	0.010	0.001	GO:0006397
	PD						Test	0.000	0.943	2.0E+01	1.000	0.000	0.000	GO:0031251
OG0006073.fa	null	-1015.74	2096.81			1.000	Ref	0.222	0.427	1.1E+00	0.000	1.000	0.000	
	alt	-1012.97	2093.35	5.541	0.019	4.352	Ref	0.298	0.527	1.0E+00	0.000	1.000	0.000	
	alt						Test	0.005	0.061	1.1E+00	0.000	1.000	0.000	N/A
	PD	-1012.97	2101.71				Ref	0.212	0.527	1.2E+00	0.000	1.000	0.000	
	PD						Test	0.061	0.061	1.2E+00	0.000	1.000	0.000	
OG0006100.fa	null	-11514.31	23094.71			1.000	Ref	0.000	0.000	2.1E+00	0.000	0.948	0.052	
	alt	-11510.84	23089.80	6.925	0.009	2.879	Ref	0.000	0.000	2.0E+00	0.000	0.952	0.048	
	alt						Test	0.000	0.000	7.0E+00	0.000	0.952	0.048	N/A
	PD	-11510.44	23097.02				Ref	0.001	0.001	2.3E+00	0.000	0.959	0.041	
	PD						Test	0.159	0.330	1.3E+00	0.000	1.000	0.000	
OG0006105.fa	null	-2340.61	4745.81			1.000	Ref	0.000	1.000	3.5E+02	0.595	0.403	0.002	
	alt	-2335.34	4737.31	10.542	0.001	2.450	Ref	0.000	1.000	1.6E+01	0.643	0.348	0.010	
	alt						Test	0.000	1.000	8.9E+02	0.643	0.348	0.010	GO:0004531
	PD	-2335.05	4744.89				Ref	0.000	1.000	2.3E+01	0.639	0.354	0.007	GO:0006259
	PD						Test	0.597	1.000	1.4E+03	0.760	0.230	0.010	

Genes with significant evidence for relaxed natural selection

OG0003435.fa	null	-5723.30	11514.84			1.000	Ref	0.032	0.096	1.0E+00	0.254	0.746	0.000	GO:0000184 GO:0003723 GO:0005515
	alt	-5721.08	11512.41	4.440	0.035	0.355	Ref	0.002	0.104	4.3E+00	0.325	0.675	0.000	
	alt						Test	0.103	0.448	1.7E+00	0.325	0.675	0.000	
	PD	-5721.07	11520.45				Ref	0.030	0.080	1.0E+00	0.191	0.809	0.000	
	PD						Test	0.064	0.244	1.0E+00	0.090	0.768	0.141	
OG0003511.fa	null	-14315.72	28699.54			1.000	Ref	0.278	0.412	3.4E+01	0.962	0.035	0.003	N/A
	alt	-14313.40	28696.91	4.635	0.031	0.552	Ref	0.208	0.273	3.7E+01	0.035	0.962	0.003	
	alt						Test	0.420	0.489	7.3E+00	0.035	0.962	0.003	
	PD	-14313.32	28704.78				Ref	0.270	0.329	3.9E+01	0.959	0.038	0.003	
	PD						Test	0.481	0.517	1.0E+00	0.878	0.122	0.000	
OG0003562.fa	null	-3896.87	7860.07			1.000	Ref	0.141	0.301	1.0E+00	0.821	0.179	0.000	GO:0016627 GO:0022900 GO:0050660 GO:0055114 GO:0006099 GO:0016491
	alt	-3893.11	7854.56	7.527	0.006	0.000	Ref	0.125	0.226	1.0E+00	0.798	0.202	0.000	
	alt						Test	1.000	1.000	1.0E+00	0.798	0.202	0.000	
	PD	-3893.06	7862.56				Ref	0.124	0.215	1.0E+00	0.768	0.232	0.000	
	PD						Test	1.000	1.000	1.7E+00	0.549	0.054	0.397	
OG0003585.fa	null	-2061.82	4190.25			1.000	Ref	0.193	0.430	1.0E+00	0.951	0.049	0.000	GO:0016192
	alt	-2059.31	4187.26	5.031	0.025	0.000	Ref	0.145	0.328	1.0E+00	0.871	0.129	0.000	
	alt						Test	1.000	1.000	1.0E+00	0.871	0.129	0.000	
	PD	-2059.30	4195.41				Ref	0.157	0.293	1.0E+00	0.914	0.086	0.000	
	PD						Test	0.712	0.772	1.6E+00	0.069	0.526	0.405	
OG0003807.fa	null	-23542.12	47152.29			1.000	Ref	0.003	0.032	6.3E+00	0.268	0.732	0.000	GO:0005524 GO:0007018 GO:0008569 GO:0030286
	alt	-23538.77	47147.61	6.683	0.010	0.598	Ref	0.002	0.032	9.2E+00	0.334	0.666	0.000	
	alt						Test	0.021	0.128	3.8E+00	0.334	0.666	0.000	
	PD	-23538.78	47155.63				Ref	0.000	0.026	4.6E+00	0.148	0.852	0.000	
	PD						Test	0.069	0.073	1.0E+00	0.399	0.579	0.022	
OG0003810.fa	null	-3248.86	6562.08			1.000	Ref	0.071	0.256	1.0E+10	0.997	0.002	0.001	GO:0004252 GO:0006508 GO:0008236
	alt	-3246.77	6559.93	4.176	0.041	0.433	Ref	0.061	0.061	1.0E+10	0.997	0.002	0.001	
	alt						Test	0.297	0.299	2.1E+04	0.997	0.002	0.001	

	PD	-3239.88	6554.24				Ref	0.047	0.053	8.6E+01	0.000	0.999	0.001	
	PD						Test	0.533	1.000	4.2E+09	0.993	0.001	0.006	
OG0003842.fa	null	-1922.46	3909.53			1.000	Ref	0.003	0.200	1.0E+00	0.000	1.000	0.000	
	alt	-1919.86	3906.36	5.201	0.023	0.000	Ref	0.003	0.175	1.0E+00	0.000	1.000	0.000	GO:0000290
	alt						Test	1.000	1.000	1.0E+00	0.000	1.000	0.000	GO:0016787
	PD	-1919.86	3914.53				Ref	0.002	0.175	1.1E+00	0.000	1.000	0.000	
	PD						Test	1.000	1.000	1.0E+00	0.059	0.219	0.723	
OG0003921.fa	null	-4795.68	9657.61			1.000	Ref	0.137	0.203	1.0E+00	0.164	0.836	0.000	
	alt	-4793.58	9655.43	4.191	0.041	0.395	Ref	0.057	0.204	2.4E+00	0.226	0.774	0.000	
	alt						Test	0.323	0.533	1.4E+00	0.226	0.774	0.000	N/A
	PD	-4793.58	9663.50				Ref	0.131	0.179	1.1E+00	0.175	0.825	0.000	
	PD						Test	0.385	0.441	1.0E+00	0.012	0.914	0.074	
OG0004087.fa	null	-1783.32	3633.40			1.000	Ref	0.275	0.326	1.0E+00	1.000	0.000	0.000	
	alt	-1781.08	3630.95	4.492	0.034	0.000	Ref	0.238	0.256	1.1E+00	0.882	0.118	0.000	GO:0003824
	alt						Test	1.000	1.000	1.0E+00	0.882	0.118	0.000	GO:0016833
	PD	-1780.85	3638.69				Ref	0.241	0.311	1.1E+00	1.000	0.000	0.000	GO:0004419
	PD						Test	1.000	1.000	3.2E+00	0.481	0.077	0.442	
OG0004119.fa	null	-7721.93	15512.04			1.000	Ref	0.246	0.565	4.2E+01	0.964	0.035	0.001	
	alt	-7718.81	15507.82	6.225	0.013	0.032	Ref	0.238	0.302	4.7E+01	0.965	0.034	0.001	GO:0016192
	alt						Test	0.955	0.963	1.1E+00	0.965	0.034	0.001	GO:0030123
	PD	-7718.82	15515.88				Ref	0.233	0.436	5.2E+01	0.963	0.037	0.000	GO:0006886
	PD						Test	0.816	0.821	1.6E+00	0.814	0.007	0.179	GO:0030117
OG0004166.fa	null	-7873.41	15812.97			1.000	Ref	0.000	0.000	2.7E+01	0.779	0.221	0.000	GO:0005198
	alt	-7870.67	15809.49	5.491	0.019	0.261	Ref	0.000	0.000	1.7E+01	0.702	0.298	0.000	GO:0006886
	alt						Test	0.044	0.053	2.1E+00	0.702	0.298	0.000	GO:0016192
	PD	-7870.66	15817.52				Ref	0.000	0.000	1.1E+01	0.743	0.257	0.000	GO:0030130
	PD						Test	0.000	0.041	1.2E+00	0.000	0.995	0.005	GO:0030132
OG0004201.fa	null	-3262.92	6592.24			1.000	Ref	0.238	0.714	1.1E+00	0.790	0.066	0.143	GO:0005515
	alt	-3260.43	6589.30	4.972	0.026	0.000	Ref	0.225	0.524	1.0E+00	0.773	0.119	0.108	GO:0032051
	alt						Test	1.000	1.000	1.0E+00	0.773	0.119	0.108	GO:0071439
	PD	-3259.81	6596.15				Ref	0.226	0.796	1.0E+00	0.793	0.207	0.000	GO:0004423
														GO:0008484
														GO:0003824

	PD						Test	1.000	1.000	4.1E+00	0.336	0.074	0.590	
OG0004376.fa	null	-2327.26	4721.12			1.000	Ref	0.168	0.723	1.4E+01	0.961	0.034	0.005	GO:0004984 GO:0005549 GO:0007608 GO:0016020
	alt	-2325.09	4718.80	4.358	0.037	0.000	Ref	0.163	0.420	1.5E+01	0.964	0.032	0.004	
	alt						Test	1.000	1.000	1.0E+00	0.964	0.032	0.004	
	PD	-2324.93	4726.65				Ref	0.165	0.266	1.3E+01	0.972	0.023	0.005	
	PD						Test	1.000	1.000	4.0E+00	0.548	0.173	0.279	
OG0004468.fa	null	-2497.71	5059.88			1.000	Ref	0.000	0.185	4.8E+00	0.677	0.238	0.085	GO:0001522 GO:0003723 GO:0009451 GO:0009982
	alt	-2495.75	5057.99	3.915	0.048	0.000	Ref	0.000	0.000	5.4E+00	0.183	0.719	0.098	
	alt						Test	0.000	0.000	1.0E+00	0.183	0.719	0.098	
	PD	-2495.70	5066.00				Ref	0.000	0.076	5.1E+00	0.717	0.183	0.100	
	PD						Test	0.022	0.107	3.6E+00	0.404	0.596	0.000	
OG0004503.fa	null	-3604.27	7274.92			1.000	Ref	0.105	1.000	2.7E+00	0.973	0.005	0.021	GO:0016021
	alt	-3601.35	7271.10	5.845	0.016	0.141	Ref	0.000	0.102	2.7E+00	0.004	0.979	0.017	
	alt						Test	0.000	0.724	1.2E+00	0.004	0.979	0.017	
	PD	-3601.35	7279.21				Ref	0.101	0.995	2.7E+00	0.983	0.000	0.017	
	PD						Test	0.526	0.557	1.4E+00	0.013	0.798	0.189	
OG0004504.fa	null	-4683.44	9435.15			1.000	Ref	0.088	0.668	1.0E+00	1.000	0.000	0.000	GO:0006464 GO:0005524
	alt	-4680.34	9430.97	6.196	0.013	0.181	Ref	0.033	0.074	1.0E+00	0.000	1.000	0.000	
	alt						Test	0.540	0.623	1.0E+00	0.000	1.000	0.000	
	PD	-4680.34	9439.05				Ref	0.072	0.114	1.0E+00	0.958	0.042	0.000	
	PD						Test	0.000	0.478	1.0E+00	0.016	0.682	0.301	
OG0004687.fa	null	-3373.87	6814.15			1.000	Ref	0.174	0.253	8.8E+00	0.951	0.049	0.000	GO:0004553 GO:0005975 GO:0004563
	alt	-3369.75	6807.92	8.253	0.004	0.046	Ref	0.085	0.157	1.6E+01	0.078	0.922	0.000	
	alt						Test	0.892	0.918	1.1E+00	0.078	0.922	0.000	
	PD	-3369.74	6816.02				Ref	0.023	0.151	6.0E+00	0.000	1.000	0.000	
	PD						Test	0.315	0.914	4.6E+00	0.000	1.000	0.000	
OG0004708.fa	null	-2303.21	4673.01			1.000	Ref	0.000	0.019	5.7E+00	0.443	0.502	0.055	N/A
	alt	-2300.30	4669.22	5.825	0.016	0.000	Ref	0.000	0.000	6.8E+00	0.520	0.424	0.056	
	alt						Test	0.000	0.000	1.0E+00	0.520	0.424	0.056	
	PD	-2299.46	4675.71				Ref	0.000	0.000	6.0E+00	0.503	0.435	0.062	
	PD						Test	0.000	0.000	4.1E+00	0.093	0.907	0.000	

OG0004733.fa	null	-1523.96	3113.08			1.000	Ref	0.384	1.000	1.4E+01	0.723	0.156	0.121	N/A
	alt	-1521.46	3110.17	4.985	0.026	0.000	Ref	0.000	1.000	1.2E+01	0.605	0.214	0.181	
	alt						Test	0.000	1.000	1.0E+00	0.605	0.214	0.181	
	PD	-1520.86	3117.29				Ref	0.532	1.000	1.9E+01	0.753	0.136	0.111	
	PD						Test	0.000	1.000	1.1E+00	0.764	0.236	0.000	
OG0004826.fa	null	-6506.27	13080.80			1.000	Ref	1.000	1.000	3.7E+01	0.815	0.163	0.022	GO:0006508 GO:0008237 GO:0008270
	alt	-6503.98	13078.23	4.587	0.032	0.000	Ref	1.000	1.000	3.9E+01	0.800	0.176	0.024	
	alt						Test	1.000	1.000	1.0E+00	0.800	0.176	0.024	
	PD	-6502.40	13083.14				Ref	1.000	1.000	4.0E+01	0.802	0.175	0.024	
	PD						Test	0.061	0.437	1.5E+01	0.154	0.846	0.000	
OG0004896.fa	null	-2594.93	5256.41			1.000	Ref	0.000	0.000	4.2E+00	0.773	0.043	0.183	GO:0000139
	alt	-2591.93	5252.45	5.993	0.014	0.000	Ref	0.000	0.000	5.5E+00	0.797	0.042	0.161	
	alt						Test	0.000	0.000	1.0E+00	0.797	0.042	0.161	
	PD	-2591.67	5260.08				Ref	0.000	0.000	4.7E+00	0.774	0.043	0.183	
	PD						Test	0.017	0.023	1.0E+00	0.702	0.234	0.064	
OG0004939.fa	null	-5818.04	11702.30			1.000	Ref	0.001	0.109	3.0E+00	0.000	0.906	0.094	N/A
	alt	-5813.28	11694.79	9.522	0.002	0.000	Ref	0.000	0.000	4.0E+00	0.890	0.000	0.110	
	alt						Test	0.000	0.996	1.0E+00	0.890	0.000	0.110	
	PD	-5810.03	11696.34				Ref	0.001	0.097	3.2E+00	0.000	0.891	0.109	
	PD						Test	0.000	0.098	2.4E+00	1.000	0.000	0.000	
OG0004942.fa	null	-9476.27	19020.68			1.000	Ref	0.004	0.213	2.9E+01	0.874	0.125	0.001	GO:0032968 GO:0006139 GO:0003676 GO:0003677
	alt	-9473.77	19017.70	4.990	0.025	0.065	Ref	0.000	0.203	3.9E+01	0.874	0.125	0.001	
	alt						Test	0.000	0.902	1.3E+00	0.874	0.125	0.001	
	PD	-9473.67	19025.54				Ref	0.008	0.168	4.7E+01	0.882	0.117	0.001	
	PD						Test	0.069	0.245	1.0E+00	0.746	0.254	0.000	
OG0005042.fa	null	-2104.34	4273.25			1.000	Ref	0.151	0.248	1.0E+00	0.998	0.002	0.000	GO:0003824 GO:0005965 GO:0018343
	alt	-2101.24	4269.08	6.204	0.013	0.219	Ref	0.099	0.226	1.0E+00	0.936	0.064	0.000	
	alt						Test	0.603	0.722	1.0E+00	0.936	0.064	0.000	
	PD	-2101.24	4277.23				Ref	0.108	0.236	1.1E+00	1.000	0.000	0.000	
	PD						Test	0.364	0.517	1.0E+00	0.032	0.766	0.202	
OG0005045.fa	null	-3017.40	6101.20			1.000	Ref	0.261	0.330	4.9E+02	0.985	0.015	0.000	

	alt	-3014.87	6098.16	5.068	0.024	0.000	Ref	0.220	0.223	3.8E+02	0.020	0.979	0.000	GO:0016757 GO:0000030
	alt						Test	1.000	1.000	1.0E+00	0.979	0.020	0.000	
	PD	-3014.86	6106.23				Ref	0.225	0.229	5.0E+02	0.983	0.017	0.000	
	PD						Test	1.000	1.000	3.4E+02	0.276	0.724	0.000	
OG0005057.fa	null	-3525.45	7119.28			1.000	Ref	0.257	1.000	3.1E+02	0.995	0.004	0.001	GO:0045162
	alt	-3523.29	7116.99	4.316	0.038	0.000	Ref	0.239	0.244	3.6E+02	0.004	0.995	0.001	
	alt						Test	1.000	1.000	1.0E+00	0.995	0.004	0.001	
	PD	-3523.04	7124.59				Ref	0.246	0.248	5.1E+02	0.995	0.004	0.001	
	PD						Test	1.000	1.000	7.8E+00	0.781	0.059	0.160	
OG0005150.fa	null	-2917.60	5899.60			1.000	Ref	0.105	0.134	1.7E+02	0.995	0.005	0.000	GO:0006904 GO:0016192
	alt	-2914.98	5896.37	5.254	0.022	0.352	Ref	0.074	0.084	1.5E+02	0.005	0.994	0.001	
	alt						Test	0.400	0.418	5.9E+00	0.005	0.994	0.001	
	PD	-2914.98	5904.48				Ref	0.085	0.090	1.7E+02	0.993	0.006	0.001	
	PD						Test	0.330	1.000	1.9E+01	0.864	0.136	0.000	
OG0005243.fa	null	-4937.91	9944.13			1.000	Ref	0.117	0.870	2.5E+00	0.925	0.024	0.051	N/A
	alt	-4934.73	9939.79	6.361	0.012	0.066	Ref	0.112	1.000	2.4E+00	0.932	0.025	0.043	
	alt						Test	0.866	1.000	1.1E+00	0.932	0.025	0.043	
	PD	-4934.73	9947.87				Ref	0.116	0.389	2.3E+00	0.934	0.013	0.052	
	PD						Test	0.703	0.735	1.3E+00	0.035	0.709	0.256	
OG0005261.fa	null	-1433.14	2933.23			1.000	Ref	0.001	0.156	9.5E+00	0.000	1.000	0.000	GO:0003725
	alt	-1431.11	2931.22	4.059	0.044	0.000	Ref	0.000	0.137	1.8E+01	0.000	1.000	0.000	
	alt						Test	1.000	1.000	1.0E+00	1.000	0.000	0.000	
	PD	-1430.41	2938.07				Ref	0.001	0.137	5.6E+00	0.000	1.000	0.000	
	PD						Test	1.000	1.000	1.6E+02	0.645	0.095	0.260	
OG0005465.fa	null	-2013.74	4090.01			1.000	Ref	0.000	0.125	2.7E+00	0.051	0.863	0.085	GO:0006269
	alt	-2011.15	4086.86	5.184	0.023	0.000	Ref	0.102	0.243	1.0E+00	0.000	1.000	0.000	
	alt						Test	1.000	1.000	1.0E+00	1.000	0.000	0.000	
	PD	-2009.22	4091.15				Ref	0.245	0.414	1.0E+00	1.000	0.000	0.000	
	PD						Test	0.000	0.085	2.2E+01	0.169	0.745	0.086	
OG0005595.fa	null	-3000.44	6067.29			1.000	Ref	0.232	0.463	1.0E+00	0.900	0.100	0.000	GO:0008495 GO:0016021
	alt	-2998.29	6065.03	4.292	0.038	0.133	Ref	0.191	0.458	1.0E+00	0.897	0.103	0.000	

	alt						Test	0.803	0.902	1.0E+00	0.897	0.103	0.000	GO:0048034
	PD	-2998.29	6073.14				Ref	0.193	0.513	1.1E+00	0.920	0.080	0.000	GO:0016765
	PD						Test	0.711	1.000	1.0E+00	0.661	0.041	0.298	GO:0006783
														GO:0031966
OG0005613.fa	null	-3318.92	6704.22			1.000	Ref	0.158	0.299	1.0E+00	0.937	0.063	0.000	
	alt	-3315.63	6699.66	6.588	0.010	0.064	Ref	0.118	0.239	1.2E+00	0.840	0.160	0.000	
	alt						Test	0.873	0.913	1.0E+00	0.840	0.160	0.000	GO:0035091
	PD	-3315.63	6707.74				Ref	0.132	0.229	1.1E+00	0.946	0.054	0.000	
	PD						Test	0.481	0.896	1.0E+00	0.068	0.778	0.155	
OG0005615.fa	null	-1706.58	3477.87			1.000	Ref	0.054	0.295	1.0E+00	0.999	0.001	0.000	
	alt	-1704.33	3475.43	4.490	0.034	0.225	Ref	0.014	0.042	1.0E+00	0.000	1.000	0.000	GO:0005524
	alt						Test	0.385	0.489	1.0E+00	0.000	1.000	0.000	GO:0004672
	PD	-1704.33	3483.62				Ref	0.042	0.283	1.2E+00	1.000	0.000	0.000	GO:0006468
	PD						Test	0.252	0.372	1.0E+00	0.022	0.791	0.188	
OG0005644.fa	null	-5011.99	10092.31			1.000	Ref	0.000	0.546	3.0E+00	0.583	0.212	0.205	
	alt	-5008.76	10087.86	6.464	0.011	0.000	Ref	0.000	0.995	3.1E+00	0.739	0.000	0.261	GO:0006260
	alt						Test	0.000	1.000	1.0E+00	0.739	0.000	0.261	GO:0055114
	PD	-5008.49	10095.40				Ref	0.092	0.349	3.9E+00	0.404	0.439	0.156	GO:0004748
	PD						Test	0.199	0.327	1.0E+00	0.976	0.024	0.000	GO:0005524
OG0005676.fa	null	-6700.20	13466.58			1.000	Ref	0.128	0.203	2.1E+00	0.000	1.000	0.000	GO:0004813
	alt	-6695.83	13459.86	8.734	0.003	0.000	Ref	0.143	0.199	2.4E+00	0.206	0.794	0.000	GO:0000166
	alt						Test	1.000	1.000	1.0E+00	0.794	0.206	0.000	GO:0005524
	PD	-6695.82	13467.90				Ref	0.187	1.000	1.1E+00	1.000	0.000	0.000	GO:0005737
	PD						Test	0.122	1.000	1.8E+00	0.000	1.000	0.000	GO:0006419
OG0005724.fa	null	-4050.28	8168.89			1.000	Ref	0.385	1.000	1.9E+02	0.996	0.004	0.000	GO:0003676
	alt	-4048.31	8166.97	3.942	0.047	0.000	Ref	0.337	1.000	1.5E+02	0.998	0.002	0.001	GO:0004812
	alt						Test	1.000	1.000	1.0E+00	0.998	0.002	0.001	GO:0043039
	PD	-4048.31	8175.05				Ref	0.000	0.342	1.8E+02	0.005	0.994	0.001	
	PD						Test	1.000	1.000	1.9E+01	0.665	0.335	0.000	
OG0005812.fa	null	-1248.09	2563.25			1.000	Ref	0.027	1.000	4.5E+00	0.913	0.000	0.087	GO:0000387
	alt	-1245.07	2559.27	6.048	0.014	0.000	Ref	0.000	0.000	9.1E+00	0.367	0.571	0.062	GO:0032797

	alt						Test	0.000	0.000	1.0E+00	0.367	0.571	0.062	
	PD	-1244.17	2565.75				Ref	0.000	0.126	5.4E+00	0.902	0.000	0.098	
	PD						Test	0.000	0.998	3.5E+00	1.000	0.000	0.000	
OG0005865.fa	null	-3658.92	7384.23			1.000	Ref	0.063	0.167	1.3E+01	0.244	0.751	0.005	GO:0005524
	alt	-3656.62	7381.64	4.615	0.032	0.142	Ref	0.038	0.169	1.2E+01	0.253	0.742	0.004	GO:0042626
	alt						Test	0.629	0.776	1.4E+00	0.253	0.742	0.004	GO:0055085
	PD	-3656.61	7389.73				Ref	0.091	0.156	1.2E+01	0.311	0.685	0.004	GO:0016021
	PD						Test	0.483	0.716	1.4E+00	0.095	0.838	0.067	GO:0016887
														GO:0009055
OG0005903.fa	null	-1343.70	2748.15			1.000	Ref	0.104	0.720	1.1E+00	1.000	0.000	0.000	GO:0020037
	alt	-1340.83	2744.47	5.726	0.017	0.000	Ref	0.078	0.082	2.5E+00	1.000	0.000	0.000	GO:0051536
	alt						Test	1.000	1.000	1.0E+00	1.000	0.000	0.000	GO:0009055
	PD	-1340.09	2751.21				Ref	0.078	0.679	1.1E+00	1.000	0.000	0.000	GO:0006099
	PD						Test	1.000	1.000	4.8E+01	0.146	0.000	0.854	GO:0016491
														GO:0055114
OG0006067.fa	null	-4657.17	9380.60			1.000	Ref	0.041	0.147	1.4E+00	0.269	0.731	0.000	GO:0051537
	alt	-4654.75	9377.78	4.829	0.028	0.420	Ref	0.017	0.146	3.2E+00	0.364	0.636	0.000	
	alt						Test	0.181	0.445	1.6E+00	0.364	0.636	0.000	GO:0005515
	PD	-4654.75	9385.84				Ref	0.038	0.118	1.0E+00	0.246	0.754	0.000	GO:0072546
	PD						Test	0.250	0.333	1.0E+00	0.013	0.965	0.021	
OG0006115.fa	null	-6960.19	13988.58			1.000	Ref	0.037	1.000	3.2E+00	0.942	0.019	0.039	
	alt	-6956.93	13984.07	6.516	0.011	0.274	Ref	0.045	1.000	3.3E+00	0.951	0.019	0.030	GO:0004525
	alt						Test	0.428	1.000	1.4E+00	0.951	0.019	0.030	GO:0006396
	PD	-6956.92	13992.09				Ref	0.036	1.000	2.6E+00	0.951	0.002	0.047	GO:0003723
	PD						Test	0.281	0.324	1.2E+00	0.046	0.782	0.172	GO:0016075
OG0006138.fa	null	-4147.71	8363.74			1.000	Ref	0.0523	0.194	1.017	0.331	0.669	0	
	alt	-4144.78	8359.90	5.860	0.015	0.253	Ref	0.019	0.192	2.5E+00	0.392	0.608	0.000	GO:0004674
	alt						Test	0.366	0.658	1.3E+00	0.392	0.608	0.000	GO:0005524
	PD	-4144.77	8367.97				Ref	0.045	0.164	1.0E+00	0.342	0.658	0.000	GO:0006468
	PD						Test	0.334	0.463	1.0E+00	0.060	0.786	0.155	GO:0004672
														GO:0035556
OG0006139.fa	null	-1710.10	3487.00			1.000	Ref	0.236	0.239	4.6E+01	0.005	0.994	0.001	GO:0002098
	alt	-1710.10	3489.05	0.001	0.981	1.025	Ref	0.238	0.239	4.6E+01	0.005	0.994	0.001	GO:0016300
	alt						Test	0.229	0.231	5.1E+01	0.005	0.994	0.001	GO:0008168

OG0006250.fa	PD	-1710.08	3497.21				Ref	0.240	0.256	4.7E+01	0.993	0.006	0.001	
	PD						Test	0.176	0.245	1.5E+01	0.226	0.774	0.000	
	null	-9301.98	18672.11		1.000		Ref	0.068	0.143	4.7E+02	0.997	0.003	0.000	
	alt	-9295.05	18660.24	13.878	0.000	0.305	Ref	0.000	0.058	7.6E+02	0.002	0.998	0.000	
	alt						Test	0.000	0.419	7.6E+00	0.002	0.998	0.000	GO:0005515
	PD	-9295.04	18668.28				Ref	0.049	0.058	7.7E+02	0.003	0.997	0.000	
	PD						Test	0.036	0.418	1.7E+00	0.000	1.000	0.000	

Table S5.4 Single copy orthologue orthogroups which displayed significant evidence for either diversifying selection (branch and branch tests) or a shift in selection intensity (RELAX test). Annotations performed using InterProScan version 5.47-82.0, see Methods section 2.4 for details. If a test was insignificant, it has been marked with an “N/A” to highlight the overlap of significance for each test on each orthogroup. *Some orthogroups did not produce any reliable function annotation, likely due to genetic distance from species used to produce our current annotation databases.

Orthogroup	Branch test (LRT)	Branch result (ω)	Branch site (LRT)	RELAX LRT	RELAX result (k)	Annotation
OG0003409	6.37	0.05	N/A	6.56	4.41	nucleoside transporter
OG0003419	8.76	999.00	N/A	0.00	N/A	Unknown function*
OG0003425	4.53	0.01	N/A	3.33	N/A	Unknown function*
OG0003430	5.96	0.04	N/A	0.00	N/A	Fibronectin type III domain
OG0003435	4.42	0.32	N/A	4.44	0.35	Unknown function*
OG0003461	4.94	0.00	N/A	0.65	N/A	Unknown function*
OG0003498	5.99	0.32	N/A	4.89	16.26	Unknown function*
OG0003511	2.05	N/A	N/A	4.64	0.55	Unknown function*
OG0003533	0.04	N/A	N/A	4.84	35.96	Unknown function*
OG0003562	9.93	1.33	N/A	7.53	0.00	domain superfamily
OG0003585	3.57	N/A	N/A	5.03	0.00	Longin domain
OG0003611	3.85	0.55	N/A	0.91	N/A	N-terminal domain
OG0003615	6.79	0.81	6.55	3.34	N/A	Unknown function*
OG0003633	7.29	0.03	N/A	5.04	2.55	receptor 158/179
OG0003668	0.32	N/A	5.04	1.08	N/A	Heat shock
OG0003700	1.08	N/A	9.11	3.95	4.14	SNARE homology domain
OG0003710	11.49	1.07	N/A	8.28	2.34	Unknown function*
OG0003750	4.12	1.42	4.15	3.41	N/A	Unknown function*
OG0003761	0.83	N/A	7.12	2.40	N/A	Unknown function*
OG0003762	5.67	0.00	N/A	1.97	N/A	Unknown function*
OG0003776	6.53	0.25	N/A	4.78	12.30	Unknown function*
OG0003806	6.35	0.00	N/A		N/A	ATPase, C-terminal domain
OG0003807	6.94	0.09	N/A	6.68	0.60	Dynein heavy
OG0003810	0.08	N/A	N/A	4.18	0.43	Peptidase site domain
OG0003842	7.51	999.00	N/A	5.20	0	Scavenger mRNA decapping
OG0003856	3.49	N/A	7.52	6.20	4.66	Transcriptional regulator

OG0003859	6.71	999.00	N/A	3.79	N/A	Unknown function*
OG0003876	6.83	0.00	N/A	3.56	N/A	Enolase domain
OG0003896	5.07	0.00	N/A	1.28	N/A	Unknown function*
OG0003900	4.15	999.00	N/A	2.40	N/A	Protein domain site
OG0003921	4.10	0.42	N/A	4.19	0.40	Unknown function*
OG0003956	4.17	0.00	N/A	4.11	32.93	Cytidine and deoxycytidylate
OG0003971	6.31	0.34	N/A	5.88	10.76	PPM-type phosphatase domain
OG0003985	4.22	999.00	N/A	1.76	N/A	Cytochrome c-like domain
OG0004002	5.27	2.93	N/A	4.69	3.77	C-terminal domain
OG0004053	13.26	0.69	13.52	12.09	4.27	Unknown function*
OG0004077	0.56	N/A	6.85	6.37	2.76	Methyltransferase domain
OG0004087	4.95	1.75	N/A	4.49	0	Pyruvate carboxyltransferase
OG0004110	1.32	N/A	5.65	0.72	N/A	hydrolase fold
OG0004119	5.25	0.87	N/A	6.23	0.03	ABC transporter
OG0004120	4.63	3.86	8.63	0.00	N/A	Unknown function*
OG0004166	5.66	0.04	N/A	5.49	0.26	Clathrin; heavy repeat
OG0004167	3.90	0.00	N/A	4.76	23.12	Unknown function*
OG0004182	4.46	2.90	8.83	3.36	N/A	Zinc finger
OG0004199	0.04	N/A	N/A	5.12	8.72	Unknown function*
OG0004201	5.40	2.41	N/A	4.97	0	Sulfatase, putative site
OG0004206	4.46	999.00	N/A	3.97	6.93	Uncharacterised conserved protein UCP013899, metal
OG0004207	5.75	3.32	N/A	2.74	N/A	PAN/Apple domain
OG0004227	5.72	999.00	N/A	6.20	4.26	alpha domain superfamily
OG0004248	4.20	1.61	N/A	0.08	N/A	cofactor biosynthesis
OG0004260	6.55	0.00	N/A	7.38	50.00	RNA polymerase Rpb1
OG0004282	0.83	N/A	5.01	0.05	N/A	tRNA-dihydrouridine synthase
OG0004292	10.38	0.00	N/A	10.31	50.00	Unknown function*
OG0004303	4.96	999.00	N/A	4.44	13.82	(WD) repeats
OG0004340	3.95	0.00	N/A	0.03	N/A	Unknown function*
OG0004365	6.16	0.00	N/A	6.13	50.00	Unknown function*
OG0004376	4.66	1.84	N/A	4.36	0	Insect olfactory receptor

OG0004387	4.83	999.00	N/A	4.73	18.07	Snurportin-1_C site
OG0004388	4.25	0.00	N/A	0.90	N/A	protein S6
OG0004411	6.58	0.00	N/A	6.47	29.41	Mitochondrial glycoprotein
OG0004414	5.30	999.00	N/A	0.49	N/A	sulfhydryl oxidase domain
OG0004430	6.42	999.00	N/A	1.28	N/A	hydrolase fold
OG0004449	7.11	2.99	N/A	6.60	4.02	Methyltransferase domain
OG0004468	0.18	N/A	N/A	3.92	0.00	Pseudouridine synthase
OG0004482	0.82	N/A	4.26	0.70	N/A	Unknown function*
OG0004490	0.06	N/A	N/A	5.43	2.39	Phospholipase site domain
OG0004503	4.18	0.60	N/A	5.84	0.14	Sodium: neurotransmitter symporter
OG0004504	4.54	0.59	N/A	6.20	0.18	Tubulin-tyrosine ligase/Tubulin polyglutamylase
OG0004620	0.24	N/A	10.73	8.24	4.50	ERCC4 domain
OG0004637	1.93	N/A	N/A	4.59	4.30	Ribosomal protein
OG0004641	4.59	0.00	N/A	0.10	N/A	Metalloenzyme, LuxS/M16 peptidase-like
OG0004660	0.40	N/A	4.30	0.04	N/A	Leucine-rich repeat
OG0004686	4.31	0.00	N/A	5.27	37.88	N-acetylmuramoyl-L-alanine; amidase
OG0004687	3.67	N/A	N/A	8.25	0.05	hydrolase family domain
OG0004693	4.85	999.00	N/A	1.63	N/A	THAP-type zinc finger
OG0004708	6.91	0.00	N/A	5.83	0.00	Zinc finger
OG0004721	6.13	0.54	N/A	4.27	19.43	S4 protein domain
OG0004733	3.76	N/A	N/A	4.99	0.00	Immunoglobulin-like
OG0004763	5.62	999.00	N/A	1.33	N/A	Unknown function*
OG0004767	0.00	N/A	5.26	2.37	N/A	ABC transporter
OG0004785	5.16	999.00	N/A	2.34	N/A	Unknown function*
OG0004795	5.05	0.00	N/A	2.71	N/A	Mitochondrial membrane translocase subunit Tim29
OG0004809	6.32	999.00	N/A	0.00	N/A	Zinc finger
OG0004820	6.59	0.00	N/A	3.96	3.64	Zinc finger
OG0004826	1.51	N/A	N/A	4.59	0	Aminopeptidase domain
OG0004829	7.16	999.00	8.98	6.10	2.91	Serine proteases, trypsin
OG0004851	0.03	N/A	N/A	8.20	50.00	Aldo/keto reductase
OG0004864	4.46	0.03	N/A	1.66	N/A	Cadherin-like domain superfamily

OG0004874	5.02	0.00	N/A	5.07	43.52	Zinc finger
OG0004881	4.36	0.85	N/A	3.60	N/A	Rab-GTPase-TBC domain
OG0004889	0.46	N/A	10.38	0.31	N/A	Unknown function*
OG0004896	5.25	0.08	N/A	5.99	0.00	Unknown function*
OG0004900	4.94	999.00	N/A	3.37	N/A	Protein kinase domain
OG0004939	13.68	0.00	N/A	9.52	0.00	Zinc finger
OG0004942	1.59	N/A	N/A	4.99	0.07	Unknown function*
OG0004947	1.59	N/A	10.31	1.34	N/A	Zinc finger
OG0004955	5.24	0.00	N/A	5.39	28.81	Unknown function*
OG0004961	0.13	N/A	18.12	1.63	N/A	F-box domain
OG0004962	3.97	0.77	42.44	11.53	2.27	PDZ domain
OG0004980	3.49	N/A	N/A	4.32	3.71	Pyridoxamine 5'-phosphate oxidase; split barrel
OG0004992	4.11	0.00	N/A	4.11	30.52	Pig-F; GPI biosynthesis protein
OG0005000	5.08	0.40	N/A	9.15	12.55	Unknown function*
OG0005005	4.36	0.06	N/A	3.47	N/A	Unknown function*
OG0005019	6.65	1.16	N/A	2.54	N/A	Fibronectin type III
OG0005042	5.50	0.58	N/A	6.20	0.22	Unknown function*
OG0005045	5.50	2.03	N/A	5.07	0.00	GPI mannosyltransferase
OG0005057	4.50	1.81	N/A	4.32	0.00	Sodium channel and clathrin linker
OG0005068	5.23	999.00	N/A	2.80	N/A	Walker family site
OG0005076	5.73	999.00	N/A	3.55	N/A	Ankyrin
OG0005107	2.04	N/A	N/A	3.92	10.67	Unknown function*
OG0005128	0.78	N/A	7.13	4.33	5.70	Superoxide binding domain
OG0005143	4.78	0.18	N/A	3.13	N/A	PPM-type phosphatase domain
OG0005150	4.11	0.37	N/A	5.25	0.35	Sec1-like protein domain
OG0005163	4.83	999.00	N/A	3.53	N/A	Dephospho-CoA kinase
OG0005215	4.73	0.34	9.87	3.42	N/A	Ribosomal protein
OG0005228	3.59	N/A	N/A	4.07	2.39	Unknown function*
OG0005243	7.56	1.02	N/A	6.36	0.07	Zinc finger
OG0005260	6.74	0.41	N/A	6.21	6.40	membrane receptor
OG0005261	5.50	999.00	N/A	4.06	0.00	YrdC-like domain

OG0005290	6.01	1.70	N/A	4.42	2.68	Glycosyl transferase, ALG6/ALG8
OG0005297	6.41	999.00	N/A	0.02	N/A	Janus superfamily (Ocnus)
OG0005306	5.44	0.00	N/A	0.22	N/A	Zinc finger
OG0005341	4.36	999.00	N/A		N/A	Zinc finger
OG0005366	5.36	0.76	N/A	3.34	N/A	Unknown function*
OG0005374	3.89	0.00	N/A	0.51	N/A	Unknown function*
OG0005394	3.64	N/A	N/A	5.70	50.00	Tudor domain
OG0005430	6.82	999.00	N/A		N/A	SOCS domain
OG0005446	4.49	999.00	N/A		N/A	Unknown function*
OG0005449	1.23	N/A	N/A	5.50	17.23	Ankyrin domain
OG0005453	0.24	N/A	6.60	3.99	4.65	Serine carboxypeptidase; S28
OG0005465	7.58	1.49	4.86	5.18	0.00	DNA subunit, eukaryotic/archaeal
OG0005529	0.49	N/A	5.09	3.42	N/A	Unknown function*
OG0005534	8.69	0.25	N/A	11.81	7.67	DENN repeat domain
OG0005546	3.26	N/A	N/A	4.22	30.06	S-adenosylmethionine binding site
OG0005584	8.58	0.38	N/A	5.93	5.29	Leucine-rich repeat
OG0005595	3.54	N/A	N/A	4.29	0.13	IX farnesyltransferase
OG0005596	4.18	999.00	N/A	0.07	N/A	Tetratricopeptide-like helical domain superfamily
OG0005613	6.68	0.84	N/A	6.59	0.06	RUN domain
OG0005615	4.51	0.47	N/A	4.49	0.23	Protein domain site
OG0005619	4.47	0.00	N/A	2.39	N/A	Unknown function*
OG0005644	5.75	0.20	N/A	6.46	0.00	Ribonucleotide reductase
OG0005667	0.21	N/A	28.37	9.06	13.94	Unknown function*
OG0005674	5.07	2.54	N/A	3.68	N/A	Unknown function*
OG0005676	10.28	1.58	N/A	8.73	0.00	tRNA class domain
OG0005694	2.50	N/A	N/A	6.23	3.27	Unknown function*
OG0005695	0.06	N/A	N/A	4.42	1.70	methyltransferase
OG0005724	7.53	3.10	N/A	3.94	0	non-lysosomal ceramidase,
OG0005730	5.97	999.00	N/A	2.85	N/A	Unknown function*
OG0005778	5.58	0.08	N/A	5.24	9.11	Unknown function*
OG0005792	0.03	N/A	7.69	0.00	N/A	Unknown function*

OG0005809	5.61	0.00	N/A	0.00	N/A	Unknown function*
OG0005812	7.73	0.00	N/A	6.05	0.00	of Cajal bodies-associated protein 8
OG0005816	1.87	N/A	6.53	6.24	5.62	SET domain
OG0005826	0.32	N/A	4.01	0.37	N/A	Unknown function*
OG0005865	4.92	0.72	N/A	4.62	0.14	ABC transporter
OG0005903	7.28	999.00	N/A	5.73	0.00	iron-sulfur binding domain
OG0005914	4.75	0.00	N/A	0.06	N/A	P-type ATPase,
OG0005919	7.49	0.00	N/A	7.49	24.26	Unknown function*
OG0005933	0.12	N/A	3.97	3.71	N/A	Unknown function*
OG0005937	3.98	0.37	N/A	3.75	N/A	Cold-shock; DNA-binding domain
OG0005964	5.67	0.00	N/A	2.91	N/A	Cation efflux
OG0005972	6.13	0.03	N/A	9.77	50.00	Unknown function*
OG0005995	4.02	0.00	N/A	3.13	N/A	Unknown function*
OG0006028	5.18	0.00	N/A	6.47	50.00	Ubiquitin
OG0006053	7.76	0.22	N/A	7.76	0.28	NatC auxiliary subunit
OG0006059	3.88	2.08	N/A	3.43	N/A	Unknown function*
OG0006067	4.68	0.35	N/A	4.83	0.42	protein complex subunit
OG0006073	5.39	0.05	N/A	5.54	4.35	Myb/SANT-like DNA-binding domain
OG0006085	9.93	999.00	7.22	2.61	N/A	Unknown function*
OG0006100	7.15	0.37	N/A	6.92	2.88	Unknown function*
OG0006105	3.10	N/A	30.41	10.54	2.45	Deoxyribonuclease II site
OG0006115	4.53	0.35	N/A	6.52	0.27	Ribonuclease III domain
OG0006126	11.97	999.00	4.34	3.24	N/A	Unknown function*
OG0006138	5.67	0.47	N/A	5.86	0.25	Protein kinase
OG0006189	1.72	N/A	N/A	4.34	0.19	Unknown function*
OG0006210	3.92	0.93	5.48	0.01	N/A	RNA polymerase II subunit GRINL1; fringe-like
OG0006223	4.14	1.14	9.95	0.17	N/A	Unknown function*
OG0006246	4.42	0.93	N/A	0.09	N/A	S1 domain
OG0006250	4.97	0.27	N/A	13.88	0.31	(WD) repeats superfamily domain

Chapter 6 – General discussion



Thesis summary

Mutualisms are widespread, spanning the full tree of life, and ecologically important. A key area of mutualism diversity is mutualistic dependence. Understanding the factors that drive mutualistic dependence and its impact is of high importance since levels of dependence has important effects on the mutualism stability, the context-dependency of mutualisms, and likelihood of extinction through several direct and indirect effects (Chomicki et al. 2020a). Mutualistic dependence even impacts the evolution of traits unrelated to the mutualistic interaction, with for instance ant-plant mutualisms impacting the evolution of flower size (Burin et al. 2024). Mutualistic dependence has been described as having two axes, (i) dependence and (ii) specialisation. Dependence refers to the level of fitness lost when a mutualistic partner is removed, ranging from facultative to obligate, obligate mutualists cannot survive and/or reproduce in the absence of their mutualist partner. Specialisation refers to the number of species that a mutualist engages with (or has the potential to engage with), generalist mutualists engage with several species whereas specialist usually engage with one or a few species (Chomicki et al. 2020a). A framework for describing species interactions by Lewis makes an additional distinction, the category “Interdependence” had two criteria the first is “degree”, is the same spectrum of “dependence” described in Chomicki et al. (2020) however the second “mode” describes the mechanism of dependence (Lewis 1985).

Most of the work studying the genomic consequences of mutualism are on bacterial endosymbionts. Bacterial endosymbionts experience drastic reductions in genome size (McCutcheon and Moran 2010, Moran and Bennett 2014, Archibald 2015), many gene losses (McCutcheon and Moran 2010, Bennett and Moran 2015), are AT biased (McCutcheon and Moran 2010) and an increase in rates in molecular evolution (Moran 1996, McCutcheon and Moran 2012). Comparatively, there have been few studies on macro (non-microbial) mutualisms (Lutzoni and Pagel 1997, Rubin and Moreau 2016, Ametrano et al. 2022). Of the two studies that used genome scale data one found that molecular evolution increased following the shift to mutualism (Rubin and Moreau 2016) whereas the other found it decreased (Ametrano et al. 2022).

This thesis focuses on the ant genus *Philidris* due to its diverse mutualistic strategies, ranging from non-mutualist populations, facultative generalist ant-garden ants and the obligate specialist species *Philidris nagasau*. This wide variety in mutualistic behaviour between closely related species allows us to assess the genomic consequences of shifting mutualist strategy. Using *Philidris* as a model system, this thesis aimed to test how

mutualistic dependence impacts genome evolution. At the same time, it also delved into the evolution of plant farming by ants.

In chapter 2, I performed a systematic review and phylogenetic comparative analyses to ask the following questions: 1) how many types of plant cultivation mutualisms exist, and where are they located geographically? (2) how taxonomically diverse are they? (3) how do these plant cultivation systems compare in relation to taxonomic diversity, presence of domatia and food rewards, specificity and dependence? (4) How do they compare to the conceptualisation of insect agriculture (Mueller et al. 2005)? I identified three type of ant/plant agricultural symbioses, which convergently evolved in the Neotropics, and South-East Asia and Fiji, but all show various divergences. I find that unlike most other kinds of farming mutualisms, ants farm plants for nesting space rather than for food. Reflecting on ant-garden mutualisms in comparison to the highly specialised and obligate mutualism between *P. nagasau* and *Squamellaria* prompted me to ask whether ant-garden ants are true agriculturalists too. I conclude that the Mueller framework was overly restrictive and excludes many potentially informative species in the pursuit of understanding the evolution of agriculture and proposed a new more inclusive eco-evolutionary framework to describe different levels of farming behaviour. By focusing on evolutionary processes, this framework has the potential to yield new insights in the evolution of insect agriculture.

In Chapter 3, I assembled and annotated 40 *Philidris* genomes, two from PacBio HiFi reads and 38 from Illumina reads to build the comparative genomic and phylogenomic framework for *Philidris*. I compare three different annotation software, and find that a new machine-learning based approach, Helixer (Holst et al. 2023) performed best. A first obvious question that this dataset allowed to test is whether the evolution of obligate dependence in *Philidris nagasau* is associated with a reduction in genome size or GC content, as in microbial obligate endosymbionts. Unlike bacterial endosymbionts, there was no difference in genome size or average GC content between non-mutualists, facultative generalists and obligate specialists. This suggests that this particular syndrome is specific to the endosymbiotic life, what Lewis (1985) would refer to as the “mode” of dependence rather than the degree of dependence. Next, I utilised these genome assemblies to build a genome-wide phylogeny of *Philidris*, consisting of 2100 single copy orthologous genes. I compared a maximum likelihood analysis of a concatenated supermatrix to a coalescent approach relying on individual gene trees, which yielded highly congruent topologies. These genome assemblies and annotations alongside the phylogeny build the foundation for the analysis in Chapters 4 and 5 as well as being an important resource for ant genomics.

In Chapter 4, I utilise the comparative genomic and phylogenomic framework built in Chapter 3 to analyse gene family expansions and contraction within *Philidris*. Specifically, I asked the following questions: 1) Does the pattern of gene expansion and contraction vary with mutualistic dependence? 2) Do genes with annotations consistent with a function in the mutualism show significant expansions and contractions following shift in mutualistic strategy? 3) What signatures of selection do mutualistic genes that have expanded or contracted show? There does not appear to be clear signatures of expansions and contractions associated to all types of mutualistic dependence (non-farming, facultative farming, obligate farming). However, the shift to obligate mutualism in *P. nagasau* is associated with the highest number of expansions and contractions compared to all other species. Amongst the significantly expanding and contracting gene families include orthogroups containing olfactory receptor (OR) genes, gustatory receptor (GR) genes and genes relating to the neurotransmitter glutamate. Following this result, I investigated signatures of selection amongst OR genes and find 35 with *P. nagasau* branches that are under diversifying selection as well as 22 orthogroups that are experiencing intensifying selection and 5 that are experiencing relaxed natural selection. The key changes in the odour and gustatory receptor landscape in *Philidris nagasau* strongly suggest that many sensory genes control many aspects of the mutualism. For instance, the selection of seeds by *Philidris nagasau*, which is highly species-specific (Chomicki and Renner, 2016), likely involves seed odour recognition. This would be similar to the situation found in Neotropical ant gardens (Youngsteadt et al. 2008).

Finally, in Chapter 5, I explore the genome-wide signatures of selection in response to shifting to obligate specialist mutualism. Specifically, I tested the following questions: 1) Is the shift to obligate dependency associated to a Red Queen or Red King scenario? 2) Is there a genome-wide macroevolutionary signature of shift to obligate dependence? Previous work on macro-mutualisms have found different results with one study on plant-ants *Pseudomyrmex* having higher rates of molecular evolution compared to their close non-mutualistic relatives, indicating that mutualism evolves as a classic arms race seen between host and parasite or predator and prey (Rubin and Moreau 2016), supporting the Red Queen hypothesis. In contrast, a study comparing lichenised fungi to non-lichenised relatives found evidence for decreased rates of molecular evolution (Ametrano et al. 2022), suggesting support for the Red King effect. However, Ametrano et al. highlighted difficulties in determining whether these reduced rates of molecular evolution were due to a Red King effect or due to demographic differences between lichenised and non-lichenised fungi, namely that lichenised fungi have longer generation times and generally have slower growth rates (Ametrano et al. 2022). The ancestral state of *Philidris* is likely a facultative generalist

(Chomicki et al., 2017), thus *P. nagasau* experienced a shift in mutualistic dependence rather than from non-mutualist to mutualist, as was being studied by the previous studies on genome evolution in mutualism. I find that *P. nagasau* has higher rates of molecular evolution compared to its sister clade (see *Materials and methods*, Chapter 5) indicating that a shift to obligate mutualism from facultative generalist mutualism follows Red Queen dynamics. The average ratio of non-synonymous to synonymous substitutions (dN/dS) was higher in *P. nagasau* compared the sister clade. Most of the 1986 single copy orthologues tested were under purifying selection, with 60% of these genes having a higher dN/dS ration in *P. nagasau* compared to the background, indicating that relaxed selection was pervasive throughout the *P. nagasau* genome ($p = 8.35E-07$). However, following using the RELAX test for shifts in selection intensity found significant evidence for relaxed selection in 60 genes and intensified selection in 45 genes. Additionally, 53 genes²⁵ that were under positive selection, and they were involved in processes such as tRNA processing, metal ion transport, nervous system development, axon guidance and signalling receptor activity. These results show that the shift to obligate dependence in *Philidris nagasau* led to genome-wide signatures of selection, that showed higher evolutionary rates (dN/dS), but also genome-wide relaxed selection, and positive selection, especially in genes consistent with sensory behaviour (Chapter 4 and 5). Genome-wide changes in signatures of selection has been found associated with major shift in species interactions such as host plant shifts in herbivorous insects (Allio et al. 2021), evolution of mutualism (Rubin and Moreau, 2016). Here, the genome-wide patterns of shifts in evolutionary signature of selection suggest that some genes have become obsolete following the shift to obligate agriculture, and some have become more important, this result mirrors the ecological observations that *P. nagasau* has gained highly specialised behaviours alongside a significant loss, namely the loss of carton nest building behaviour. One important finding of this thesis is that the evolution of obligate dependency follows a Red Queen rather than a Red King scenario. This result follows that of the evolution of mutualism in *Pseudomyrmex* ants nesting in Central American *Acacia* (Rubin and Moreau, 2016). However, it disagrees with findings examining these hypotheses at the level of traits (morphology), where there is significant support for the Red King scenario (Davis et al. 2014, Chomicki and Renner 2017b). Altogether, the emerging view is that there might not be a one size fits all mechanism governing the impact of mutualism (or mutualistic dependence) on genome evolution. The nature of the interaction appears to play an important role in determining the selection regimes. Thus, there might not be universal patterns that govern selection on ‘cooperative’ genes in mutualisms.

²⁵ In the genome-wide datasets consisting of single-copy orthologous genes, others were found in mutualism-specific genes, see Chapter 4.

Framing and understanding mutualism diversity

I began this thesis by highlighting the inconsistent use of certain terminology when discussing the vast diversity of mutualisms. Some examples that seem to cause confusion include symbiosis: which was originally introduced to describe “unlike organisms living together” regardless of the effect on fitness, therefore encompassing mutualistic but also antagonistic interactions such as host-parasite interactions (Bary 1879). However, many use symbiosis and mutualism interchangeably or even as to refer to all species interactions as symbiotic (Lewis 1985). Another source of confusion is the interchangeable use of the terms dependence and specialisation, which are correlated but separate as highlighted by Chomicki et al. (2020). The broadest aim of this thesis was to ask the question “*what are the genomic consequences of shifts in mutualistic dependence?*” specifically, the consequences of shifting from a facultative generalist ant-garden lifestyle as seen in ant-garden ants within *Philidris* to the obligate specialised farming lifestyle seen in *P. nagasau*. In Chapter 3, I find that genome size and GC content does not appear to vary between obligate specialists, facultative generalist and non-mutualists (Figure 3.4) in contrast to the radical shifts in genome size (McCutcheon and Moran 2010, Moran and Bennett 2014) and AT bias seen in endosymbiont bacteria (Van Leuven and McCutcheon 2012). While both *P. nagasau* and bacterial endosymbionts are both obligate specialists, they have the same “degree” of specificity (Lewis 1985) the “mode” is different, endosymbiotic bacteria physically cannot survive outside of their host whereas *P. nagasau* regularly leave the plant domatia to forage and engage in farming behaviour (this difference is also captured well by Lewis’s criterion “*physical contact*”, bacterial symbionts are inhabitational at the organellular level whereas *P. nagasau* is inhabitational at the organismal level. Therefore, it is not surprising that *P. nagasau* genomes have evolved differently following a shift to obligate specialised mutualism compared to endosymbiotic bacteria, because other aspects of their biology and ecology are different. These results suggest that the striking features of genome evolution of endosymbiotic microbes are largely symptoms of their unique lifestyle, more than signatures of obligate dependence. While comparative genomic work in more systems is needed, these results suggest that obligate dependence in macro-mutualisms is encoded distinctly than in endosymbiotic microbes – a key knowledge gap in our understanding of mutualism (Chomicki et al., 2020).

On a similar note, our genome-wide analyses yielded similar results to a study on mutualistic plant-ants *Pseudomyrmex*, we find increased rates of molecular evolution, a small portion of genes under positive selection, most genes were under purifying selection and there were signals of pervasive relaxed selection. Are these changes signatures of a shift towards obligate, specialised symbiotic mutualism? Possibly. However, as highlighted by Rubin and

Moreau these results also mirror genomic changes in obligate, specialised, symbiotic parasites (Lutzoni and Pagel 1997, Yoshizawa and Johnson 2003, Bromham et al. 2013, Rubin and Moreau 2016). Therefore, it may be that this is a syndrome of obligate, specialised symbiosis rather than being specific to mutualism.

These two examples also highlight the importance of thoroughly and accurately classifying interactions that we study, especially if we are aiming to use our study systems as models to study species interactions overall.

Genome evolution of farmers? The difficulties of studying behaviour

Farming has evolved many times in non-human species (Mueller et al. 2001, Aanen et al. 2002, Silliman and Newell 2003, Hata and Kato 2006, Brock et al. 2011, Pion et al. 2013, Branstetter et al. 2017, Vanderpool et al. 2018). The obligate specialised agriculture between *Philidris nagasau* and *Squamellaria* was described in 2016 (Chomicki and Renner 2016a) and while there has been a great amount of work studying intricacies of this mutualism since (Chomicki et al. 2016, 2020b, Chomicki and Renner 2019a, Pu et al. 2021, Diarra and Osborne-Naikatini 2023), there are still many unknowns.

The comparative genomic analysis of *Philidris* allowed to ask what the genomic basis of obligate farming might be. The most characteristic feature of the obligate farming ant *Philidris nagasau* is its drastic evolution of sensory genes, with both massive loss and large gains of olfactory and gustatory receptors and shifts to positive selection in 35 OR orthogroups. However, linking genomic changes to shift in behaviour remains a major challenge, and calls for further experimental approaches. Below, I briefly discuss two axes for future research in this system.

Seed recognition and specificity

Philidris nagasau is highly specialised in that it only collects six species of one genus. In fact, the two clades of *Philidris nagasau* (Vanua Levu island clade and Taveuni island clade, Chapter 3) typically grow monoculture of a single species, and rarely polycultures of up to three species (Chomicki and Renner, 2016). In both islands, generalist, non-farmed *Squamellaria* (*S. wilkinsonii* in Vanua Levu and *S. jebbiana* in Taveuni; (Chomicki and Renner 2016b)) are sympatric with farmed *Squamellaria*, and in some instances even grow on the same tree. Despite this, *Philidris nagasau* only cultivates and nest within species from the farmed *Squamellaria* clade. *Squamellaria* seeds do not have a food reward (such as an elaiosomes) and yet the seeds are still highly attractive to *P. nagasau*. The Neotropical antgarden ant *Camponotus femoratus* has a preference for the plant *Peperomia macrostachya*

(Youngsteadt et al. 2008). *Peperomia macrostachya* seeds contain the volatile compound methyl 2-hydroxy-6-methylbenzate [methyl 6-methylsalicylate (6-MMS)], to determine whether this chemical was the attractant a spatially controlled two-choice olfactometer assay was performed and confirmed the hypothesis that volatile compounds mediated the mutualism between *C. femoratus* and *P. macrostachya* (Youngsteadt et al. 2008). Given that there are expansions in gene families in *P. nagasau* related to both olfactory receptors (ORs) and gustatory receptors (GRs) as well as evidence for positive selection in ~10% of the tested OR orthogroups it seems likely that this is seed odour also mediate mutualism between *P. nagasau* and *Squamellaria*. Future work should focus on characterising the chemical components of farmed *Squamellaria* seeds and performing similar experiments to determine which, if any, chemical compounds attract *P. nagasau* to seeds. Another theoretical possibility would be to create transgenic ants, for instance using CRISPR–Cas9, and “knockout” putative ORs and GRs and test if this effects the attractiveness of *Squamellaria* seeds in *P. nagasau*. However, while transgenic clonal raider ants (*Ooceraea biroi*) have been developed (Trible et al. 2017, Hart et al. 2023) it is highly unlikely that this would be possible for *P. nagasau* due to the difficulties of keeping this species in captivity. Alternatives could involve cross-species complementation, that is, introducing specific *P. nagasau* ORs or GRs in a transformable insect system and performing experimental assays on those transformants.

Disease protection

Following experiments wherein ants were excluded from farmed and non-farmed *Squamellaria* it was observed that in the absence of *P. nagasau* farmed *Squamellaria* become overcome with fungal disease (Chomicki and Renner 2019a) suggesting that *P. nagasau* is reducing disease in *Squamellaria*. The fact that non-farmed *Squamellaria* do not seem to depend on ants for disease resistant suggests that farmed *Squamellaria* may have has been selected for growth over defence, similar to what is seen in many crops grown by humans known as the growth-defence trade off (Derbyshire et al. 2024). Leafcutter ants are known to prevent disease in their fungal crop both via physical “weeding” (Currie and Stuart 2001) and via the rearing of bacteria that produce anti-microbial compounds (Currie et al. 2006). Ongoing work suggests that both mechanisms might be at play in the *Squamellaria*/*P. nagasau* system (Pu et al., 2021; Diarra and Osborne-Naikatini 2023; G. Chomicki and U. Diarra, pers. comm. June 2024).

Conclusions

Plant-cultivation by ants has evolved repeatedly and varies in level of dependence and specialisation. The genus *Phildris* encompasses the full spectrum of variation with non-

mutualist populations, facultative generalist ant-garden ants and the highly specialised, obligate plant agriculture seen in the Fijian ant *Philidris nagasau*. In this thesis, I built a genomic basis to study this mutualism by assembling and annotation 40 genomes (two from PacBio HiFi reads and 38 from Illumina reads) and constructed a highly support phylogeny. Using this framework, I investigate gene family expansions and contractions and found that the obligate specialist *P. nagasau* experiences the most expansions and contractions compared to the facultative-generalists and non-mutualists. Several of the expanding and contracting orthogroups contained olfactory receptor (OR) genes, therefore we investigated signatures of selection on these genes and found that 12% were under positive selection on the *P. nagasau* branch. These results point to the possibility that the mutualism between *P. nagasau* and *Squamellaria* is likely mediated by olfactory cues. Finally, we perform genome-wide signatures of selection tests and find that the average rates of molecular evolution are higher in *P. nagasau* compared to the background as is the ratio of nonsynonymous to synonymous mutations. The diversity of genome-wide signature of selection illustrates this major and unique shift in species interactions.

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