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Dynamics of Salticid-Ant Mimicry Systems

Thesis submitted by

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ABSTRACT

Mimicry in arthropods is seen as an example of evolution by natural selection through predation pressure. The aggressive nature of ants, and their possession of noxious chemicals, stings and strong mandibles make them unfavourable prey for many animals. The resemblance of a similar-sized arthropod to an ant can therefore also protect the mimic from predation. *Myrmarachne* is an ant-mimicking salticid spider genus, whose species associate closely with their model ant species. The behavioural reactions of *Myrmarachne* to ants were analysed, including instances when there was contact between the spider and the ant. In Townsville the salticid *Cosmophasis bitaeniata* and one *Myrmarachne* species associate with *Oecophylla smaragdina* workers. The *Myrmarachne* mimics the ant visually, and *Cosmophasis bitaeniata* mimics the cuticular hydrocarbons of the *O. smaragdina* worker ants. *Cosmophasis* and *Myrmarachne* also mimic ants through certain types of behaviour, such as the "antennal illusion" and bobbing the opisthosoma up and down. The behaviour of both salticids to *O. smaragdina* was compared. This *Myrmarachne* was also studied with a hemipteran mimic of *O. smaragdina*, *Riptortus serripes*, to see whether the salticid could discriminate between the potentially dangerous ant and its hemipteran mimic. The history of the evolutionary dynamics between *Myrmarachne* and the model ant species were studied by analysing molecular phylogenies of the two animal taxa.

In a confined space, *Myrmarachne* species displayed versatile reactions to sympatric ants that depended on factors such as the position of the ant and the distance between the *Myrmarachne* and the ant. *Myrmarachne* also show interspecific differences in their reactions to ants. All *Myrmarachne* species avoided contact with the ants whenever

possible. Even when there was contact between the two, *Myrmarachne* managed to avoid being attacked by the ant. *Cosmophasis bitaeniata* also avoids contact with ants. *C. bitaeniata* and *Myrmarachne* had the same reaction types to ants, but actions occurred at different frequencies. Overall, there were more similarities than differences between the ways these two salticids interacted with *O. smaragdina* worker ants, even though *Myrmarachne* and *C. bitaeniata* have different methods of mimicking the ants. As for the types of behavioural mimicry, there was a significant difference between *Myrmarachne* species, as well as between the two salticid genera. When *Myrmarachne* was presented with another morphological ant mimic (the alydiid bug *Riptortus serripes*), the spiders' reactions differed from those displayed towards the ants. These differences indicate that *Myrmarachne* can distinguish the ant and the bug using visual cues (perhaps through the structure of the mouthparts, or the way the two insects move around). So behaviourally, *Myrmarachne* is a versatile genus apparently under strong selection pressure and showing a high rate of differentiation and speciation. The phylogenetic study also reflects strong selection pressure, resulting in highly polymorphic species. *Myrmarachne* species have undergone adaptive radiation and speciation as they evolved towards resembling their different model ant species. Therefore the behavioural and evolutionary dynamics of these salticids and their model ants represents a case of plasticity and versatility by the salticids.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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RESEARCH QUESTIONS

Mimics are animals that live in sympatry with their models, either to gain protection from predators or to take advantage of their models. In the case of ant-mimicking salticid spiders, the close association means that the spiders are in danger of being attacked by the ants. This raises several questions about the dynamics of salticid-ant mimicry systems, such as:

- Given the fact that ant-associating salticids are preyed upon less frequently than other salticids, are there behaviours and reactions by the ant-associating salticids towards the ants allowing for a safer association between the two?
- And how much do these behaviours and reactions vary between species of one genus, as well as between genera of salticids displaying different types of ant-mimicry?
- Are these ant-mimicking salticids able to recognise ants by distinguishing between the potentially dangerous ants and other (harmless) ant-mimicking arthropods?
- And given the strong selection pressure on ant-mimics exerted by the ants and potential predators, how have these ant-mimics evolved with regards to their model ants, and how has the behaviour of ant-mimics been affected by these pressures?

Chapter 1 : Introduction

Interactions between mimics and their models have fascinated biologists through the ages. Although Darwin observed and documented mimicry across taxa, his explanation for mimicry tended to drift towards sexual selection (Darwin, 1859). Wallace opposed him in this, interpreting the function of mimicry as one of protection (Vorzimmer, 1970). In fact, Wallace (1891) gave a great deal of credit for this insight to Bates (1862) and his work on mimicry in butterflies. Bates' definition of mimicry as "resemblance in external appearance, shapes and colours between members of widely distinct families" was revised over the years, and in 1963 the following definition was agreed upon at the International Zoological Congress: "mimicry is the close resemblance of one organism to another which, because it is unpalatable and conspicuous, is recognised and avoided by some predators at some times" (Wickler, 1968).

Four main types of mimicry have been identified: Batesian, Müllerian, Wasmannian and aggressive mimicry (Rettenmeyer, 1970), although there is some controversy regarding the names and divisions of mimicry systems (Pasteur, 1982). Batesian mimicry (Bates, 1862) works on the principle that a palatable mimic gains protection from predators by resembling an unpalatable model and being confused with the latter by potential predators. Müllerian mimicry is similar to Batesian mimicry, except that it involves both model and mimic not being very palatable. In addition, in Batesian mimicry the benefits of the mimic are coupled with a fitness cost to the model, whereas in Müllerian mimicry both the mimic and the model benefit from the interaction (MacDougall & Dawkins, 1998). Wasmannian mimicry occurs when the mimicry helps the mimic live in association with the model, a dynamic that Wasmann himself studied extensively

(Wasmann, 1925 in Rettenmeyer, 1970). Poulton (1890) first spoke about aggressive mimicry as the kind where the mimicry occurs in order to avoid arousing suspicion in the model, in a situation where the mimic takes advantage of its model in some sort of parasitic or predacious way (Rettenmeyer, 1970). Aggressive mimicry is sometimes also referred to as Peckhamian mimicry, named after E. G. Peckham.

Ant-mimicry in salticids is mainly thought to be a form of Batesian mimicry, since the spiders tend to be associated with ants possessing potent chemicals or stings (Rettenmeyer, 1970). As a consequence, the spiders gain protection from predators that specialise in catching spiders (such as hummingbirds and certain species of solitary wasps) as well as from predators that avoid preying on ants (Peckham, 1889 in Rettenmeyer, 1970). According to McIver and Stonedahl (1993) ants are also a good model for Batesian mimicry because they are abundant, conspicuous, well-armed and distasteful. In addition to Batesian mimicry, ant-mimicry can also be associated with Wasmannian mimicry, since Wasmann's work concentrated on resemblances facilitating the mimic living with its host, as is the case in many ant-mimicking systems (Wasmann, 1925 in (Rettenmeyer, 1970)). Wasmannian mimicry is linked to the situation where an organism both resembles and lives in association with its model, and where the mimicry is targeted at deceiving the ant model.

Myrmecomorphy (as defined by Donisthorpe, 1972 in (Cushing, 1997)), originally termed myrmecoidy) describes arthropods mimicking ants morphologically and/or behaviourally. Myrmecophily (or "ant-loving") has several different definitions. Hölldobler and Wilson (1990) defined a myrmecophile as "an organism that must spend at least part of its life cycle with ant colonies", whereas Wheeler's (1900) definition is

“an insect nest mate or parasite of ants, harboured in their nests, either cared for by the ants or preying upon the ants or their brood”. So the association of a myrmecophile to its ant hosts could be parasitic or commensal. However, myrmecophily is a definition of the behavioural state of the myrmecophile, rather than an ecological term. An arthropod that spends at least part of its life cycle with ant colonies can more appropriately be termed an inquiline. McIver and Stonedahl (1993) state that the resemblance to the ant model in myrmecomorphs can be morphological, behavioural, chemical or textual, and myrmecophiles are usually convergent in chemical and/or textual characters that make a close relationship with the ants more possible. Often there also is some sort of communication between the ants in a colony and the arthropod guest, and Hölldobler (1971) describes this as some sort of chemical and mechanical language.

Both myrmecophily and myrmecomorphy are widespread amongst arthropods. Myrmecomorphy occurs in more than 2000 species in over 200 genera of arthropods, and it has evolved at least 70 times. Myrmecomorphy has evolved at least 15 times in the spiders (at least four times in the Clubionidae, three times in Salticidae, and also in Theridiidae, Araneidae, Thomisidae, Gnaphosidae, Zodariidae and Eresidae) (McIver & Stonedahl, 1993). Among spiders typical morphological ‘modifications’ include a constriction in the middle of the carapace and a shiny opisthosoma shaped similarly to the ant’s abdomen; the constricted carapace gives the spider the appearance of a three-segmented body like that of ants, whereas the shiny look of the opisthosoma - obtained through the presence of hairs or scale-like setae – and its shape further increase the mimic’s resemblance to the ant (McIver & Stonedahl, 1993).

Characteristics such as body shape, colouration and ant-like movements make *Myrmarachne* species remarkable ant mimics, which is why they were the focal organisms used in this study. *Myrmarachne* is a very speciose genus, comprising over 200 recognised species worldwide, most of which live in the tropics (Proszynski, 2003). Formally, *Myrmarachne* Macleay, 1838 is a genus of jumping spiders (Salticidae) classified as Pluridentati under the classification of Simon (1897-1903) (Todd Davies & Zabka, 1989). *Myrmarachne* are described as "ant-like spiders ranging from about 3.0 to 9.0 mm in length" (Wanless, 1978), and a feature of the genus is the remarkable similarity between *Myrmarachne* species and a range of ant species, making *Myrmarachne* an archetypal example of a myrmecomorphic arthropod.

Edmunds (1993) found that relative to the abundance of all the salticids in one habitat, the ant-mimicry in *Myrmarachne* species reduces their risk of being preyed on by the wasp *Pison xanthopus*. This indicates that there is some protective value for the spiders in mimicking ants, confirming *Myrmarachne* species as Batesian mimics. Many *Myrmarachne* species vary markedly in coloration during their development. Mimics that look different during various instar changes have been termed transformational mimics (Mathew, 1935). Edmunds (1978) studied three common *Myrmarachne* species in Ghana, and found that the younger instars of each species mimicked different ant genera and species to the adults. He found a positive association between ant models and the spider mimics both for young and adult spiders, implying that there is an assortative association, with each stage specialising in being near its own model. Most *Myrmarachne* species are also sexually dimorphic (Wanless, 1978), the main feature being the enlargement of the male chelicerae. Nelson and Jackson (2006) have found that the enlarged chelicerae of the males increase their resemblance to ants carrying a

"parcel" in their mandibles. Thus the male *Myrmarachne* with enlarged chelicerae are compound mimics, since they mimic an ant-parcel combination (Nelson & Jackson, 2006).

Studying *Myrmarachne* behaviour – in particular intraspecific interactions - Jackson (1982) found that male *M. lupata* use different courtship tactics depending on where the female is located and what phase of maturity she is at. Being salticids, *Myrmarachne* courtship relies on vision so that the females see the body posture as well as palpal and leg movement of the male. Usually, the female spends most of her time inside a retreat, whereas males can be found out in the open more often. This is because the males will go and find female retreats to mate with the resident female spider. Salticid retreats are usually easily identified by the silk structure, those of females having an opaque, woolly appearance, whereas males' retreats have a thinner, sheet-like appearance (Jackson, 1982). *Myrmarachne* retreats have been recorded in leaf-litter (Edmunds, 1978), or on the bottom surface of large, waxy leaves (Jackson, 1982). Studies on feeding in *Myrmarachne* (and other salticids) have shown the main source of food is small insects, although *Myrmarachne* have also been found to feed on various other food sources such as pollen and nectar (Jackson, et al., 2001). Although they are ant-mimics living in close proximity to ants, *Myrmarachne* do not feed on their models (Edmunds, 1978; Jackson, 1986). Prey and predatory behaviour of *Myrmarachne* was studied by Jackson (1986) and Jackson and Willey (1994). Like other typical salticids *Myrmarachne* are active diurnal hunters that do not build webs for prey capture, *Myrmarachne* species rely on vision to capture their prey (Forster, 1977). Jackson (1986) found however, that *Myrmarachne* species' predatory behaviour differs from that typically observed in salticids. Whereas salticids characteristically leap on their prey after a set sequence of

behaviours (as described by Forster (1982)), *Myrmarachne* species capture prey by approaching it and tapping it with their first pair of legs (referred to as legs I), and then lunge onto the prey. This behaviour is thought to be carried out to keep up an ant-like appearance (Jackson & Willey, 1994).

Little is known about actual interactions between *Myrmarachne* species and their model ants. Jackson (1986) reports *M. lupata* tapping the antennae of ants with its front legs (legs I). Otherwise, *Myrmarachne* have been reported to try to avoid any contact with ants (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2004). However, the behaviour of *Myrmarachne* around, and interactions with, their model ants has not been described in detail. In this study the reactions of several *Myrmarachne* species to various ants were investigated in the laboratory; the outcomes are described in Chapter 2. The main findings presented in Chapter 2 include the interspecific variations between *Myrmarachne* in their interaction with ants. The main factors affecting the behaviour of *Myrmarachne* were the ant species encountered and the position of the ant as seen by the spiders' main eyes.

Previous studies of *Myrmarachne*-spider interactions have shown that contact between the spider and the ant occurs infrequently. However, because of the inherent dangers arising from contact between the *Myrmarachne* and the ant, questions about the nature of the contact and its outcomes were raised, leading to a separate analysis of those times when contact does occur between the *Myrmarachne* and the ant. The results, presented in Chapter 3, show that most contacts are between the antennae of the ant and the leg I of the *Myrmarachne*, and usually results in the *Myrmarachne* running away.

Myrmecophily is the close association with ants by another group of selected animals. Some well-documented examples of myrmecophily in insects include certain lycaenid butterflies, aphids, and ptinid, scarabaeid and staphylinid Coleoptera, to name but a few. These associations with ants range from mutualism to parasitism (Pierce, et al., 2002). For example, many Lycaenidae exhibit ant-dependent egg-laying (Fraser, et al., 2002), and the relationships of the caterpillars to the ants can range from mutualism to parasitism, depending on the species. Several aphids are known to live in association with ants in a mutualistic relationship, and certain aphid species have been shown to develop better when attended by ants (Flatt & Weisser, 2000; Stadler, et al., 2002). Amongst coleopterans, the Ptinidae have relatively many myrmecophilous species, since myrmecophily seems to have evolved in this family three times in the New World alone (Philips, 2000). A large number of Scarabaeidae have also been found to display myrmecophily (Vaz-de-Mello, et al., 1998). Other coleopterans that are commonly myrmecophilous are members of the Staphylinidae (Danoff-Burg, 1994).

An example of a myrmecophilous spider is *Attacobius attarum* (Corinnidae), which lives with the leaf-cutting ant *Atta sexdens* (Formicidae) and preys on the immature stages of the ant (Erthal & Tonasca, 2001). Spiders can also take advantage of living with ants by eating the prey the ants have caught. One spider species that does this is the myrmecophilic spider *Gamasmophora maschwitzi* (Oonopidae) living in colonies of the South East Asian army ant *Leptogenys distinguenda* (Formicidae) (Witte, et al., 1999). The green ant (*Oecophylla smaragdina*) myrmecophile *Cosmophasis bitaeniata* (Salticidae) is a chemical mimic, and gets accepted in the ants' colonies due to having the same cuticular hydrocarbons, acquired from the colony (Allan & Elgar, 2001; Elgar & Allan, 2004). *Cosmophasis bitaeniata* only slightly resembles green ants visually in

that it bobs its greenish abdomen up and down in a manner similar to that of *Oecophylla smaragdina* (Allan, et al., 2002).

In this study *Cosmophasis bitaeniata* was used as a comparison to *Myrmarachne*, to see how different ant-associating salticids react to their model ant. This study is presented in Chapter 4, and shows that there are many similarities between *Myrmarachne* and *Cosmophasis* with regards to their interactions with – and reactions to – ants, despite the two salticids' different modes of mimicry.

The strategies of ant mimicry are commonly divided into morphological, chemical and behavioural mimicry. Behavioural mimicry usually reinforces the morphological similarity since the mimic has added more visual stimuli associated with the model. An example of behavioural and morphological mimicry used together in one structure is the mimicry of ants' antennae by the spiders' first pair of legs. Reiskind (1977) gives the example of *Castianeira memnonia* (Clubionidae) having bright yellow ends to the first pair of legs, just like *Pachychondila obscuricornis* (Formicidae), its model ant, which has yellow antennal ends. The form also plays a role in making the mimicry of an antenna by a leg more accurate, in that sometimes the distal end of the leg is slightly expanded into a club. Alternatively, the terminal segment of the spider's first pair of legs may be pigmented to give it a "shortened" appearance, to resemble shorter antennae. The common behavioural mimicry associated with spider legs resembling ants' antennae was termed "antennal illusion" by Reiskind (1977), when studying the ways in which different clubionid and salticid spiders carried out ant-mimicry. "Antennal illusion" is the action by which the spider lifts its first pair of legs, usually with the femur held close to the cephalothorax, and produces a waving motion (similar

to that of ants' antennae) from the patella-tibia joint. The posture of the first pair of legs varies between ant-mimicking spiders, but a general pattern is such that the femur is held close to the prosoma, and the segments mimicking the funiculi lie almost parallel to each other. In addition, the legs are moved in a manner resembling the "searching" action of ants' antennae (Reiskind, 1977). Jackson and Drummond (1974) also describe antennal illusion in a salticid spider, noting intersexual differences in how the spider moves its first pair of legs. However, the reason for this intersexual difference is not known.

Several myrmecomorphs and myrmecophiles mimic another behaviour common to ants, namely raising the 'gaster' and 'bobbing' it up and down. In ants, raising the gaster is believed to be an example of ritualised behaviour serving as a form of communication between ants. For example, to recruit nest-mates to a site where they are needed to help in defending the colony, the African weaver ant *Oecophylla longinoda* adopts a posture by which the gaster is raised, and the ant elevates itself on stilt-legs with the antennae held straight (Hölldobler, 1999). This might aid the release of pheromones to attract other worker ants. In the case of *Polyrhachis laboriosa*, raising the gaster has been shown to be a ritualised display used during interspecific competition to dissuade other ants from attacking (Mercier & Dejean, 1996; Mercier, et al., 1997). In each of these cases, a raised gaster in an ant is an aggressive signal.

Both *Myrmarachne* and *Cosmophasis* carry out some form of antennal illusion, as well as "bobbing" their opisthosoma – a display closely similar to the raised gaster in ants. In Chapter 5, a closer analysis of *Myrmarachne*'s antennal illusion and opisthosomal "bobbing" is described, with a comparison to the actions of *Cosmophasis bitaeniata*.

Myrmecomorphy in Hemiptera has also evolved several times, in particular in the families Miridae and Alydidae (McIver & Stonedahl, 1993). For example, the mirid bug *Barberiella* sp. from central America has been described as being a mimic of the ant *Camponotus planatus* (Jackson & Drummond, 1974). Within the family Alydiidae, genera such as *Hyalimenus* (Oliveira, 1985) and *Riptortus* (Mathew, 1935; Kumar, 1966) are remarkable ant mimics.

In Townsville, the nymphal stages of the pod-sucking bug *Riptortus serripes* are myrmecomorphic mimics of the green tree ant *Oecophylla smaragdina*. *Riptortus serripes* was used to examine how *Myrmarachne* reacts to the ant-mimicking bug in contrast to its model ant. This study – making up Chapter 6 – shows that the bug and the ant, although similar in appearance, do not cause the same reactions in *Myrmarachne*. This means that the shape and colours of an animal are not the only factors affecting *Myrmarachne*'s reactions to that animal.

The association between *Myrmarachne* and their ant models can also be studied in evolutionary terms, by comparing phylogenetic trees of the spiders and the ants and testing for coevolution and co-speciation. The information contained in molecular data can be an independent source for constructing phylogenies other than morphological and ethological characters (Hausdorf, 1999). This is why several studies have been carried out using mitochondrial as well as nuclear gene fragments for reconstructing phylogenies of various taxa. For example, the nuclear protein-coding gene Elongation factor-1α (EF-1α) (Hedin & Maddison, 2001a), the mitochondrial 12s rRNA gene (Vink, et al., 2002), and the mitochondrial cytochrome oxidase 1 gene (cox 1) (Hedin &

Maddison, 2001b) are some commonly used genes for constructing phylogenies. Phylogenetic studies carried out so far range from general ones such as the araneomorph spider phylogeny, looking at eight species (Hausdorf, 1999), to more specialised studies such as the one by Vink *et al.* (2002), which constructs phylogenetic trees for Australasian wolf spider genera. The jumping spider phylogeny constructed by Maddison and Hedin (2003b) shows that ant mimicry has arisen at least four times in salticids. For the salticid genus *Habronattus*, phylogenetic reconstruction of 94 species was done using EF-1α sequences (along with other DNA sequences) (Maddison & Hedin, 2003a).

These studies all help as guidelines for the molecular phylogeny constructed for *Myrmarachne* in Chapter 7. In this case, the gene fragments used for the spiders were the following mitochondrial genes: cox 1, the cytochrome B and a fragment spanning from the 16s to the NADH dehydrogenase subunit 1. *Cosmophasis bitaeniata* was used as an outgroup for rooting the *Myrmarachne* phylogenetic trees. The molecular phylogenetic trees constructed showed intraspecific polymorphisms of *Myrmarachne*, and helped in determining species homogeneity.

To establish the phylogeny of the model ants, animals were collected and identified. For most ants identification was done to genus level using the key in Shattuck (1999). Chris Burwell and Rudy Kohout identified voucher specimens of the ants deposited at the Queensland Museum, Brisbane. So far, important phylogenies of ants have been constructed by Baroni-Urbani *et al* (1992), Bolton (2003) and Johnson *et. al* (2003). Some of these phylogenies (e.g. (Baroni Urbani, et al., 1992; Bolton, 2003)) are at higher taxonomic levels than required in this study, whereas others do not contain all

the necessary taxonomic groups for this study (e.g. (Johnson, et al., 2003)). That is why a whole new phylogenetic tree of the ants in this study had to be constructed (and also outlined in Chapter 7), and compared to existing phylogenies.

With both model ant and *Myrmarachne* phylogenies constructed, the question of how the different *Myrmarachne* species evolved with respect to their model ant species can be answered. Coevolution (and co-speciation) occurs when two separate taxonomic groups diverge in a similar pattern due to micro- and macroevolutionary processes, and is usually associated with parasites and their hosts (eg (Brooks, 1985, 1988; Paterson, et al., 1993) and less so in the case of mimicry. A review by Mallet (1999) looks at coevolution in Müllerian mimicry (where the model and the mimic are both unpalatable) and one possible scenario (which was not supported in Mallet's review) would be that the two species coevolved in a mutual convergence. The other case, which is more common in Batesian mimicry (and would therefore be the expected one in the case of *Myrmarachne* and their ant models), is unilateral advergence of the mimic to the model species (Turner, 1995 in (Mallet, 1999)). Chapter 7 of this thesis contains a comparison of the *Myrmarachne* and model ant phylogenies constructed from molecular characters, looking at the evolutionary dynamics of the two groups. The main finding here was that there is no evidence of co-speciation between the model ants and the spider mimics.

The four main species of *Myrmarachne* found in Townsville, and used in this study proved to be undescribed. With their relative identities determined, selected morphological characters that are taxonomically informative were found. Identifying species based on morphological characters is quicker and easier than using molecular

techniques, and has been done for centuries. Most commonly used characters in spiders presently include male and female reproductive organ structure, tarsal claws, trichobothrial arrangement and others (Coddington & Levi, 1991). In the case of *Myrmarachne* characters that have been found useful include the male's chelicera to body length ratio, cheliceral dentition, the coloration of the legs, and the detailed structure of the female reproductive organ (the epigyne). Males of certain *Myrmarachne* species have greatly enlarged chelicerae, a character thought to have evolved due to sexual selection (Wanless, 1978; Jackson, 1982). The enlarged chelicerae of the males are a form of compound mimicry, since the males resemble ants carrying a parcel in their mandibles (Nelson & Jackson, 2006). Males with enlarged chelicerae are still protected from predators that avoid ants, but are more vulnerable against myrmecophagic predators (Nelson & Jackson, 2006).

Another character that is under evolutionary selection pressure is coloration (Oxford & Gillespie, 1998). The coloration of the legs seems to be consistent within *Myrmarachne* species (unlike the coloration of the rest of the body). The structure of the epigyne is also different between the *Myrmarachne* species, but in some cases the differences can only be identified after dissection of the epigyne and examination under a high magnification. The discovery/determination of the morphological characters useful for identification of the four new species used in this study make up Chapter 8. Chapter 8 also contains a formal description of the four *Myrmarachne* species from Townsville.

To summarise, this thesis investigates model-mimic dynamics from different perspectives. The first part consists of behavioural studies focusing on the behaviour of ant-mimicking salticids in the presence of ants. More specifically, Chapter 2 is a study

of the behavioural reactions of *Myrmarachne* to sympatric ants, including both the model and non-model species. Chapter 3 describes instances of contact between the *Myrmarachne* and their sympatric model ants, looking at various causes for – and consequences of – contact. The following chapter (Chapter 4) compares the behaviours of a *Myrmarachne* species and a *Cosmophasis* species to their common model ant, *Oecophylla smaragdina*. The mimicry of ants' antennae (waving of the first pair of legs in spiders) known as antennal illusion is carried out by several ant-mimicking spiders, and is analysed in more detail in Chapter 5. Non-spider ant-mimics, namely *Riptortus serripes* nymphs, are also included in the study, to see how a *Myrmarachne* species reacts to other arthropods that resemble ants; this study makes up Chapter 6. Apart from behavioural studies, the mimic-model system is also evaluated using phylogeny. In Chapter 7, phylogenetic trees of the mimic spiders are mapped onto those of the model ant species; these mappings show that models and mimics most likely evolved separately. For this project, the focus ant-mimicking salticid genus used is *Myrmarachne*. This thesis also includes a taxonomic chapter (Chapter 8), which is doubled as a taxonomic manuscript describing the four main *Myrmarachne* species from Townsville that were used in this study.

Mimicry is a fascinating topic for naturalists, evolutionary biologists and geneticists, since it is the product of evolution by natural selection through predatory pressure. So the evolution of mimics is expected to have been (and still be) driven by strong natural forces. Mimicry also involves a large behavioural component; in other words the mimics have in most cases had to modify their behaviour to be able to survive in their ecological niche. Therefore the behavioural ecology of mimics is worth studying, keeping in mind their evolutionary history. The documented cases of mimicry in nature

are vast, even when looking at selected taxa such as the Salticidae. However, relatively few studies take a holistic approach to mimicry, incorporating behavioural, ecological and evolutionary dynamics. This is what was carried out during the course of this project, focussing on the ant-mimicking genus *Myrmarachne*. As *Myrmarachne* is a speciose genus whose members display specialised behaviours and appear to be obligate associates with a diversity of ant species, the *Myrmarachne*-ant relationships provides a rich yet delimited system in which to study mimic-model interactions (behavioural, ecological and evolutionary).

Chapter 2 : Interactions of four ant-mimicking salticids (Araneae: Salticidae: *Myrmarachne*) with sympatric ant species

Abstract

Ant-mimicry in salticids is commonly found in nature, especially in tropical species. *Myrmarachne* is a widespread, specialised ant-mimicking salticid genus whose species associate closely with the ant species they mimic. Because ants generally react aggressively to other arthropods in their vicinity they present a threat to salticids that live close by. By keeping a *Myrmarachne* and an ant in a confined space and analysing the *Myrmarachne*'s behaviour, this study shows how *Myrmarachne* species respond to the presence of ants. Responses depend on factors such as the position of the ant and the distance between the *Myrmarachne* and the ant. Different ant species elicit varying responses. In addition to displaying behavioural plasticity, *Myrmarachne* also show conditional interspecific differences in their reactions to ants.

2.1 Introduction

Myrmarachne Mcleay, 1838 is a genus of ant-mimicking jumping spiders (Araneae: Salticidae). Resemblance to ants is both morphological and behavioural (McIver & Stonedahl, 1993). Morphological similarities between *Myrmarachne* and model ants include coloration, apparent dermal texture and body shape such as a constricted carapace and an elongated body. Behavioural mimicry involves both generally moving around like ants and an "antennal illusion", which involves the spider lifting the first pair of legs and waving them around giving the impression of a pair of antennae, while walking on the remaining 3 pairs of legs (Reiskind, 1977). These ant mimics also move their opisthosoma up and down in a "bobbing" movement, similar to the abdominal "bobbing" movement of ants, which ants use to recruit nestmates (Hölldobler, 1999) or to dissuade other ants from attacking (Mercier, et al., 1997). *Myrmarachne* is thought to be a Batesian mimic of ants (Edmunds, 1978; Jackson, 1982). Batesian mimics are palatable animals that gain protection from predation by resembling an unpalatable species (Bates, 1862). For the mimicry to be effective the model and mimic must be sympatric, allowing for predators to learn avoiding the model 'type' (Edmunds, 1974).

When *Myrmarachne* and ants meet in nature, *Myrmarachne* tend to avoid the ants by moving away from them (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2005). Ants are generally aggressive towards arthropods that do not belong to their colony (Hölldobler & Wilson, 1990). Whether this aggression affects salticids' survival directly is not clear. There have been shown to be lower numbers of hunting spiders in areas where ant colonies forage, probably because the spiders avoid the aggressive behaviour of foraging ants, rather than as a result of ant predation on spiders (Halaj, et al., 1997). However, a study by Nelson *et. al* (2004) has shown that ants do prey on salticids, but

less so if they are ant mimics. So whereas most cursorial spiders tend to avoid being in the vicinity of ant colonies, some spiders have evolved to live close to ants. Being Batesian mimics, this is the case for *Myrmarachne*.

Many species of *Myrmarachne* show a positive association with their model ant species in nature, meaning that they live closer to their model ant colony than to those of other ants (Edmunds, 1978). In general, each *Myrmarachne* species has its own ant model, and some *Myrmarachne* species as they grow sequentially mimic several ant species of different sizes (Edmunds, 1978).

The intra- and interspecific interactions of salticids, as well as ecological functions such as prey capture are largely dependent on the salticids' visual acuity. Salticids have remarkable visual abilities, unparalleled in arthropods of similar size (Blest, 1985; Land, 1985). Salticids have four pairs of eyes: the posterior-lateral (PL), posterior-median (PM), anterior-lateral (AL) and anterior-median (AM). The PL, PM, and AL eyes are thought to serve mainly as motion detectors. The AM eyes on the other hand work in different ways to produce images with structural and spatial acuity (Land, 1985). The images then allow the salticid to distinguish between jumping-spider prey and other prey by using certain cues (Harland & Jackson, 2000). With discriminatory distances of up to 47 body lengths, salticids can also distinguish between prey and conspecific rivals (Harland, et al., 1999).

This study focuses on the dynamics of *Myrmarachne* reactions to sympatric ant species. As with predatory versatility (Jackson & Pollard, 1996), the underlying question here is how variable the reactions of *Myrmarachne* are and how they depend on factors such as

visual cues, the relative distances separating the spider and ant, and the type of ant encountered. In essence do the spiders discriminate between ant species? And are there differences in the reaction frequencies or even reaction types between the *Myrmarachne* species?

2.2 Materials and methods

This study was undertaken on the Townsville campus of James Cook University (19˚ 13' S, 146˚ 48' E) and involves the four most common locally occurring *Myrmarachne* species and the dynamics of their interactions with sympatric ants, focusing on the reactions of the *Myrmarachne*. The reactions to the ants were analysed together with independent variables related to the position and motion of the ant in relation to the *Myrmarachne*. The variables were recorded by analysing videotapes showing *Myrmarachne* and ants together.

Myrmarachne were filmed with their model ant species as well as with a non-model ant species occurring in sympatry. Sympatric species were chosen by collecting ants that occurred within a 10 meter radius of where the *Myrmarachne* was found. Mimic-model associations were determined visually, by seeing which sympatric ants most closely resembled each *Myrmarachne* species.

2.2.1 Videotape recording

One *Myrmarachne* and one ant were placed in a 10 cm diameter, plastic petri-dish and the interactions recorded using a low light, high resolution video camera connected to a Panasonic NV-L25 HQ PAL video recorder. The apparatus allowed for 50 fields per second time resolution. Recordings were made for between one and three hours depending on how active the spider and the ant were. One hour recordings were the standard length, but if there were insufficient interactions in that time, then the recordings were extended to three hours. The 10 cm diameter plastic petri-dish was chosen as the study area to have a controlled, standardised environment. The size of the petri-dish is large enough for the animals to have space to move in, and small enough to

ensure frequent encounters between the two animals inside. It also allows the investigator to control what species encounter each other inside the space, without having interference from other species and/or environmental variables.

Myrmarachne and ants were collected and used immediately whenever possible. Each *Myrmarachne* was used with two different ant species in sequence, always with a minimum of 5 minutes between recordings to allow the *Myrmarachne* to settle down, and changing the petri-dish to avoid any chemical cues from the previous ant affecting the next ant used. The order of filming of the ants with the *Myrmarachne* (whether the model or the non-model ant was used first) was done at random to avoid possible bias from the spider getting used to being in the petri dish.

2.2.2 Videotape analysis

Analyses of the recordings were carried out using a SVHS player connected to a computer with a Miro DC 30 video digitising card, using the program Adobe Premiere (version 4.2). The instances of an interaction between the *Myrmarachne* and the ant were digitized. Some variables were recorded from the digitized video, while others were taken from the frame of the interaction, which was exported to ImageTool version 3.00 (© 1995-2000, The University of Texas Health Science Centre in San Antonio) to measure distances and look at positions.

In this study, an interaction between a *Myrmarachne* and an ant is defined as starting at the moment at which the *Myrmarachne* shows a definite response (or change in behaviour brought about by an external stimulus) to the ant. For every interaction, the following variables were recorded:

The *Myrmarachne* species: A, B, D or F and sex of spider: male (m) or female (f)

The ant genus: *Opisthopsis*, *Polyrhachis*, *Tetraponera* and *Oecophylla*

Motion spider: whether the *Myrmarachne* was moving (m) or stationary (s)

Motion ant: whether the ant was moving (m) or stationary (s)

The distance at which the *Myrmarachne* reacted to the ant

The position of the ant when *Myrmarachne* saw it: a = the head front-on, b = the head side-on, c = the thorax or abdomen side-on, d = the abdomen from behind, e = the ant was behind the spider

The primary *Myrmarachne* reaction (described in Table 2.1)

Table 2.1: Description of *Myrmarachne* reactions

Reaction	*Description*	*Reaction group*	*Description*
0	No reaction, just looking	Y	Stationary
1	Jump backwards (x1)	B	Backward-moving
2	Jump backwards (x2)	B	Backward-moving
3	Jump backwards (x3)	B	Backward-moving
4	Turn around and run away	B	Backward-moving
5	Turn around and walk away	B	Backward-moving
6	Touch ant (non-aggressive)	R	Contact
7	Attack ant	R	Contact
8	Side-stepping	G	Forward-moving
9	Jump to the side	G	Forward-moving
10	Move backwards (not jumping)	B	Backward-moving
11	Run forwards	G	Forward-moving
12	Follow ant visually by turning	Y	Stationary
13	Jump forwards	G	Forward-moving
14	Move to the side and walk/run forwards	G	Forward-moving
15	Open chelicerae (males only)	na	

The first column contains the numbers used for representing the different *Myrmarachne* reactions (described in the second column). The third and fourth columns display the "reaction groups" the reactions were placed in to simplify analyses.

The activity levels of ants (and possibly of *Myrmarachne*) vary between species. To account for this variation, an index, named the Total Motion Index (or TMI) was developed. This index is used as the variable accounting for interspecific differences of ant activity. The TMI is calculated as:

(number of interactions/minute) x (1+ Psm) x (1+ Pam)
where Psm and Pam are the proportions of movement of the spider and ant respectively.

The more the *Myrmarachne* and the ant move around, the higher the likelihood of interactions and subsequently the higher the TMI. So generally, a high TMI shows a more active state of the *Myrmarachne*, the ant, or both. Including the TMI in analyses means that the movements in a *Myrmarachne*'s surrounding (including its own movements) are accounted for as a possible variable of influence on its reactions.

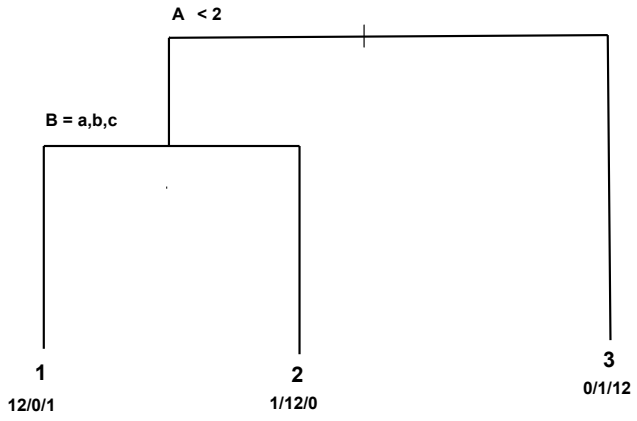
2.2.3 Data analysis

The program R version 2.1.1 (R_Development_Core_Team, 2005) was used for analysing the data. First, a recursive partitioning tree (rpart package in R (Therneau, et al., 2005)) was constructed using the reactions as the response variable, and *Myrmarachne* species, *Myrmarachne* sex, ant species, distance, position and TMI as the predictor variables. A second tree was constructed by collapsing the reactions into four generalised groups. Recursive partitioning tree analysis belongs to the "Classification and Regression Tree" (CART) analysis method, first popularised by Breiman *et. al.* (Breiman, et al., 1984). This method involves partitioning data sets into similar groups, thus predicting the correlation of one or more independent variables to a categorical

dependent variable by building decision trees. This method of data analysis has increased in popularity in many areas of science (for examples see (Lindbladh, et al., 2002; Lehmann, et al., 2003; Karels, et al., 2004)) Another method termed "bagging" (bootstrap aggregating) has been added to CART analysis as a method of stabilising the trees built. Bagging involves calculating a value termed the "out of bag estimated error", which basically gives a value to indicate the stability of the tree constructed by the CART analysis (Breiman, 1996).

2.2.4 Interpretation of regression trees

Despite widespread use in other fields of data analysis, Classification and Regression Trees have been little used in biology – hence their interpretation probably needs explanation. Below is a simple regression tree display and notes on the interpretation.



This tree involves two independent descriptor variables, A and B. A is measured on a ratio scale and represents a 'regression' component, whereas B is a classification variable with distinct categories 'a', 'b', 'c', 'd', ... In use the analysis repeatedly iterates through the data to find values of the independent variables that best partition the dependent variables (here '1', '2', '3'). The earlier (upper) nodes separate the data

more strongly, the lower nodes in the sequence separate the data in progressively finer detail. In operation the number of nodes analysed can be controlled to provide 'big picture' or 'detailed' scenarios.

In this example the variable A might represent separation of interacting individuals, B might represent species and sex and the dependent variable might be the behaviours that occur.

At each node the values and letters shown after the independent variables indicate the best predictor(s) of the dependent variables found to the left of the node split. The split to the right consists of the remaining independent variable values (whether numerical or categorical). The terminal nodes are designated by the dependent variable that categorises the outcome of the partitionings.

The vector at the base of the dependent variables (e.g. '12/0/1') indicates the number of times each dependent variable was placed in that particular classification. So for example from the first node, the dependent variable '3' occurred 12 times with variable 'A is larger than 2' (whereas variables '1' and '2' only occurred with 'A >2' 0 and 1 times respectively). I.e. when A is greater than 2, the most likely outcome is '3'. Similarly, when A is less than 2, and B is either 'a', 'b', or 'c', the most likely outcome is '1'. The most likely outcome is '2' when variable A is less than 2, and B is a class other than a, b, or c.

ANOVAs based on linear models of TMI as a function of *Myrmarachne* and ant species, and distance as a function of *Myrmarachne* and ant species were also calculated. A chi-squared test for reaction and position frequencies was also performed.

Both the ANOVA and the chi-squared test are used to test whether there are differences between the *Myrmarachne* species.

2.3 Results

The data collected from this study allows for a wide range of possible analyses and interpretations. Sometimes a data set needs to be analysed at a broad scale to determine the general patterns, whereas at other times the reaction groups must be kept as detailed as possible, not to lose the natural complexity of behavioural systems. This is why this study includes analysis of a data set with all the reactions, and one where the reactions are placed into four groups that are based on general movement.

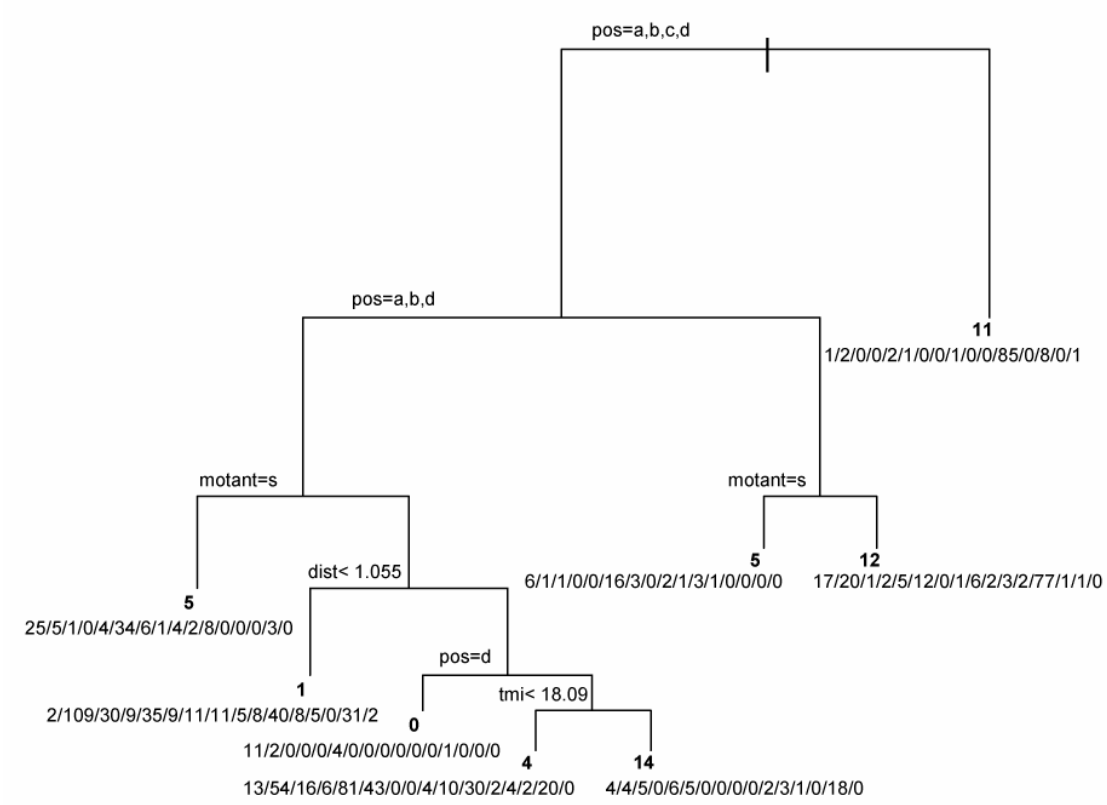


Figure 2.1: Classification tree showing all *Myrmarachne* reactions and their predictor variables
Reactions are shown at the end of the branches (reactions as described in Table 2.1). pos refers to the position the ant was in at the time *Myrmarachne* sees it ("a"= head-on, "b"= side of head, "c"= side of body, "d"= abdomen, "e"= ant is behind *Myrmarachne*), dist is the distance (in cm) between the ant and the *Myrmarachne* during the interaction, motant refers to whether the ant is moving (m) or stationary (s), and tmi is the Total Motion Index.

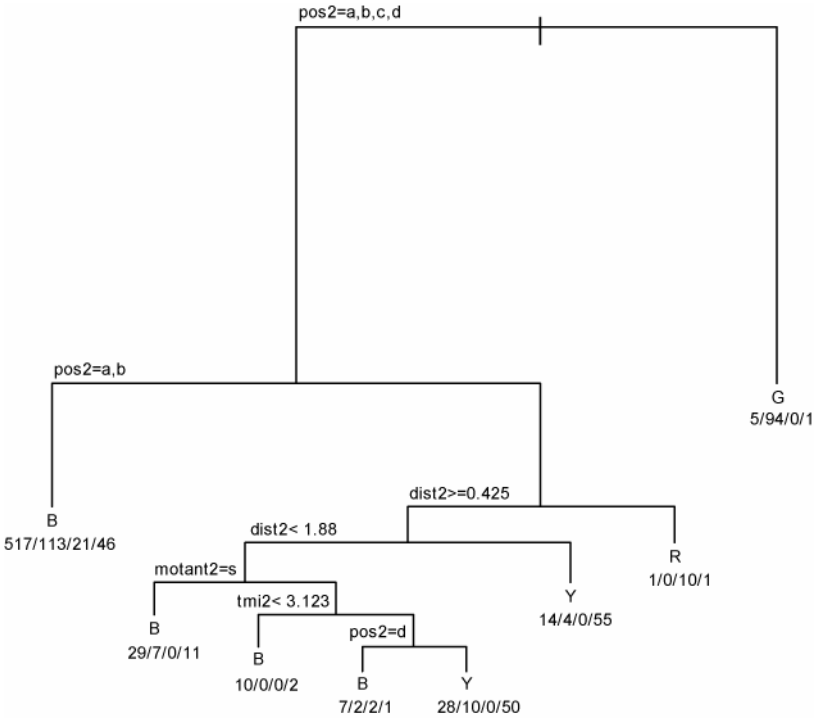


Figure 2.2: Classification tree showing *Myrmarachne* reaction groups and their predictor variables
Reaction groups are: "Y"= stationary, "B"= backward-moving, "R"= contact, "G"= forward-moving. pos refers to the position the ant was in at the time *Myrmarachne* sees it ("a"= head-on, "b"= side of head, "c"= side of body, "d"= abdomen, "e"= ant is behind *Myrmarachne*), dist is the distance (in cm) between the ant and the *Myrmarachne* during the interaction, motant refers to whether the ant is moving (m) or stationary (s), and tmi is the Total Motion Index.

The classification trees show how important certain variables are in predicting the various classes of dependent variables. The first classification tree shows all the independent variables contributing to the 15 classes of reactions. The Out-of-bag estimate of misclassification error for that particular tree was between 0.6638 and 0.63, with a mean of 0.65 (± 0.0077 standard deviations) when the analysis was run 25 times. This low value means that the overall placement of the variables as classification predictors is stable. The following explanations refer to the variables in the classification tree. The initial split on the regression tree is made when the *Myrmarachne* has an ant behind it (position e), and the most frequently associated reaction is *Myrmarachne* running forward (reaction 11). If the ant is stationary, turning and walking away is the most commonly associated *Myrmarachne* reaction. If however the *Myrmarachne* sees the ant from the side (position c), and if the ant is moving, the

reaction most closely correlated to these variables is for the *Myrmarachne* to follow the ant visually by turning, to keep the ant in the vision of its AM eyes (reaction 12). The distance between the *Myrmarachne* and the ant at the time of the interaction also has an effect on the *Myrmarachne* reaction. When the distance between the *Myrmarachne* and the ant is less than 1.06 cm, then the associated reaction is for *Myrmarachne* to take one jump backwards (reaction 1). If the distance is greater than 1.06 cm, and the spider can see the ant's head, then depending on the TMI value the *Myrmarachne* reactions correlated are either to move away sideways and keep walking, or to turn around and walk away. The second correlation tree was built with four classes of reactions, but with the same independent variables. The correlation to note on this classification tree (Figure 2.2), is the one between the position of the ant, the distance between the *Myrmarachne* and the ant, and the *Myrmarachne* response which involves making contact with the ant (type R). In other words, if an ant is in a position in which it is seen side-on or from the back (positions c and d respectively), and if the distance between the *Myrmarachne* and the ant is smaller than 0.43 cm, the associated reaction is the one where *Myrmarachne* makes contact (either aggressive or non-aggressive) with the ant. The second classification tree had an Out-of-bag estimate of misclassification error between 0.32 and 0.33, with a mean of 0.33 ± 0.0046 standard deviations, when the bagging analysis was run 25 times. The fact that this average value is lower than that of the first tree means that the fewer dependent variable classes made the classification of the independent variables more stable and reliable.

The following figures show the most important variables and their relationship with each other.

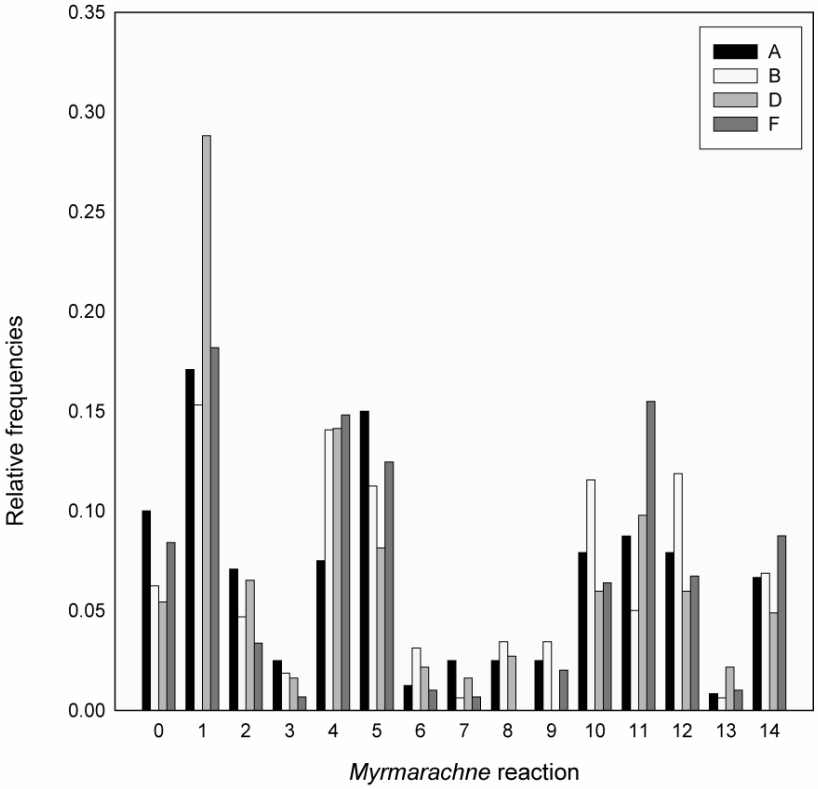


Figure 2.3: Bar chart showing the relative frequency of primary reactions for each *Myrmarachne* species ("A" "B" "D" "F"), showing that each species carries out reactions at different frequencies. Reactions are as described in Table 2.1. (χ^2_{45}= 103.9, p<0.001)

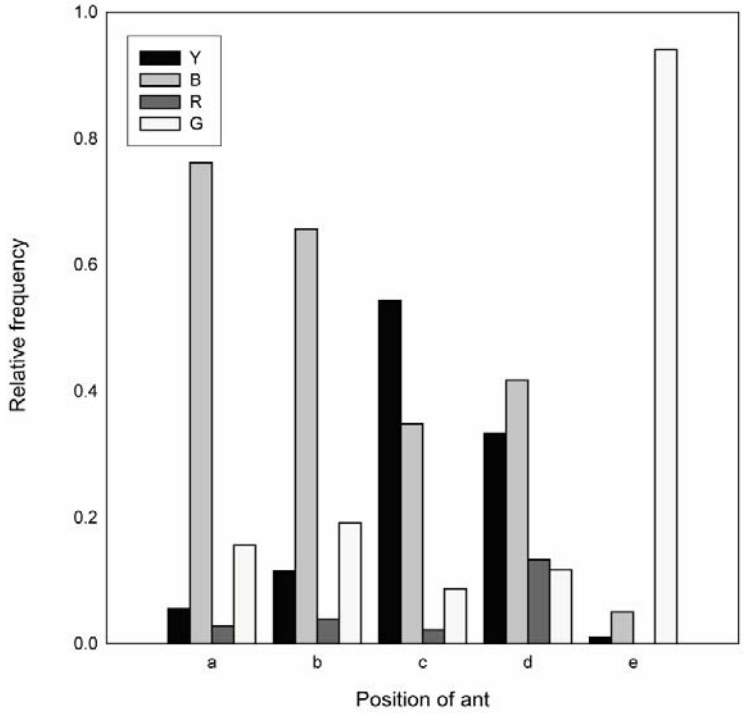


Figure 2.4: Bar graph showing the relative frequencies for each reaction group carried out by all *Myrmarachne* species following the five positions of the ant.
Reaction groups are "Y"= stationary, "B"= backward-moving, "R"= contact, "G"= forward-moving; positions are (corresponding to the part of the ant the *Myrmarachne* sees with its AM eyes): "a"= head-on, "b"= side of head, "c"= side of body, "d"= abdomen, "e"= ant is behind *Myrmarachne*. (χ^2_{12}= 30.13, p<0.01)

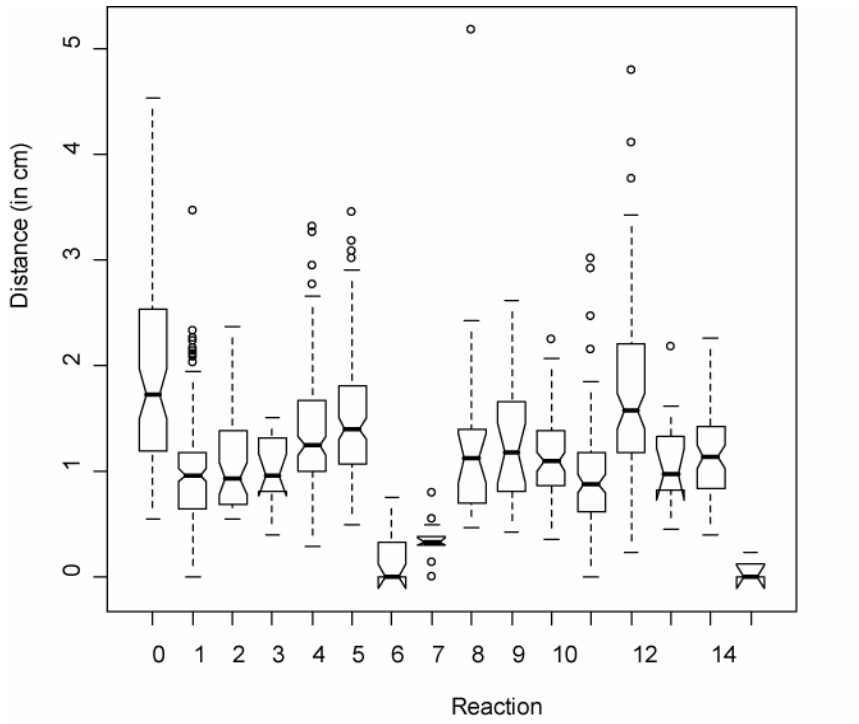


Figure 2.5: Notched boxplot of mean distances at which the *Myrmarachne* reactions occurred (reactions are as described in Table 2.1) (ANOVA: $F_{(15,1028)}= 23.17$, $p<0.001$)

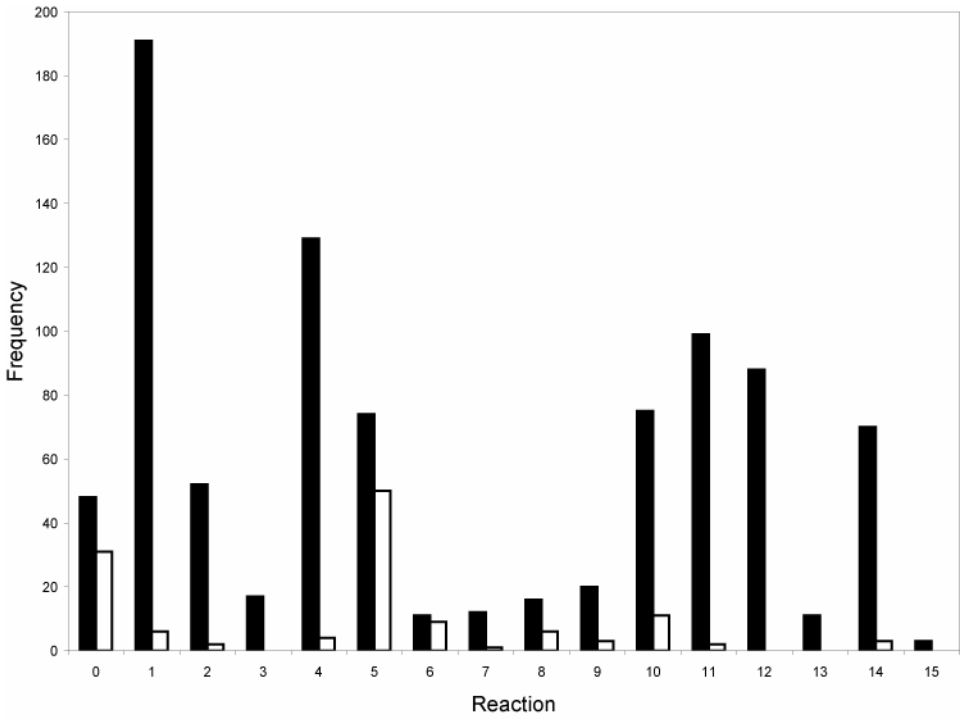


Figure 2.6: Bar chart showing the total frequency of each reaction for interactions when ants are moving or stationary for all *Myrmarachne* species.
Ant moving is represented by the black bars (m), and ants stationary by the white bars (s). Reactions are as described in Table 2.1. ($\chi^2_{15}= 229.93$, $p<0.001$)

As the classification trees show, the position of the ant when seen by the spider is one of the most important predictor variables for how the *Myrmarachne* is going to react. The frequencies of the positions the ants were in when the *Myrmarachne* saw them varied significantly, with a chi-squared value of 30.13, which gives a P value of less than 0.01 (at 12 degrees of freedom). The distances were also different depending on the *Myrmarachne* species, as found by the ANOVA, which gave an F-value of 25.87 (dof = 3/1028), making the p-value less than 0.001. The difference was also significant depending on the ant species, with an F-value of 7.23 (dof = 3/1028), and a significance value smaller than 0.001. There was also a significant interaction effect between the *Myrmarachne* and the ant species, with the ANOVA resulting in an F-value of 4.12 (dof = 9/1028), and a p-value smaller than 0.001. The TMI was also different depending on the *Myrmarachne*-ant interaction, with the ANOVA F-value being 5.22 (dof = 9/82), making the significance less than 0.001.

The frequency at which the four *Myrmarachne* species carried out each type of reaction varies significantly depending on the reaction, and in some cases it varies depending on *Myrmarachne* species, with a chi-squared value of 103.9, making the significance level less than 0.001 for 45 degrees of freedom. This was testing how much the reaction frequencies varied between the four *Myrmarachne* species. The most common reaction for all four species was to take one jump backwards (reaction 1), which occurred in 18.9 percent of the cases. It follows that two and three jumps are progressively less common (5.2 and 1.6 percent respectively). Two other common reactions were turning around and either walking or running away (occurring 12.8 and 11.9 percent of the time respectively). Here there was a difference in species whether the spider walked or ran away more frequently. The spiders then do other avoidance reactions, or they stay still

and look at the ant. The less common reactions were the ones involving contact between the spider and the ant, be it aggressive (occurring 1.3 percent of the time) or non-aggressive (occurring 1.9 percent of the time) contact.

Dividing up the reactions into groups (stationary, backward-moving, contact and forward-moving reactions) gives a clearer overall view of how different factors affect the reactions of *Myrmarachne* species. The reaction of a *Myrmarachne* is related to the position the ant is in when the spider sees it. Backward-moving reactions were most frequent when the *Myrmarachne* could see the ant's head. When the ant was side-on, the most common reaction was for the spider to be stationary, whereas having the ant behind it was almost exclusively related to the spider moving forward.

As well as the ant's position at the time of an interaction, the distance between the *Myrmarachne* and the ant also affects the *Myrmarachne* reaction. When the *Myrmarachne* found itself very close up to the ant (within 0.5 cm) the most common reaction was to make contact. However, interactions at this distance did not occur frequently. More commonly interactions occurred where the separation was between 0.5 and 1.0 cm, when the *Myrmarachne* reacted mainly by moving backwards. This general trend remained until a distance of up to 3.0 cm, after which there were very infrequent (stationary) reactions. *Myrmarachne* reactions were much more frequent when the ant was moving. For cases when the ant was moving, the frequency pattern of reactions is very similar to that of the overall reactions.

When the ant was not moving, the most commonly recorded reaction of the *Myrmarachne* was turning and walking away, as well as not reacting, but just facing the ant with the AM eyes.

Table 2.2: Total Motion Index for each *Myrmarachne*-ant species combination

	A	*B*	*D*	*F*
Opisthopsis haddoni.	**6.33 ±0.81**	15.96 ±3.46	5.14 ±0.90	20.37 ±3.21
Polyrhachis near *obtusa*	10.53 ±3.92	**10.49 ±1.24**	24.81 ±4.81	9.86 ±2.62
Tetraponera punctulata	10.61 ±5.06	3.31 ±0.78	**4.75 ±0.94**	16.05 ±3.14
Oecophylla smaragdina	17.87 ±6.02	16.32 ±0.49	13.24 ±7.87	**9.85 ±1.60**

TMI values are shown as the mean ±standard error of mean for each *Myrmarachne* and ant species combination. The bold numbers show the values for each *Myrmarachne* species with its own model ant species.

The Total Motion Index (TMI) gives an indication of how much the ant and the *Myrmarachne* were moving around in the study arena (petri-dish), taking into account both animals and the frequency of their encounters. Thus the TMI can be seen as an indicator value of the activity levels. Table 2.2 shows the mean TMI value (±standard error of the mean) for each *Myrmarachne*-ant combination. The lowest value was obtained from *Tetraponera punctulata* with *Myrmarachne* species B, whereas the highest one was *Polyrhachis* near *obtusa* with *Myrmarachne* species D. *Oecophylla smaragdina* had the least spread of average values over the four *Myrmarachne* species.

2.4 Discussion

Myrmarachne have – like other salticids – remarkable visual abilities, which allow them to judge distances (Forster, 1982) and discriminate between objects such as prey and conspecific rivals (Harland, et al., 1999). As vision is an important (if not the most important) sensory channel for these spiders, one could assume that *Myrmarachne* visually detect possible threats, and react accordingly to remain safe. These reactions were investigated in this study, where the source of the threat to *Myrmarachne* was sympatric ant species. Ants react aggressively towards animals other than colony nest-mates (Hölldobler & Wilson, 1990), often killing them (Halaj, et al., 1997). To ensure their survival, *Myrmarachne* must avoid contact with ants (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2005). The avoidance of ants is also shown in these experiments, where the most common reaction by a *Myrmarachne* when it is in the vicinity of an ant is to jump backwards. This is an efficient avoidance mechanism, since the physiology of salticids is adapted to jumping. Furthermore, a jump backwards means the *Myrmarachne* can still see the ant with its anterior median (AM) eyes, which enables the spider to carry out further actions depending on the actions of the ant. For the *Myrmarachne*, to turn around and keep walking (or running) away from the ant is a method that gets it away even more effectively, but at the cost of losing the capacity to monitor the ant.

Myrmarachne responses to ants are conditional on the position of the ant at the time when the *Myrmarachne* sees it. In these experiments, the most frequent position a *Myrmarachne* sees an ant in is face-on. This happens because a *Myrmarachne* – like other salticids (Forster, 1982) – will often turn to face an approaching object to scan it with the AM eyes. The *Myrmarachne* will then move backwards (or turn around and go

in the opposite direction to the one the ant is coming from), thus avoiding contact. An ant behind a *Myrmarachne* almost exclusively elicits a running forwards reaction (which is the most straight-forward avoidance mechanism in that position). Although this scenario is not the most common one, it is the one with the strongest pattern (as was shown by the regression analysis). In contrast, when a *Myrmarachne* sees an ant from the side, its most common reaction is to stay where it is, and to keep following the ant visually. This reduces the possibility of the ant detecting the *Myrmarachne*, and therefore contact between the two.

The distance at which a *Myrmarachne* responds to the ant also determines how the former will react. The *Myrmarachne* will only make contact with an ant at very close range, which might be a last-chance resort. Usually, when the ant's head is in the field of view of the *Myrmarachne's* AM eyes, the latter will jump backwards, as this seems to be the quickest response to carry out. At relatively large distances, the *Myrmarachne* is likely to stay stationary, and just observe the movements of the ant, not drawing attention to itself.

The interspecific differences in reactions by the *Myrmarachne* species were strong enough to be detected by both the ANOVA and the χ^2 analyses. When trying to account for *Myrmarachne* behaviour, generalisations will not always apply. This interspecific variation in the genus *Myrmarachne* can be explained in terms of small-scale or local adaptation: *Myrmarachne* species may have adapted their behaviour to better suit the nature of the ants they encounter most often in nature (which has been shown by Edmunds (1978) to be their model ant species). Whether these behavioural differences are genetically determined or learned is not known. Previous studies (Edwards &

Jackson, 1994; Carducci & Jakob, 2000) have shown that the behaviour of jumping spiders is affected by their rearing.

The TMI results possibly indicate local adaptation and learning by *Myrmarachne*. The TMI is an index of the ant and the *Myrmarachne's* general movement. Certain *Myrmarachne*-ant combinations gave significantly lower TMI values than others. This could be due to the nature of the two animals. On the other hand, the *Myrmarachne* species, the ant, or both may be less responsive to certain cues (such as dermal coloration) associated with the animals they have encountered most frequently in nature. This could lead to less movement associated with an agitated state.

Myrmarachne have been shown to have higher chances of survival than non-ant associating salticids when placed with an ant (Nelson, et al., 2004; Nelson, et al., 2005). The results of our study suggest that this higher survival rate may due to *Myrmarachne*'s behavioural adaptations, as they show considerable behavioural versatility in the presence of ants. The visual acuity of *Myrmarachne* allows them to adapt their reactions according to the relative movement (mainly direction and distance) of the "threat" (in this case the ant). Salticids have been shown to use their visual acuity for prey capture, displaying versatile predatory behaviour (Freed, 1984; Jackson & Willey, 1994; Jackson & Pollard, 1996). This study shows that versatile behaviour in salticids is not limited to prey capture, but extends into areas of immediate survival (i.e. not getting killed by ants). In addition, small-scale adaptations between *Myrmarachne* species show that selection pressure, learning or both have modified *Myrmarachne* species' behaviours in the presence of different ant species.

Chapter 3 : Contact between *Myrmarachne* (Araneae: Salticidae) and ants

Abstract

Myrmarachne (Araneae: Salticidae) is an ant-mimicking genus of jumping spiders. *Myrmarachne* species live close to their model ant species, yet they avoid making contact with the ants. However, contact can be unavoidable at times, so the question is what really happens when the ant and the spider make contact. This study found that the consequence of the contact very much depends on what body parts of both animals are involved. The most common form of contact was between the ant's antennae and the spider's first pair of legs. This resulted most frequently in the *Myrmarachne* running away. In contrast, when the spider's chelicerae were involved the ant would run away more frequently. The study concludes that even when there is contact between the two, *Myrmarachne* manage to avoid being attacked by the ant, thus remaining safe.

3.1 Introduction

Ant mimicry in terrestrial arthropods is relatively common, because ants possess characteristics making them ideal models for Batesian mimicry, amongst which are their aggressive nature and their noxious taste (Rettenmeyer, 1970). Thus ant mimicry has evolved many times in both insects and spiders (for reviews see (McIver & Stonedahl, 1993; Cushing, 1997)). For Batesian mimicry to be effective, the mimic must live in the proximity of its model. However, ants tend to be aggressive towards animals not belonging to their colony, posing a threat to their mimics (Hölldobler, 1983; Hölldobler & Wilson, 1990; Halaj, et al., 1997).

Myrmarachne Mcleay, 1838 (Araneae: Salticidae) is a large genus of ant-mimicking spiders. There are over 200 *Myrmarachne* species worldwide (Proszynski, 2003), and at sexual maturity, most of them are specialist mimics of one ant species, associating closely with their particular model ant species (Mathew, 1944; Edmunds, 1978). This means that occasional contact between the *Myrmarachne* and the ant is unavoidable, despite the fact that previous studies have shown that *Myrmarachne* avoid contact with ants (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2005). This study examines instances when contact does occur between *Myrmarachne* and ants, and attempts to answer the key questions: which body parts of the ant and the spider are most frequently involved during the contact, and what the reactions to contact are. This shows what the most likely outcomes are in the instances when *Myrmarachne* cannot avoid contact with ants, and whether those instances place the *Myrmarachne* in danger.

3.2 Materials and methods

Individuals from four *Myrmarachne* species and sympatric ants were collected from locations in Townsville (19˚ 13' S, 146˚ 48' E), and brought into the laboratory, where video tape recordings were made of the behaviours of the animals. Recordings were made of one *Myrmarachne* and one ant in a 10 cm diameter plastic petri-dish using a low light, high resolution video camera connected to a Panasonic NV-L25 HQ PAL video recorder. For each recording, a new petri-dish was used to avoid chemical cues from previous ants/spiders affecting the behaviour of the following pair. Each *Myrmarachne*-ant combination was left for 1h 30 min. Later, the video tapes were analysed, recording every time contact between the *Myrmarachne* and the ant occurred. When contact did occur, the following things were recorded:

- The body part of the *Myrmarachne* making contact with the ant (a = one leg I, b = two legs I, c = chelicerae, d = prosoma, e = opisthosoma)
- The body part of the ant making contact with the *Myrmarachne* (a = one antenna, b = two antennae, c = mandibles, d = head, e = thorax or abdomen, f = leg)
- The intensity of the contact (s = soft, h = hard)
- The reaction of both the ant and the *Myrmarachne* (a = *Myrmarachne* runs away, b = ant runs away, c = both run away, d = *Myrmarachne* moves away (any movement other than running), e = ant moves away (any movement other than running), f = both move away (any movement other than running), g = ant attacks, h = no reaction).

The reactions listed in this study are not the only reactions possible, but they are the only ones observed during this experiment.

The average number of contacts was also calculated. Data analysis was carried out using the statistics program R version 2.1.1 (R_Development_Core_Team, 2005). A recursive partitioning tree was constructed using the rpart package (Therneau, et al., 2005) to find which variables were most closely correlated to which reactions. This follows the Classification and Regression Tree (CART) analysis, which was popularised by Breiman *et. al.* (Breiman, et al., 1984) as a means of partitioning data sets into similar groups. The partitioning predicts the correlation of one or more independent variables to a categorical dependent variable by building decision trees. Both "recursive partitioning tree", "decision tree" and "classification tree" are terms used interchangeably in this study.

To find out whether the number of contacts per hour was dependent on either the *Myrmarachne* or ant species, ANOVA was used. Chi-squared tests were also performed on the frequencies with which each part of the *Myrmarachne* and the ant made contact.

3.3 Results

The recursive partitioning analysis shows the strongest predictor variables for reactions of the *Myrmarachne* and the ants. At each node of the classification trees are the independent variables deemed most likely to be correlated with the dependent variable at the end of the node. The independent variables are split at the nodes, the labels showing the ones following down the left side of the tree; by default, the missing ones follow down the right side of the tree.

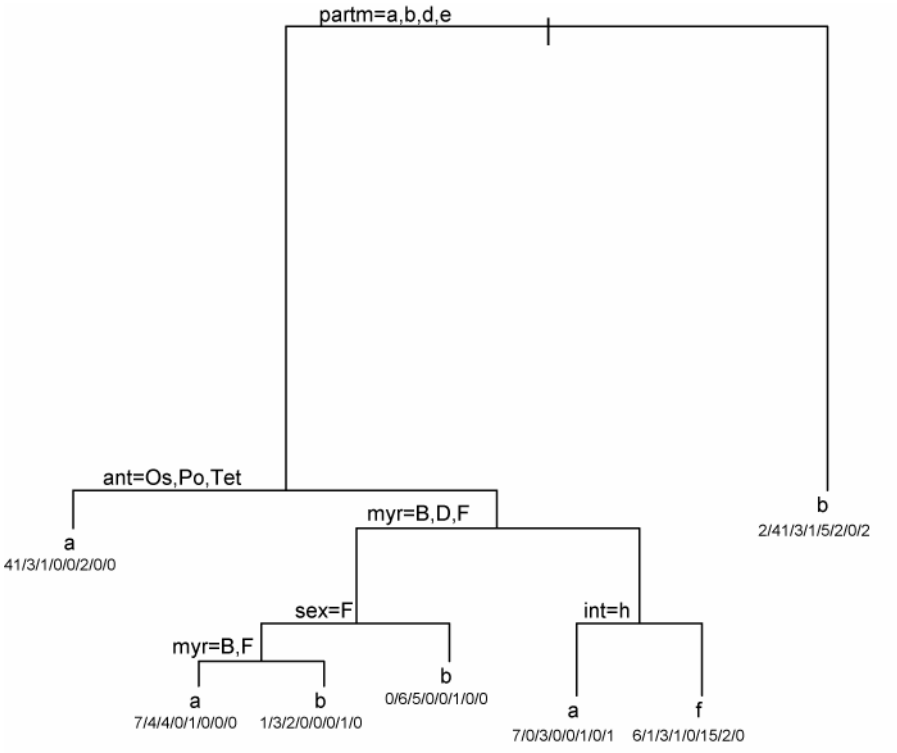


Figure 3.1: Recursive partitioning tree of *Myrmarachne* and ant reactions and all their predictor variables upon contact between the ant and the spider
Reactions are: a = *Myrmarachne* runs away, b = ant runs away, c = both run away, d = *Myrmarachne* moves away, e = ant moves away, f = both move away, g = ant attacks, h = no reaction. partm refers to the part of the *Myrmarachne* (a = one leg I, b = two legs I, c = chelicerae, d = prosoma, e = opisthosoma) touching the ant, ant is the ant species (Os = *Oecophylla smaragdina*, Po = *Polyrhachis* near *obtusa*, Tet = *Tetraponera punctulata*), myr is the *Myrmarachne* species (A, B, D or F), sex is the *Myrmarachne* sex (m = male, f = female), int refers to the intensity of the contact (s = soft, h = hard).

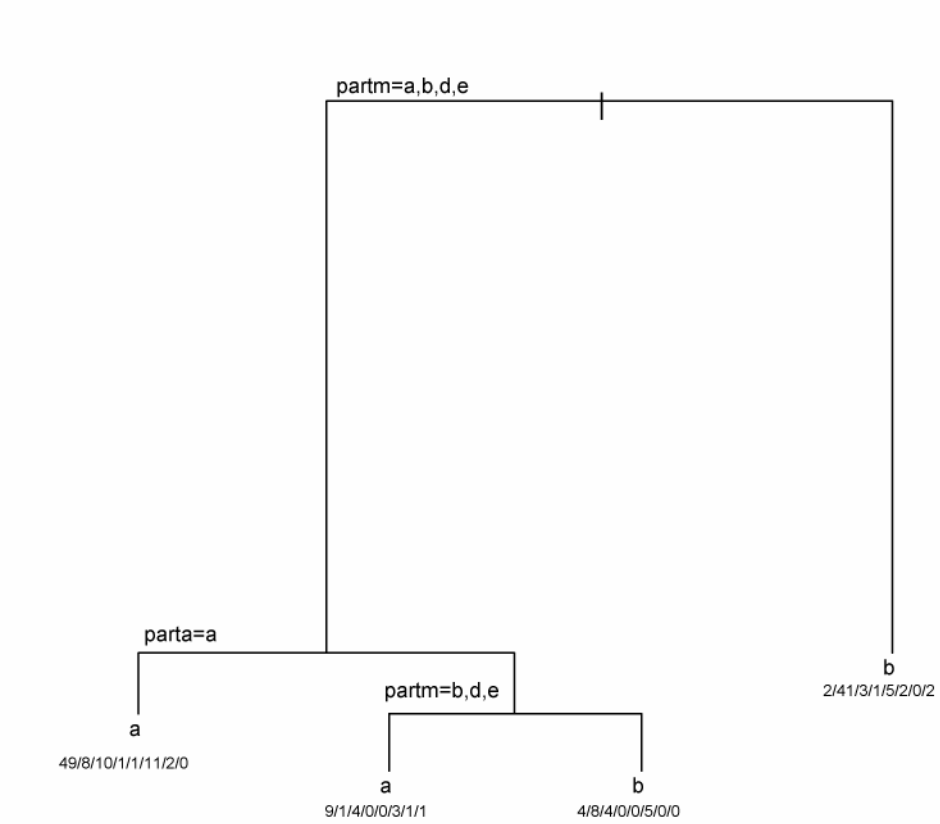


Figure 3.2: Recursive partitioning tree of *Myrmarachne* and ant reactions to contact, and their predictor variables (only body parts included)
Reactions are: a = *Myrmarachne* runs away, b = ant runs away, c = both run away, d = *Myrmarachne* moves away, e = ant moves away, f = both move away, g = ant attacks, h = no reaction; contact between *Myrmarachne* and the ant only takes into account the part of the *Myrmarachne* partm (a = one leg I, b = two legs I, c = chelicerae, d = prosoma, e = opisthosoma) and the part of the ant parta (a = one antenna, b = two antennae, c = mandibles, d = head, e = thorax or abdomen, f = leg) making contact.

The recursive partitioning trees show that the contact with *Myrmarachne*'s chelicerae to any part of the ant's body is most closely correlated with the ant running away. The ant also runs away most frequently if the *Myrmarachne*'s leg I touches any of the ant's body parts other than the antennae. If the antennae of the ant make contact with any of the *Myrmarachne*'s body parts (other than the chelicerae), the most closely correlated reaction is the *Myrmarachne* running away. The first classification tree (Figure 3.1) also shows that the reactions can be correlated to variables such as ant species, *Myrmarachne* species and *Myrmarachne* sex. For example, *Myrmarachne* running away is associated with ant species *Oecophylla smaragdina*, *Polyrhachis* near *obtusa* and

Tetraponera punctulata. If the ant species is *Opisthopsis haddoni*, female *Myrmarachne* species B and F are correlated with *Myrmarachne* running away. On the other hand, *Opisthopsis haddoni* with a male *Myrmarachne* from species B, D or F is correlated most closely with the ant running away.

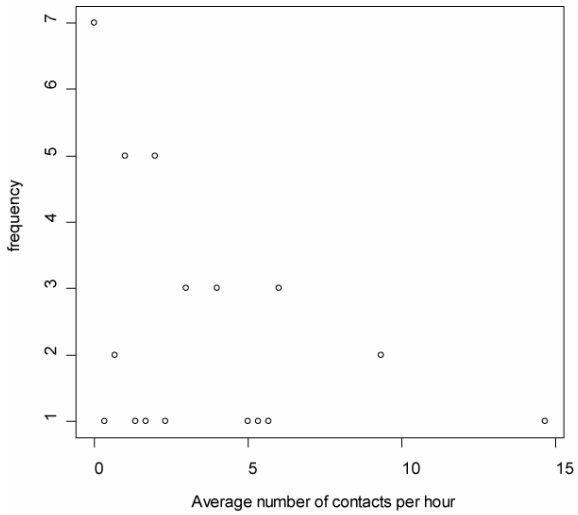


Figure 3.3: Scatterplot showing the frequency of average number of contacts per hour between all *Myrmarachne* species ("A", "B", "D" and "F") and all ant species (*Opisthopsis haddoni*, *Polyrhachis* near *obtusa*, *Tetraponera punctulata* and *Oecophylla smaragdina*).
The points are the total frequency of each *Myrmarachne*-ant combination for a particular number of contacts per hour (averaged over each *Myrmarachne*-ant combination). The graph is based on count data.

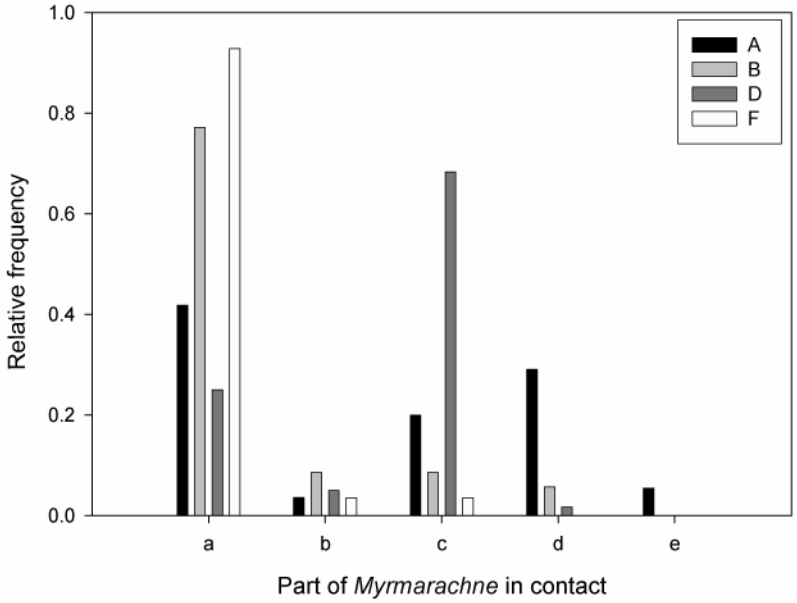


Figure 3.4: Bar chart showing relative frequency of part of *Myrmarachne* in contact with the ant for each *Myrmarachne* species (A, B, D and F)
Part of *Myrmarachne* is coded as: a = one leg I, b = two legs I, c = chelicerae, d = prosoma, e = opisthosoma. ($\chi^2_{12} = 97.83$, $p < 0.001$)

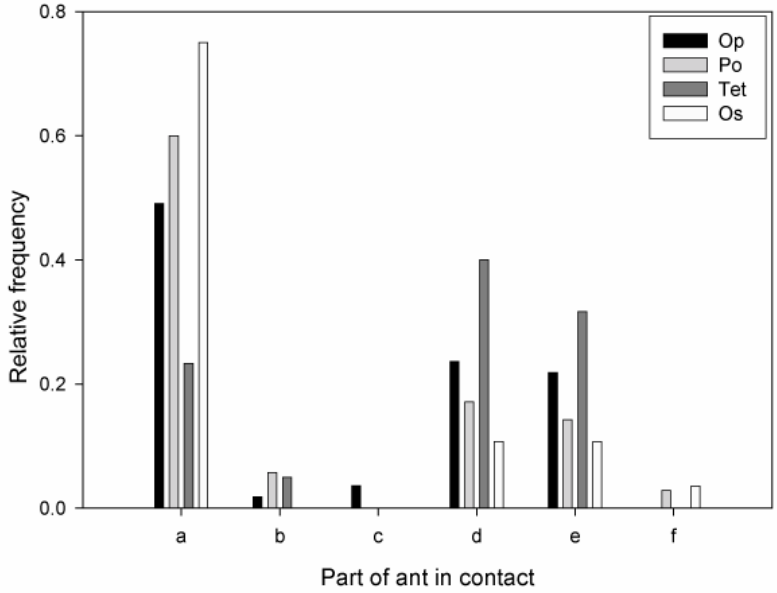


Figure 3.5: Bar chart showing relative frequency of part of the ant in contact with *Myrmarachne* for each ant species.
Parts of ant are coded as: a = one antenna, b = two antennae, c = mandibles, d = head, e = thorax or abdomen, f = leg. Ant species are coded as: Op = *Opisthopsis haddoni*, Po = *Polyrhachis* near *obtusa*, Tet = *Tetraponera punctulata*, Os = *Oecophylla smaragdina*. ($\chi^2_{15} = 37.20$, $p < 0.001$)

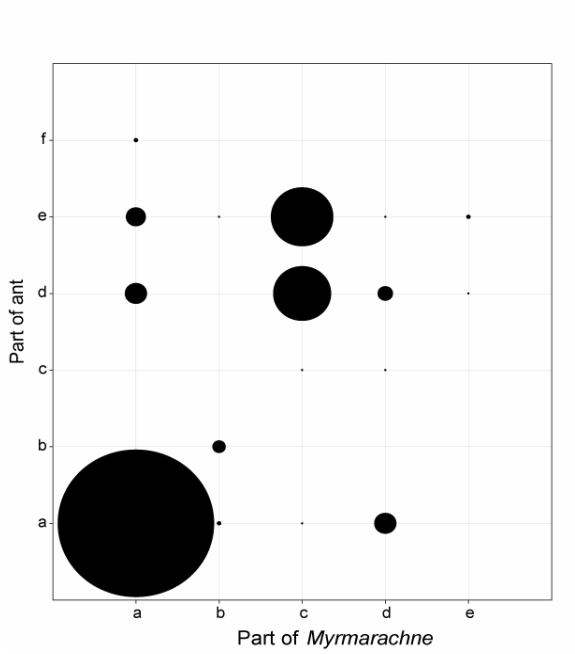


Figure 3.6: Frequencies of contact between the body part of *Myrmarachne* and the ant's body part making contact
Parts of *Myrmarachne* are coded as: a = one leg I, b = two legs I, c = chelicerae, d = prosoma, e = opisthosoma, and parts of ants are coded as: a = one antenna, b = two antennae, c = mandibles, d = head, e = thorax or abdomen, f = leg. Frequencies are indicated by the size of the bubble.

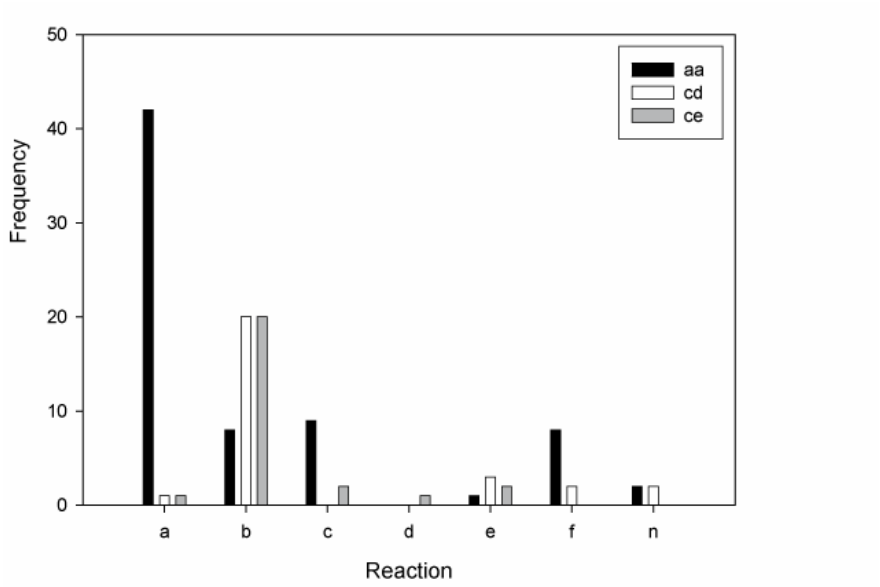


Figure 3.7: Bar chart showing the total frequencies at which the reactions of *Myrmarachne* and ants occurred following the three contact combinations
Reactions are coded as: a = *Myrmarachne* runs away, b = ant runs away, c = both run away, d = *Myrmarachne* moves away, e = ant moves away, f = both move away, g = ant attacks, h = no reaction. Contact combinations are coded as: aa = one leg I of *Myrmarachne* making contact with one antenna of the ant, cd = *Myrmarachne*'s chelicerae making contact with the ant's head and ce = *Myrmarachne*'s chelicerae making contact with the ant's thorax or abdomen. ($\chi^2_{21} = 86.76$, $p < 0.001$)

The average number of contacts per hour between a *Myrmarachne* and an ant was found to be 2.90 contacts per hour. The average number of contacts per hour is neither dependent on the *Myrmarachne* species, nor on the ant species involved, as shown by the F values for the ANOVAs, which were 1.00 (dof = 3/22) and 2.22 (dof = 3/22) respectively, with respective probabilities of 0.4163 and 0.1147. In addition, there is no discernable interaction effect of *Myrmarachne* and ant species in determining the number of contacts per hour, since the ANOVA F value for that was 0.80 (dof = 9/22) and the probability 0.6232.

Myrmarachne and ants came into contact with each other through specific parts of their bodies. The body part of *Myrmarachne* most frequently making contact with the ant was the leg I (51% of the time with one leg, and 5% with both legs I). The chelicerae

were the next most frequent *Myrmarachne* body part coming into contact with the ant (31% of the time). The next most frequent body part was the prosoma with 11% of contact and the opisthosoma with only 2% of contact to the ant. The frequencies of the *Myrmarachne* body parts making contact with the ant are significantly different between *Myrmarachne* species, with a chi-squared value of 97.83, which – with 12 degrees of freedom - gives a significance value less than 0.0001. The main difference is that species B and F made contact with their legs more frequently than species A and D, whereas species D made contact more frequently with its chelicerae than the other species.

The part of the ants that most frequently came into contact with the *Myrmarachne* was the antennae (47% of the time it was only one, and 3% of the time it was two antennae). The next most frequent ant body part was the head (26%) followed by the rest of the body – abdomen and thorax – (22%). Both the mandibles and the legs made contact with the *Myrmarachne* only 1% of the time each. There was a significant difference in the frequencies of ant body parts making contact with the *Myrmarachne* between the four ant species, with a chi-squared value of 37.20, 15 degrees of freedom and a p-value of 0.001. The main difference is that *Oecophylla smaragdina* made the most contact with one antenna, whereas *Tetraponera punctulata* had most contact with its head, thorax and abdomen.

The reactions to coming into contact with each other of the *Myrmarachne*, the ant or both were similar from both groups. The most frequent reaction to contact was the *Myrmarachne* running away (35% of events), followed by the ant running away (33% of events). Both the ant and the *Myrmarachne* ran away at the same time 12 % of

interactions, and similarly – 2 % of interactions – they both moved away together (a category of movement excluding running). One percent of events, the *Myrmarachne* only moved away from contact on its own, as opposed to the 3% of the ant moving away. The ant attacked the *Myrmarachne* (aggressively moving towards it) on 3 occasions (2% of interactions). No visible reaction to the contact from either animal could be seen in 2% of interactions. The chi-squared test for differences between *Myrmarachne* species in the frequencies of the results showed that there is a significant difference between *Myrmarachne* species, with a chi-squared value of 86.76, which – with 21 degrees of freedom – gives a p-value less than 0.0001.

The most frequent contact between *Myrmarachne* and the ant occurred between the leg I of *Myrmarachne* and one antenna of the ant (39% of contact). The second most frequent contact was between *Myrmarachne*'s chelicerae and the ant's head and the third most frequent contact was *Myrmarachne*'s chelicerae and the ant's thorax/abdomen. Each one of these combinations elicited reactions at different frequencies. For example, the leg I of the *Myrmarachne* and the antenna of the ant touching resulted most frequently in the *Myrmarachne* running away. On the other hand, the *Myrmarachne*'s chelicerae making contact with either the ant's head or its thorax or abdomen was most closely associated with the ant running away. In nature, similar observations have been made, where female *Myrmarachne* were "pushing" ants (mainly small ones from the genus *Crematogaster*) that got too close to the *Myrmarachne*'s retreat, presumably endangering the salticid's eggs. The *Myrmarachne* "pushes" using its chelicerae, but the action does not involve any biting. Rather, it is a very quick jerky forward movement towards the target, making strong contact (FSC personal observation).

3.4 Discussion

Previous studies have shown that contact rarely occurs between *Myrmarachne* and ants (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2005). When there is an interaction between an ant and a *Myrmarachne*, contact only makes up 3.17 % of the salticid's total reactions to the ant when they are both in a confined space (data from Chapter 2). This study has shown that when contact between an ant and a *Myrmarachne* does occur, it is most likely to be between one of the ant's antennae and one of the *Myrmarachne*'s front legs. Both groups of animals have been reported to use those body parts for different reasons. Ants' antennae are a sensory organ used in chemical communication, usually between workers of the same colony (Hölldobler & Wilson, 1990). However, if the ants detect a chemical from an animal other than their nestmates, they are likely to react aggressively, as a defence mechanism (Hölldobler & Wilson, 1990). *Myrmarachne* have also been shown to use their legs I for tapping insects such as moths as a part of their prey capture technique (Jackson, 1986). However, *Myrmarachne* are not generally known to prey on ants (Jackson & Willey, 1994), so their use of the first pair of legs during contact with ants is not likely to be for predatory purposes. This study has shown that *Myrmarachne* are most likely to run away following contact between their leg I and the ant's antenna, probably because of the inherent danger of the ants reacting aggressively.

The other frequent point of contact between the *Myrmarachne* and the ant is the chelicerae of the former making contact with the head, thorax or abdomen of the other. Ants that get too close to *Myrmarachne* do occasionally get "attacked" by the salticid, and as shown by this study, when the *Myrmarachne*'s chelicerae make contact with the ant, the most common reaction is the ant running away.

Although ants are said to be aggressive, and a potential danger to other animals of similar size (Halaj, et al., 1997; Nelson, et al., 2004; Nelson, et al., 2005), this study has shown that ants are only aggressive towards the *Myrmarachne* following 2% of all instances of contact. In addition, the ants' aggression never resulted in any harm done to the *Myrmarachne*. This is supported by the fact that myrmecomorphic salticids (such as *Myrmarachne*) have a high rate of survival when compared to other types of salticids that encounter ants, possibly due to some form of behavioural mimicry (Nelson, et al., 2004; Nelson, et al., 2005). The fact that *Myrmarachne* run away from ants, or "push" them with their chelicerae suggests that the salticid has developed these mechanisms for avoiding serious injury or death from the ants. The fact that *Myrmarachne* never really attack ants (the way they would prey) also suggests that their behaviour is matched to the ants' in that the *Myrmarachne* do not elicit an aggressive response from the ants.

Chapter 4 : A comparison of the interactions of two ant-associating jumping spiders (Araneae: Salticidae) with their common model ant species

Abstract

Myrmarachne and *Cosmophasis* are both jumping spiders that mimic ants. In North Queensland there is a *Myrmarachne* species, and a species of *Cosmophasis* that both associate with the green tree ant *Oecophylla smaragdina*. *Myrmarachne* mimics the ant visually, and *Cosmophasis bitaeniata* mimics the cuticular hydrocarbons of the *O. smaragdina* worker ants. In this study similarities and differences in how the two spiders interact with workers from model ant colonies were examined. *Myrmarachne* and *C. bitaeniata* had the same reaction types to the ant, but actions occurred at different frequencies. The two salticids reacted at similar distances to the ant, and the position of the ant when seen by the salticids was only slightly different. Another similarity between the *Myrmarachne* and the *C. bitaeniata* was that they both avoided coming into contact with the ant. Overall, there were more similarities than differences between the way the two salticids interact with *O. smaragdina* worker ants, even though *Myrmarachne* and *C. bitaeniata* have different methods of mimicking *O. smaragdina* workers.

4.1 Introduction

Many arthropods mimic ants through morphological, behavioural or chemical resemblances. Ant-mimicry is beneficial for reasons ranging from protection from potential predators (Batesian mimicry), to being able to take advantage of the ant colony in some way (aggressive mimicry) (see review by Rettenmeyer (1970)). Morphological and behavioural mimicry of ants is also known as myrmecomorphy, whereas myrmecophily describes the case where arthropods associate closely with ants. The occurrence of both myrmecomorphy and myrmecophily in arthropods has been reviewed by McIver and Stonedahl (1993) (in general), and by Cushing (1997) (focusing on spiders).

Of the spider families, the jumping spider family (Araneae: Salticidae) is the one containing the highest number of recognized species (5027) grouped in 550 genera (Platnick, 2005). Both myrmecomorphy and myrmecophily can be found in salticids (Cushing, 1997). Within the Salticidae *Myrmarachne* McLeay, 1835 is a genus comprised solely of myrmecomorphic species. These animals are considered to be Batesian mimics of ants (Wanless, 1978; Edmunds, 1993). There are over 200 named species of *Myrmarachne* world wide, and many more that have yet to be named or described (Proszynski, 2003).

Myrmarachne plataleoides (O. P.-Cambridge) found in India and Malaysia, is a known mimic of the green tree ant - *Oecophylla smaragdina* – workers (Mathew, 1944). In North Queensland, Australia there is another species of *Myrmarachne* that mimics *O. smaragdina* and lives in the proximity of the ant colonies (referred to as *Myrmarachne*

sp. F in this study; voucher specimens can be found in the Queensland Museum, Brisbane, with reference numbers s66651 and s66652).

In North Queensland another salticid that associates with the green ant is *Cosmophasis bitaeniata* (Keyserling, 1882). The association with the ants makes *C. bitaeniata* a myrmecophile, but although there is a slight similarity in coloration between *C. bitaeniata* and *O. smaragdina*, the salticid does not bear the same physical resemblance to the ants as the *Myrmarachne* species. The mimicry of *O. smaragdina* by *Cosmophasis bitaeniata* has been found to be mainly chemical in that the spider mimics the cuticular hydrocarbons specific to the green ant colony with which it associates (Allan & Elgar, 2001; Allan, et al., 2002). This is considered to be a form of aggressive mimicry, whereby the chemical signature of the ant colony allows the salticid to enter the ants' nest undisturbed where it preys on the ant larvae (Allan & Elgar, 2001; Allan, et al., 2002). However, *Cosmophasis bitaeniata* avoids contact with the aggressive worker ants (Allan & Elgar, 2001).

Myrmarachne also tend to avoid direct contact with ants (Mathew, 1944; Edmunds, 1978; Halaj, et al., 1997; Nelson, et al., 2005), as these react aggressively towards animals that are not part of their colony (Hölldobler, 1983; Hölldobler & Wilson, 1990). So studies on both *Myrmarachne* and *Cosmophasis* reactions to ants have shown that both species avoid direct contact with ants using their vision.

The aim of this study is to determine the similarities and differences in the reactions of two ant-associating salticids to their model ant, by looking at the reactions of both the *Myrmarachne* and the *Cosmophasis* species to the green tree ant *O. smaragdina*. Since

one of the spiders is a chemical mimic and the other is a visual mimic, the question is whether they react in a similar manner to the same ant, or whether they have developed different behavioural strategies for dealing with the ant.

4.2 Materials and methods

Salticids were videotaped as they interacted with the ants. Videotape recording was carried out by placing one *Myrmarachne* sp. F or one *Cosmophasis bitaeniata* and one *Oecophylla smaragdina* worker ant in a 10 cm diameter plastic petri-dish and recording by using a low light, high resolution video camera connected to a Panasonic NV-L25 HQ PAL video recorder. The apparatus allowed for 50 fields per second resolution. Recordings were made for one hour.

Myrmarachne sp. F, *Cosmophasis bitaeniata*, and ants were collected and used immediately whenever possible. The spiders and ants were collected from the same tree whenever possible, to ensure that the spiders and ants were likely to be ones that would encounter each other in nature, and to ensure that the colony odour of the *C. bitaeniata* would be the same one as that of the ant, thus avoiding an aggressive reaction from the latter. Each *Myrmarachne* sp. F–ant or *Cosmophasis bitaeniata*–ant combination was carried out in a new petri-dish, to avoid the possibility of chemical cues from a previous experiment influencing the outcomes of the following experiment.

Analyses of the recordings were carried out using a SVHS player connected to a computer with a Miro DC 30 video digitising card, using the program Adobe Premiere (version 4.2).

In this study, an interaction between a salticid and an ant is defined as the point in time at which the salticid shows a definite response (or change in behaviour brought about by an external stimulus) to the ant. For every interaction, the following variables were recorded:

Motion spider: whether the salticid is moving (m) or stationary (s)

Motion ant: whether the ant is moving (m) or stationary (s)

The distance at which the salticid reacts to the ant

The position of the ant when salticid sees it: a = the head front-on, b = the head side-on, c = the thorax or abdomen side-on, d = the abdomen from behind, e = the ant is behind the spider

The primary salticid reaction (see Table 4.1)

Table 4.1: Primary salticid reactions

Reaction	*Description*
0	No reaction, just looking
1	Jumps backwards (x1)
2	Jumps backwards (x2)
3	Jumps backwards (x3)
4	Turns around and run away
5	Turns around and walk away
6	Touches ant (non-aggressive)
7	Attacks ant
8	Side-steps
9	Jumps to the side
10	Moves backwards (not jumping)
11	Runs forwards
12	Follows ant visually by turning
13	Jumps forwards
14	Moves to the side and walk/run forwards
15	Opens chelicerae (males only)
16	Follows ant
17	Moves towards ant

The activity levels of ants and spiders vary between individuals and species. To account for this variation, an index, named the Total Motion Index (hereafter TMI) has

been developed. This index is used as the variable accounting for interspecific differences of ant activity. The TMI is calculated as:

(number of interactions/minute) x (1+ Psm) x (1+ Pam)

where Psm and Pam are the proportions of movement of the spider and ant respectively.

The more the spider and the ant move around, the higher the likelihood of interactions and subsequently the higher the TMI. So generally, a high TMI shows a more active state of the salticid, the ant or both. Including the TMI in analyses means that the movements in a salticid's surrounding (including its own movements) are accounted for as a possible variable influencing its reactions.

All of the data analysis was carried out using the program R version 2.1.1 (R_Development_Core_Team, 2005), with the vegan package (Oksanen, et al., 2005) for carrying out a Mantel test with Pearson's product-moment correlation of the distance matrices of the *Myrmarachne* sp. F and *C. bitaeniata* data sets. Recursive partitioning analysis – a version of CART (Classification and Regression Tree Analysis), which was popularised by Breiman *et. al.* (1984) was also carried out with the rpart package (Therneau, et al., 2005). CART analysis groups and divides independent variables based on their predictive value of the dependent variables. An ANOVA was carried out on the data to find any differences in the distance at which the two salticids reacted to the ant. ANOVA was also used to find differences in the TMI. Chi-squared tests were applied do the data on distance, motion and reaction frequencies of the salticids to *O. smaragdina*.

4.3 Results

The Mantel test shows whether there is a significant linear correlation between two distance matrices. The r-value for the Mantel test on distance matrices using Pearson's product-moment correlation between *Myrmarachne* sp. F and *Cosmophasis bitaeniata* based on 1000 permutations is 0.03898, with a significance of 0.087. This means that there is a weak correlation between the distance matrices of *Myrmarachne* sp. F with ants and *C. bitaeniata* with ants.

The following figures show trees constructed by CART analysis. The dependent variables are shown at the end of the trees' branches, whereas the nodes show the dependent variable with the greatest predictive value, with the categories (or values) indicating the "decision" of following the node split to the left.

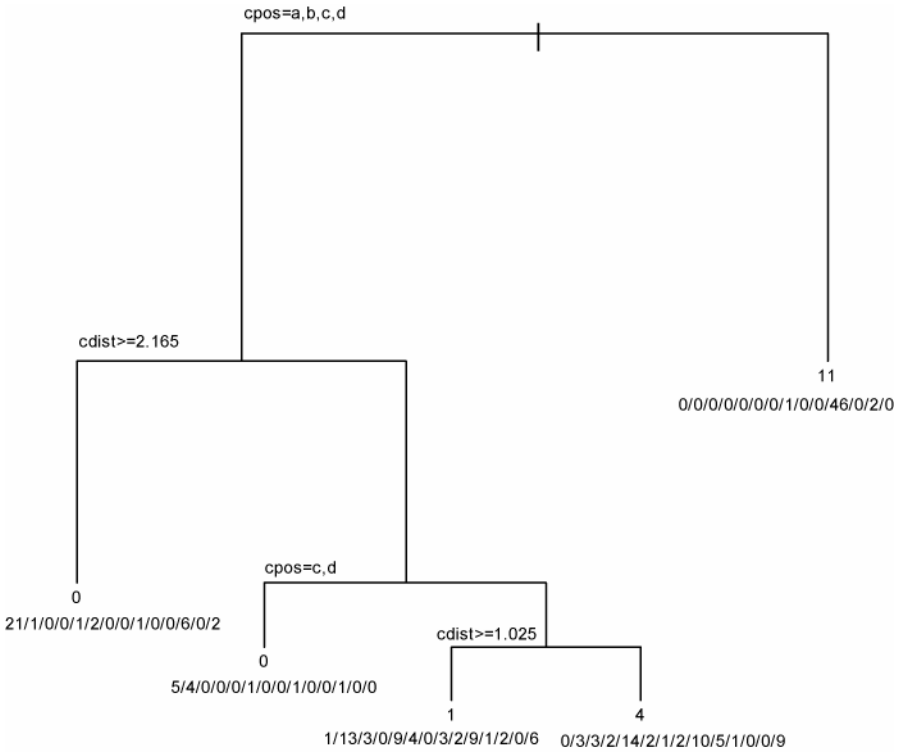


Figure 4.1: Recursive partitioning tree of *Cosmophasis bitaeniata* reactions to the ant.
The maximum depth was set to 4 and the reactions are at the end of the branches. cpos refers to the position the ant was in when seen by *Cosmophasis* ("a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant/bug is behind *Cosmophasis*), and cdist is the distance (in cm) between *Cosmophasis* and the ant at the time of the spider's reaction.

Figure 4.2: Recursive partitioning tree of *Myrmarachne* sp. F reactions to the ant.
The maximum depth was set to 4 and the reactions are at the end of the branches. mpos refers to the position the ant was in when seen by the spider ("a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant/bug is behind *Myrmarachne* sp. F), mdist is the distance (in cm) between the spider and the ant at the time of the spider's reaction, mtmi is the Total Motion Index value for the particular *Myrmarachne* /ant combination and mma is whether the ant was stationary (s) or moving (m).

The following Figures show the outcomes of separate variables in this study.

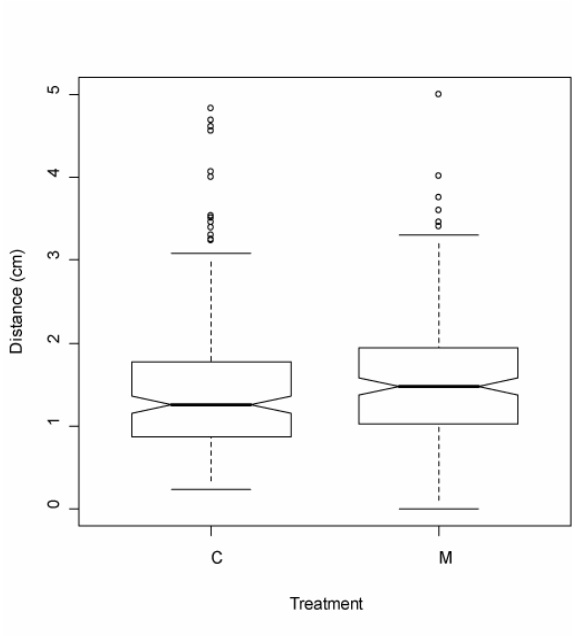


Figure 4.3: Notched boxplot of mean distance (in cm) between *Cosmophasis bitaeniata* (C) and the ant, and *Myrmarachne* sp. F (M) and the ant
(ANOVA: $F_{(1,398)} = 0.168$, $p = 0.682$).

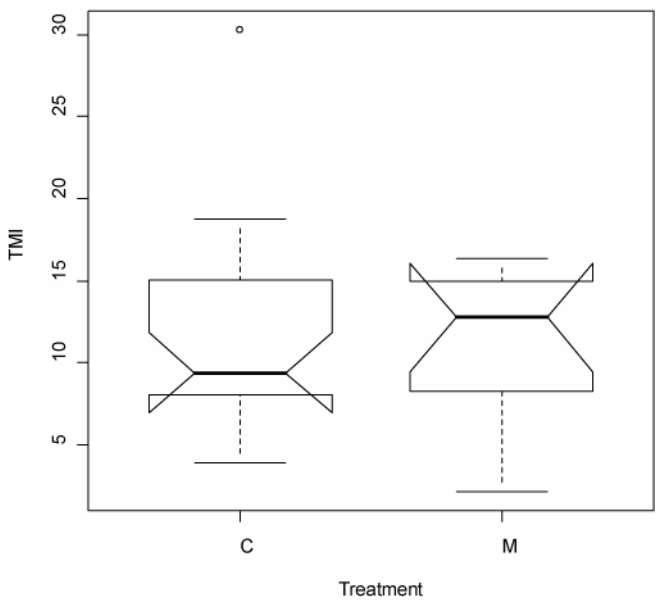


Figure 4.4: Notched boxplot of mean TMI (Total Motion Index) value between *Cosmophasis bitaeniata* (C) and *Myrmarachne* sp. F (M).
(ANOVA: $F_{(1,28)} = 0.113$, $p = 0.739$).

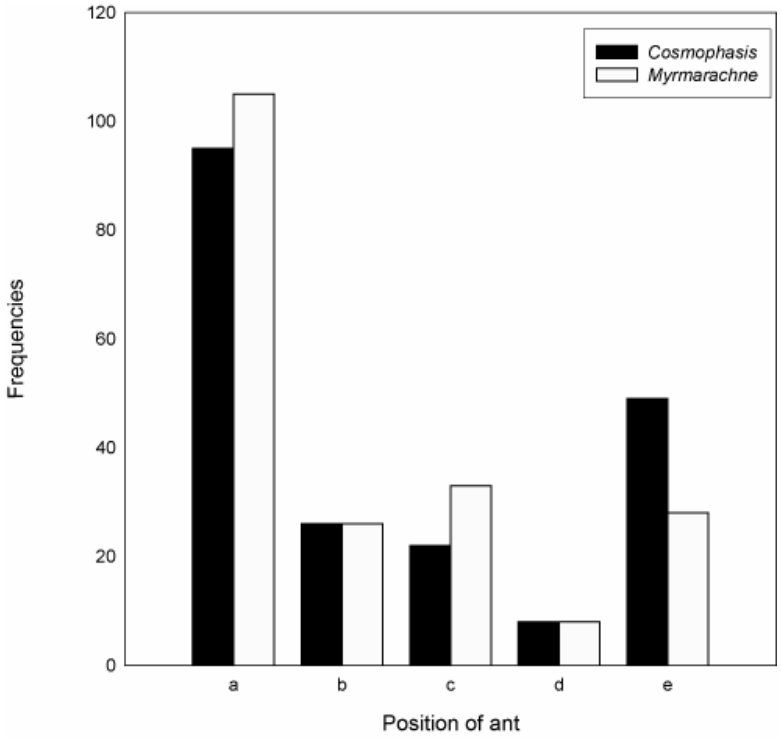


Figure 4.5: Bar chart showing total frequencies of each position the ant was in when seen by the two spiders, *Cosmophasis bitaeniata* and *Myrmarachne* sp. F.
Cosmophasis bitaeniata is represented by the black bars, and *Myrmarachne* sp. F by the white bars. Positions of the ant are coded as: "a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant is behind the spider. ($\chi^2_4 = 8.43$, $p = 0.077$).

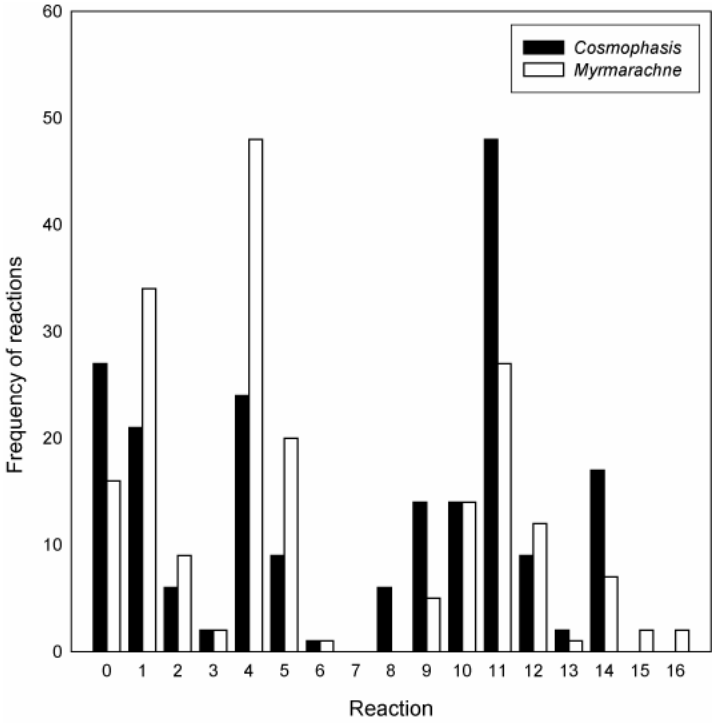


Figure 4.6: Bar chart showing the total frequencies for each reaction carried out by *Cosmophasis bitaeniata* and *Myrmarachne* sp. F when seeing the ant in any position.
Reactions are as described in Table 4.1. *Cosmophasis bitaeniata* is represented by the black bars, and *Myrmarachne* sp. F by the white bars. ($\chi^2_{16} = 43.73$, $p < 0.001$).

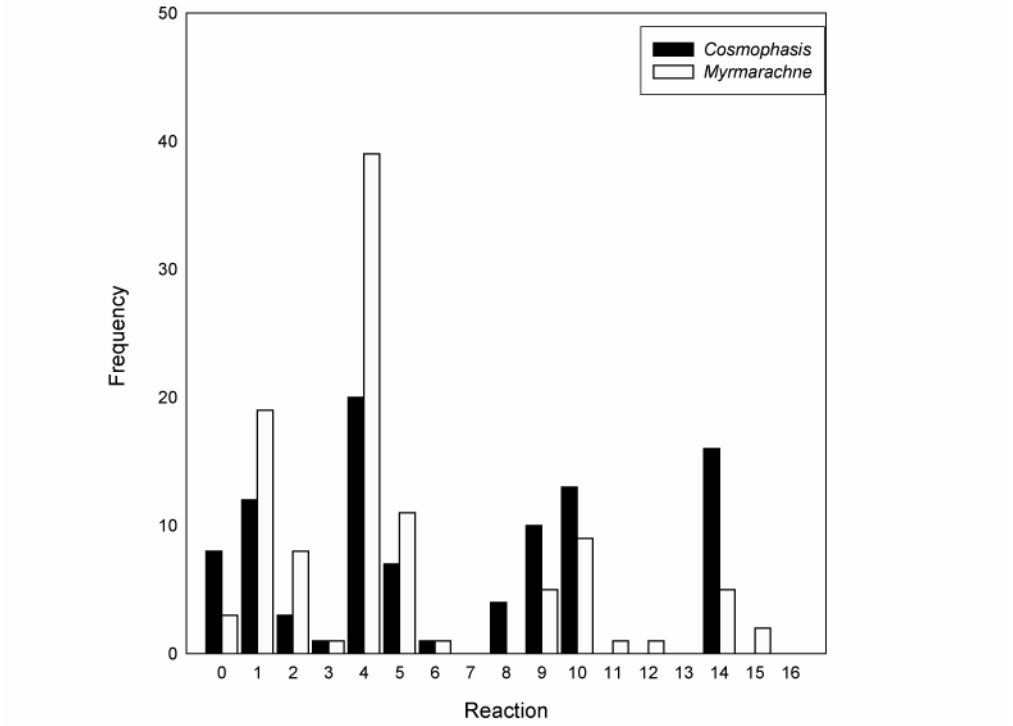


Figure 4.7: Total frequency of each reaction carried out by *Cosmophasis bitaeniata* and *Myrmarachne* sp. F when the ant was seen by the spider head-on (position "a").
Cosmophasis bitaeniata is represented by the black bars, and *Myrmarachne* sp. F by the white bars. ($\chi^2_{16} = 28.86$, $p = 0.025$).

The recursive partitioning analysis showed that for both salticids the first split of variables distinguishes when the ant is behind the spider, and the spider runs forwards. After that, *Cosmophasis bitaeniata* not reacting (reaction "0") is correlated to two separate variables: a distance to the ant greater than 2.17 cm, or seeing the ant's body side-on or from behind (positions "c" and "d"). If however, the ant's head is seen front- or side-on, and the distance is smaller than 1.03 cm, the most likely *C. bitaeniata* reaction is to take a jump backwards. If in the same scenario the distance to the ant is greater than 1.03 cm, then the most closely correlated reaction of *C. bitaeniata* is to turn around and run away. The movement of the ant is the next most important predictor variable for *Myrmarachne* sp. F reactions, where a stationary ant and a TMI smaller than 8.67 are correlated with no reaction, but a stationary ant and a TMI greater than 8.67 are correlated with *Myrmarachne* sp. F turning around and walking away. *Myrmarachne* sp. F turning and running away is correlated with a moving ant and a distance smaller than 2.36 cm, or a moving ant, and a distance greater than 2.36 cm, and seeing the ant front-on or from the rear. In the last scenario, if the ant is seen side-on (either the head or the body); the correlated *Myrmarachne* sp. F reaction is to follow the ant visually by turning.

The mean distance between *Cosmophasis bitaeniata* and *O. smaragdina* at the time of the salticids' reaction is not significantly different to that between *Myrmarachne* sp. F and the ant, meaning that the two salticids react at approximately the same distance to the ant, since the ANOVA F-value was 0.168 (with 1,398 degrees of freedom), giving a significance of 0.682. The same applies to the average TMI of either salticid with *O. smaragdina*, where the ANOVA F-value was 0.113 (dof = 1,28), and the significance 0.739, which shows that overall there is a similar degree of general movement in the

Petri-dish. When looking at motion as a variable, there is a significant difference between the frequency of movement of *Cosmophasis* and *Myrmarachne* sp. F, since Pearson's Chi-squared test with Yates' continuity correction gave a chi-squared value of 46.81, which makes $p<0.0001$. *Myrmarachne* moves during 85.5% of the interactions, whereas *C. bitaeniata* moves less frequently, namely 53.5% of interactions. The movement of the ant is also significantly different depending on the salticid the ant is with, giving a chi-squared value of 12.82, and a significance value less than 0.001. The ant moves during 96% of the interactions when it is in the vicinity of *C. bitaeniata*, as opposed to 85% of the interactions when it is with *Myrmarachne* sp. F.

The frequencies of each position the ant is in when it is seen by each salticid are not highly significant, since the chi-squared value is 8.43 and the degrees of freedom are four, which gives a p-value of 0.077. The main difference is that *Myrmarachne* sp. F saw the ant more frequently front-on and side-on to the body than *C. bitaeniata*, whereas *C. bitaeniata* was more frequently in front of the ant during an interaction than *Myrmarachne* sp. F. The reactions of the two salticids to *O. smaragdina* were carried out at significantly different frequencies to each other ($\chi^2_{16} = 43.73$, $p < 0.0002$). Both salticids carried out more avoidance than other reactions to the ant, but whereas *Myrmarachne* sp. F jumps backwards, and turns around more frequently than *C. bitaeniata*, the latter tends to use sideways avoidance more often than *Myrmarachne* sp. F. Contact with the ant was 0.5% of the total reactions for both salticids, meaning that contact with *O. smaragdina* is an equally infrequent reaction for both *Myrmarachne* sp. F and *C. bitaeniata*. When considering the salticid reactions to seeing the ant in each position separately, the only position under which the reaction frequencies are significantly different is position “a” ($\chi^2_{16} = 28.16$, $p < 0.025$).

4.4 Discussion

Cosmophasis bitaeniata and *Myrmarachne* sp. F are both ant-mimicking salticids that associate with the same ant, *Oecophylla smaragdina*, in North Queensland, Australia. *Myrmarachne* sp. F is a visual, and *C. bitaeniata* a chemical mimic of the ant. This means that *O. smaragdina* workers might not see the difference between a *Myrmarachne* sp. F and an individual of their own colony, but would probably notice when making contact with the spider and finding different chemical compounds to the ones on their nestmates' cuticles. On the other hand, due to the chemical mimicry of *O. smaragdina* cuticular hydrocarbons, *C. bitaeniata* can enter the ants' nest undetected (Allan & Elgar, 2001). The different approaches to mimicry could mean that the behaviour of the spiders is different.

The weak correlation between the distance matrices of the two salticids' interactions with the ants, is probably caused by of the different frequencies at which the salticids carry out each reaction. The analyses of each variable separately show that in fact there are many similarities between *Myrmarachne* sp. F and *C. bitaeniata*'s interactions with *O. smaragdina*. For example, both spiders react at a similar distance to the ant, meaning that the response distances of the two salticids are similar. In both cases the frequencies of the ant's positions when the spiders see it are only slightly different. *Myrmarachne* sp. F sees the ant front-on more often than *C. bitaeniata* does, possibly because *Myrmarachne* sp. F is slightly more efficient at turning around so that anything approaching falls in the field of vision of the AM eyes. And although the individual frequencies of movement of both spider and ant are different, the TMI – which gives an overall value for motion in the Petri-dish – is not significantly different for the two salticids and the ant.

Considering the reactions of *C. bitaeniata* and *Myrmarachne* sp. F separately, there is a significant difference between the reaction frequencies of the two salticids. This is also the case when the ant is front-on to the spiders. However, the reaction types of the two salticids are similar, except that *Myrmarachne* sp. F favours backwards-moving reactions and *C. bitaeniata* moves to the side more frequently. Again, this may be related to the mechanical properties of the body structures of the two salticids.

A worker ant from any *O. smaragdina* colony making contact with *Myrmarachne* sp. F is expected to attack it, since *Myrmarache* sp. F have a "foreign" chemical signature to the *O. smaragdina* ants. This is not necessarily the case for *Cosmophasis*, which mimic cuticular hydrocarbons of specific *O. smaragdina* colonies, a phenomenon allowing it to enter the ants' nest to prey on their larvae (Elgar & Allan, 2004). However, *C. bitaeniata* avoids contact with *O. smaragdina* workers just as much as *Myrmarachne* sp. F. The finding that both salticids avoid contact with the ant supports previous studies (Mathew, 1944; Edmunds, 1978; Allan & Elgar, 2001). So perhaps *Cosmophasis* only allows the ants to make contact with it when it is absolutely necessary, such as when *Cosmophasis* enters the ants' nest to prey on the larvae, where there is limited space to move out of the ants' way.

In conclusion, the differences between the two salticids can be explained in terms of body structure and manoeuvrability coming from fine mechanical differences, such as the length of the legs in relation to the body. The similarities show that the visual abilities of the two spiders are similar, and that they both react in the safest way to avoid being attacked or even killed by an *O. smaragdina* worker. So in the end, the different

strategies and purposes of ant-mimicry adopted by the two salticids do not have a significant effect on how the spiders interact with their model ants.

Chapter 5 : Behavioural mimicry in ant-mimicking spiders (Araneae: Salticidae) from North Queensland

Abstract

Both the ant-mimicking spiders of the genus *Myrmarachne* (Araneae: Salticidae) and *Cosmophasis bitaeniata* (Araneae: Salticidae) imitate ants through certain types of behaviour, such as the "antennal illusion" and moving the opisthosoma up and down. In this study, the behavioural mimicry of four different sympatric *Myrmarachne* species and of the sympatric *C. bitaeniata*, which shares a model with one of the *Myrmarachne* species, was examined. The behavioural mimicry was found to be very variable, and mainly conditional on the other behaviours of the salticid at any time. There was a significant difference in the behavioural mimicry activity of *Myrmarachne* and *C. bitaeniata*, as well as differences between the *Myrmarachne* species. The occurrence of interspecific differences is a strong indication that selection pressure is acting on behavioural mimicry, as it is on other traits that determine the resemblance of a mimic to its model.

5.1 Introduction

Ant mimicry in arthropods can be morphological, chemical or behavioural, or a combination of any of these (Rettenmeyer, 1970; McIver & Stonedahl, 1993). Many spider families – such as the Salticidae and Clubionidae – contain species or genera of ant mimics (for review see (Cushing, 1997)). One of the major morphological differences between ants and spiders is that the former have three pairs of legs and one pair of antennae, whereas the latter have four pairs of legs and no antennae. Thus predators of spiders may be able to distinguish between the two arthropods by the presence or absence of antennae. Spiders overcome this difference and thus increase their resemblance to ants through the phenomenon that has been termed "antennal illusion" (Reiskind, 1977). Antennal illusion is a term describing several different components. Antennal illusion involves the modification of the spider's first pair of legs in various ways, giving them a closer resemblance to the antennae of ants. These modifications include colour and pattern, form, posture and movement (Reiskind, 1977). For example, some spiders have black distal ends to their legs, giving them a shortened appearance. Alternatively, the antennal segmentation is mimicked by a darkening of the bases of tibial spines in some spiders (Reiskind, 1977). The mimicry of antennal movement involves the spider lifting its first pair of legs and waving them about (as ants would their antennae), while walking on its remaining three pairs of legs. This is done by holding the femur close to the prosoma, and having the segments mimicking the funiculi lie almost parallel to each other (Reiskind, 1977). The combination of structural modifications and of peculiar behaviours to emphasise the structures, together generates a remarkable gestalt illusion of an ant to human, and presumably to other, visual systems. These modifications all fall under the general term "antennal illusion".

Antennal illusion has been observed in several lineages of spiders, including members of the clubionid genera *Castianeira* (e.g. (Reiskind, 1977)) *Myrmecotypus* (e.g. (Jackson & Drummond, 1974; Reiskind, 1977)) and *Myrmecium* (Oliveira, 1988), as well as in salticid genera such as *Sarinda* (Jackson & Drummond, 1974), *Synemosina* (Reiskind, 1977) and *Myrmarachne*, a genus of Batesian ant-mimics (e.g. (Reiskind, 1977; Edmunds, 1978; Jackson, 1982, 1986)).

Myrmarachne have also been observed moving their opisthosoma up and down in a manner similar to gaster bobbing in ants (Edmunds, 1978; Jackson, 1982, 1986). In ants this motion is thought to be a ritualised behaviour for aggression, as demonstrated in *Polyrhachis laboriosa*, where a raised gaster is used by an ant to deter other ants from attacking it (Mercier & Dejean, 1996; Mercier, et al., 1997). *Oecophylla longinoda* is another ant said to raise its gaster in an aggressive context, namely to recruit nest-mates to a site where they are needed to help in an attack (Hölldobler, 1999). In ants this action might aid the release of pheromones to attract other worker ants. Either way, in ants such as *P. laboriosa* and *O. longinoda* a raised gaster is associated with aggression, and *Myrmarachne* have adopted this movement as part of their behavioural mimicry.

In Townsville, North Queensland, there are at least four different species of *Myrmarachne*, all of which carry out the antennal illusion, as well as moving their opisthosoma up and down. *Cosmophasis bitaeniata*, another salticid that mimics ants, also lifts its first pair of legs, but in a much less pronounced manner to *Myrmarachne* (personal observation). However, *C. bitaeniata* clearly moves its opisthosoma up and

down, a motion which in this study will be referred to as “bobbing” (“twitch abdomen” in Jackson (1982)). This study addresses the following questions:

1. Does the distance from an ant, or the position an ant is in, affect whether or not the *Myrmarachne* and *Cosmophasis bitaeniata* wave their first pair of legs, bob their opisthosoma or do both at the same time?
2. Is there a difference between *Myrmarachne* and *Cosmophasis bitaeniata* in the frequency at which they carry out antennal illusion and/or bobbing the opisthosoma?
3. Is there a difference in the frequency at which antennal illusion and/or bobbing the opisthosoma are carried out between the *Myrmarachne* species (and sex) used in this study?
4. Do the frequency and the amplitude at which the *Myrmarachne* wave their first pair of legs depend on other factors such as *Myrmarachne* movements, *Myrmarachne* species or *Myrmarachne* gender?
5. Do *Myrmarachne* carry out their antennal illusion/opisthosomal bobbing at different frequencies depending on whether or not they are reacting to an ant?

In addition to finding out how the behavioural mimicry relates to other traits, all these questions ultimately reflect the evolutionary dynamics of behavioural mimicry in ant-mimicking salticids. A comparison of the two salticid genera will show how their behavioural mimicry is related to their ecological niches. In contrast, a comparison between species of one genus (*Myrmarachne*) will indicate whether there is selection pressure on traits such as antennal illusion and opisthosomal “bobbing”, since interspecific differences in traits are a result of evolutionary differentiation.

5.2 Materials and methods

Video tape recordings made of a *Myrmarachne* or a *Cosmophasis bitaeniata* interacting with an ant inside a study area, a 10 cm diameter plastic petri-dish, were analyzed to determine the dynamics of all interactions. *Myrmarachne* were recorded both with their model and other sympatric ant species, whereas *C. bitaeniata* was recorded with its model *Oecophylla smaragdina* only. Recordings were made for 1 hour, on each occasion using a different pair of animals as well as a new petri-dish, thus avoiding possible chemical cues from a previous encounter affecting the behaviour of the animals in the subsequent one. The recording was carried out using a low light, high resolution video camera connected to a Panasonic NV-L25 HQ PAL video recorder. The subsequent analysis was done using a SVHS player connected to a computer with a Miro DC 30 video digitising card, using the program Adobe Premiere (version 4.2). Analysis of the recordings involved two separate stages:

1. For each salticid/ant combination, twenty instances (the first ten at the start of the recording and the next ten in the middle of the recording) when the spider reacted to the presence of the ant were analysed. The analysis involved noting whether the spider was waving its first pair of legs, lifting them up without waving them, and/or bobbing its opisthosoma. These states will be referred to more generally as "behavioural mimicry". The categories used to classify behavioural mimicry are shown in Table 5.1. At the same time, the behaviour of the spider was recorded, as well as its distance from the ant and the position the ant was in relative to the spider. As a measurement of the activity levels between a salticid and an ant, the Total Motion Index (TMI) was calculated and used. The calculation is as follows:

(number of interactions/minute) x (1+ Psm) x (1+ Pam)

where Psm and Pam are the proportions of movement of the spider and ant respectively.

This part of the study also allows for a comparison between *Myrmarachne* and *C. bitaeniata*.

Table 5.1: Behavioural mimicry carried out by *Myrmarachne* and *Cosmophasis bitaeniata*

Behaviour	***Explanation***
None	neither waving the antennae nor bobbing the opisthosoma
AI	waving of the first pair of legs only
Bob	only bobbing the opisthosoma
AI+bob	waving the first pair of legs and bobbing the opisthosoma at the same time
Lift	lifting the first pair of legs without the up-and-down movement

2. The recordings of *Myrmarachne* with ants (not including *C. bitaeniata*) were analysed by taking ten measurements per hour (every 6 minutes) of the frequency at which the first pair of legs was waved (cycles per second), and the amplitude (in mm) of the leg movement. When measuring the frequency, one cycle was taken to be the movement of the leg from one point through both top and bottom extremities and back to the starting point. The amplitude was measured from the highest apex of the tip of leg I to the substrate. At the same time, other variables were recorded, namely whether or not the *Myrmarachne* was moving, whether it was bobbing its opisthosoma and whether or not it had the ant in its field of vision (i.e. it was facing the ant with its anterior median eyes) at that particular point in time. Again, these variables were recorded to find any possible correlations to the antennal illusion. This part of the study was also carried out to find interspecific differences in *Myrmarachne* with regards to the "style" of the antennal illusion, i.e. the average speed and amplitude of the leg waving. The data was also recorded in terms of behavioural mimicry (as in part 1.) to get a comparison of

the frequencies of behavioural mimicry types between a general situation and instances when the *Myrmarachne* was reacting to the ant.

The data analysis was carried out in the program R version 2.1.1 (R_Development_Core_Team, 2005), using the rpart package (Therneau, et al., 2005) to build recursive partitioning (or classification) trees to find the variables most closely associated with the different groups of antennal illusion and bobbing. Classification tree analysis was popularised by Breiman *et. al.* in 1984 (Breiman, et al., 1984), and has since been used in various areas of science (e.g. (Lindbladh, et al., 2002; Lehmann, et al., 2003; Karels, et al., 2004).

ANOVAs and chi-squared tests were also carried out to find out interspecific differences between *Myrmarachne* species as well as *C. bitaeniata* and *Myrmarachne*.

5.3 Results

The classification trees constructed show which variables are the most important ones for predicting the different classes (or values) of the independent variables. The dependent variables are shown at the end of the trees' branches, whereas the independent variables are shown at each node, indicating the classes or values that are most closely correlated with the branch splitting off to the left. The following trees were constructed using the following data sets: 1. the groups of behavioural mimicry during the *Myrmarachne*'s reactions to the ants, 2. the groups of behavioural mimicry during the *Cosmophasis*' reactions to the ants, 3. the frequency and amplitude data of the leg waving in *Myrmarachne*, with variables such as motion of the spider included.

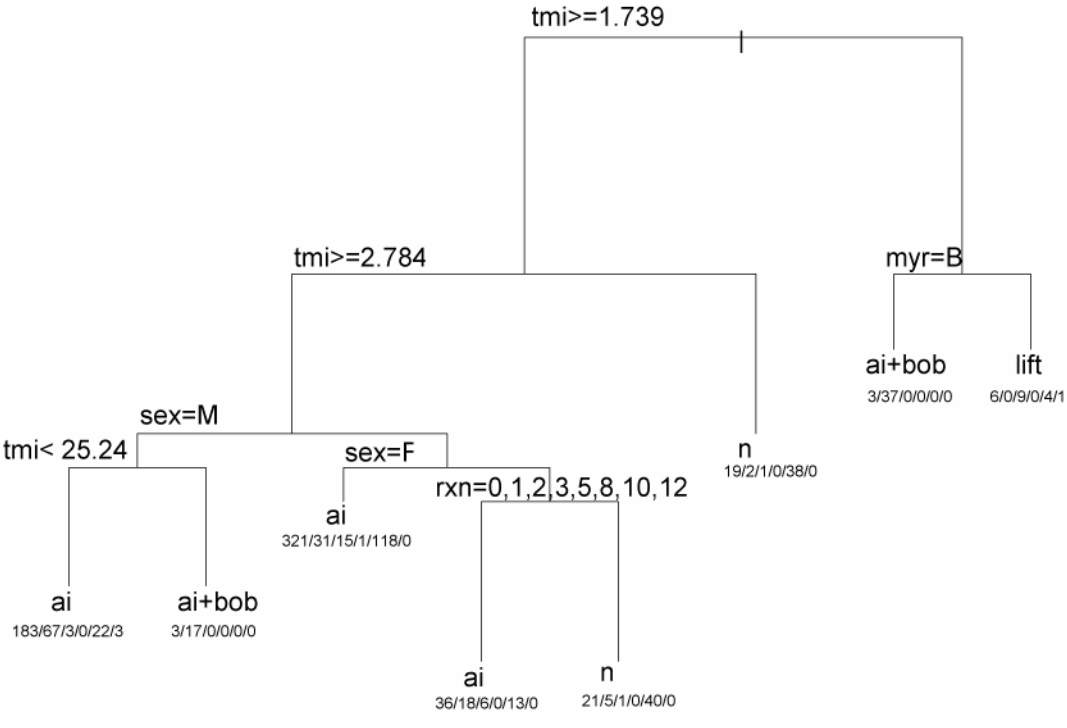


Figure 5.1: Recursive partitioning/classification tree of *Myrmarachne* behavioural mimicry profile and correlated variables during *Myrmarachne*'s interactions with ants.
Behavioural mimicry is coded as: n = neither waving the antennae nor bobbing the opisthosoma; ai = waving of the first pair of legs only; bob = only bobbing the opisthosoma; ai+bob = waving the first pair of legs and bobbing the opisthosoma at the same time; lift = lifting the first pair of legs without the up-and-down movement. tmi refers to the Total Motion Index, myr is the *Myrmarachne* species, sex is the *Myrmarachne*'s sex (M=male, F=female), rxn is the reaction category of *Myrmarachne* to the ant. The numbers under the dependent variables represent the number of times (count data) each class was placed in that particular partitioning sequence.

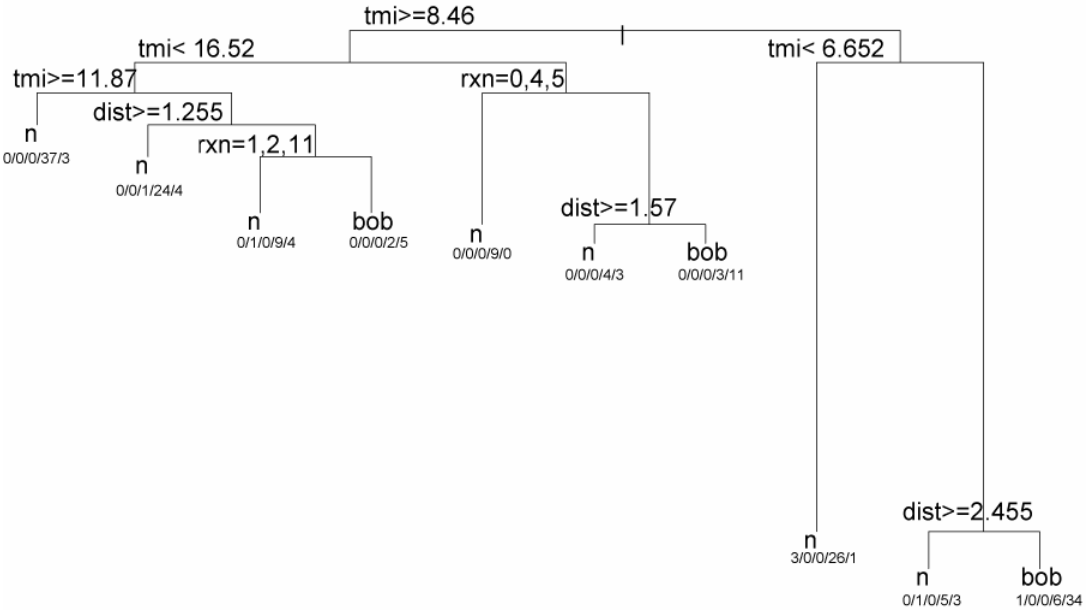


Figure 5.2: Recursive partitioning/classification tree of *Cosmophasis bitaeniata* behavioural mimicry profile and related variables during its interactions with ants.
Behavioural mimicry is coded as: n = neither waving the antennae nor bobbing the opisthosoma; ai = waving of the first pair of legs only; bob = only bobbing the opisthosoma; ai+bob = waving the first pair of legs and bobbing the opisthosoma at the same time; lift = lifting the first pair of legs without the up-and-down movement. tmi refers to the Total Motion Index, rxn is the reaction category of *C. bitaeniata* to the ant, dist is the distance (in cm) between the spider and the ant at the time of the spider's reaction. The numbers under the dependent variables represent the number of times (count data) each class was placed in that particular partitioning sequence.

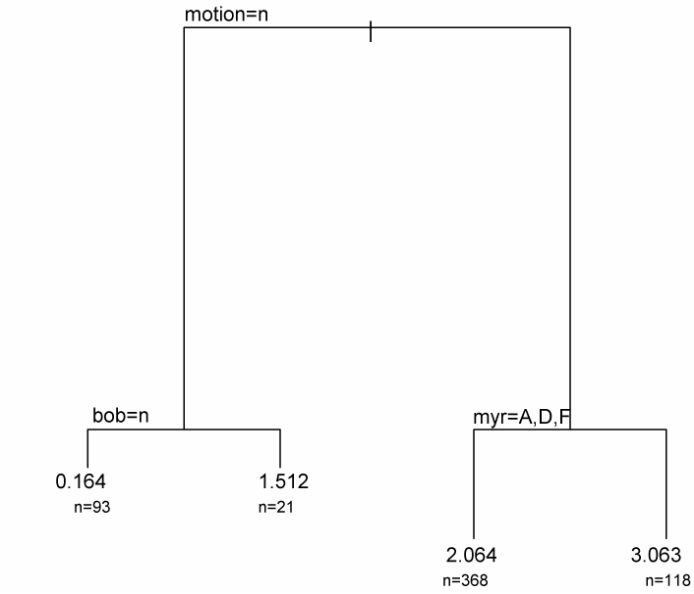


Figure 5.3: Recursive partitioning tree of frequency (cycles/second) of leg I waving by all *Myrmarachne* species, and the variables that are most closely correlated with a particular frequency value.
"motion" refers to whether the *Myrmarachne* was moving (y) or not (n), "bob" refers to whether the *Myrmarachne* was moving its opisthosoma up and down (y) or not (n), "myr" is the species of *Myrmarachne* (A, B, D and F). The letter n at the end of the frequency value represents the number of times that frequency value was placed in correlation with the predictor variables.

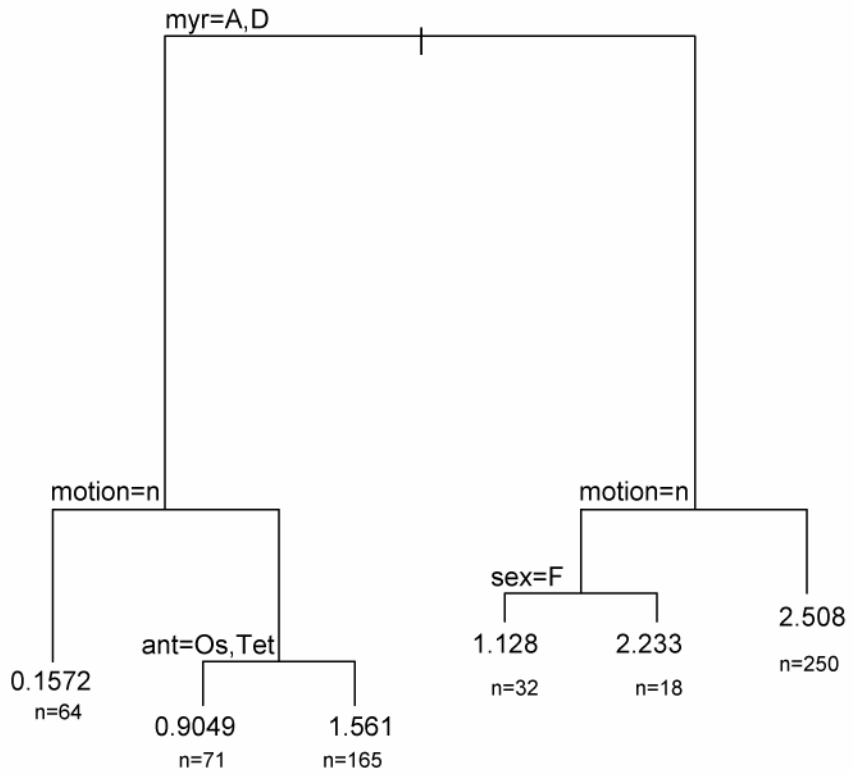


Figure 5.4: Recursive partitioning tree of amplitude (mm) of leg I waving by all *Myrmarachne* species. "myr" is the species of *Myrmarachne* (A, B, D and F), "motion" refers to whether the *Myrmarachne* was moving (y) or not (n), "sex" is the sex of *Myrmarachne* (M=male, F=female), and "ant" refers to the ant species (Os = *Oecophylla smaragdina*, Tet = *Tetraponera punctulata*) present in the Petri-dish with the spider. The letter n at the end of the amplitude value represents the number of times that amplitude value was placed in correlation with the predictor variables.

The main interpretations of the recursive partitioning trees will be given here. For both recursive partitioning trees of the interactions with ants of *Myrmarachne* and *Cosmophasis bitaeniata*, the Total Motion Index (TMI) is the highest independent variable in the tree, indicating that the TMI is correlated to the type of behavioural mimicry strategy (i.e. antennal illusion and/or opisthosomal bobbing). In addition, the recursive partitioning tree of the *Myrmarachne* behavioural mimicry shows that both the *Myrmarachne* species and the sex of the spider are factors closely correlated to the

Myrmarachne's preference in antennal illusion and/or bobbing. And as far as *Myrmarachne* reactions to the ants go, the general trend is that the legs are waved during reactions that do not involve running forwards. A closer look at the *Cosmophasis bitaeniata* recursive partitioning tree shows that this salticid either shows no behavioural mimicry, or it bobs its opisthosoma, usually at a high TMI, and a small distance to the ant.

The recursive partitioning trees of the frequency and amplitude of the *Myrmarachne*'s leg waving show the values of the respective variables and the most closely associated independent variables. The tree showing the frequency of leg I movements indicates that the lowest frequencies are most closely associated with the *Myrmarachne* neither moving nor bobbing its opisthosoma. When *Myrmarachne* moves, the next variable that partitions the data is the *Myrmarachne* species, where species B waves its legs at a higher frequency than the three other species. Similarly, *Myrmarachne* species is a predictor variable for the amplitude of leg movement. Plus, the amplitude is generally larger when the *Myrmarachne* are moving. The sex of the *Myrmarachne* also shows up as a predictor variable, where males are more closely associated with a larger amplitude value. A larger amplitude value is also associated with the presence of either *Opisthopsis haddoni* or *Polyrhachis* near *obtusa*, as opposed to the other two ant species, *Oecophylla smaragdina* and *Tetraponera punctulata*

The following graphs show the relationship between variables and antennal illusion characteristics both in *Myrmarachne* and *Cosmophasis bitaeniata*.

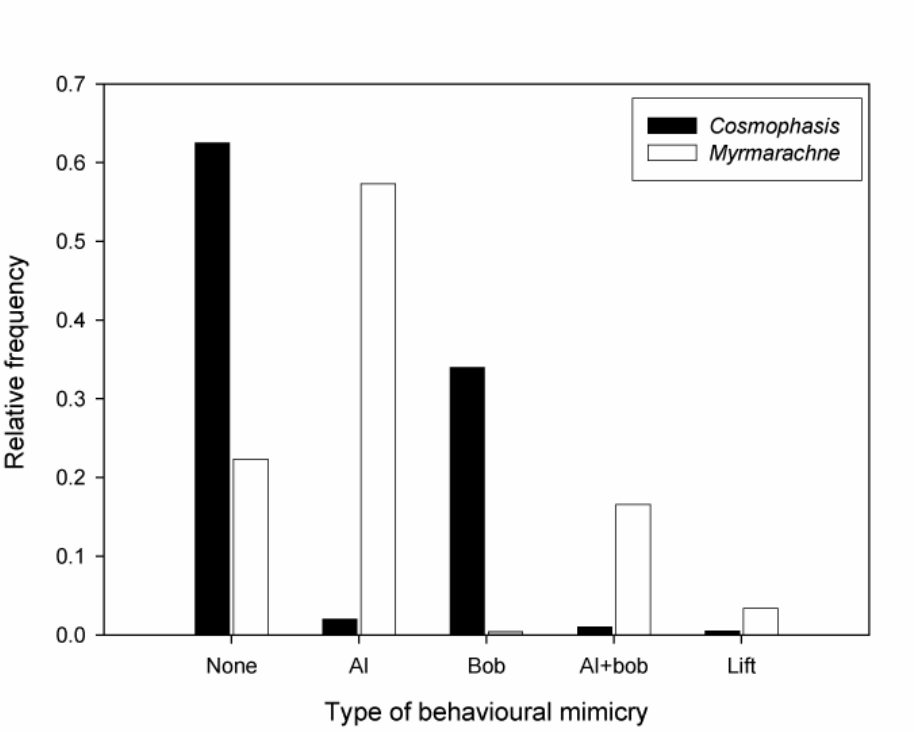


Figure 5.5: Bar chart showing the relative frequencies of the type of behavioural mimicry of *Myrmarachne* versus *Cosmophasis bitaeniata*.
Myrmarachne is represented by the white bars, and *Cosmophasis bitaeniata* by the black bars. (χ^2_4 = 46.13, p <0.0001)

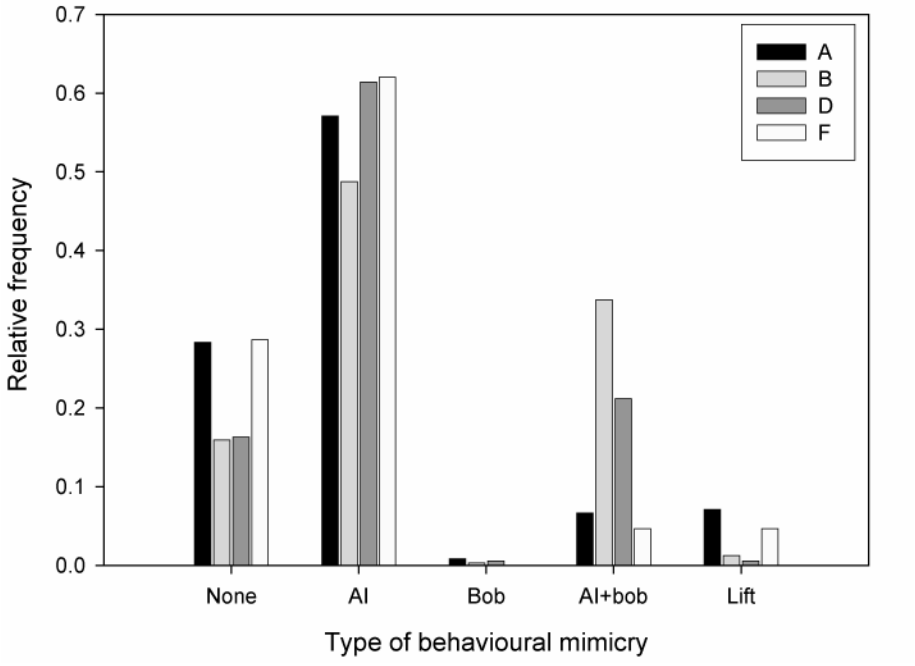


Figure 5.6: Relative frequencies of the type of behavioural mimicry of all four *Myrmarachne* species. (χ^2_{12} = 142.69, p <0.0001)

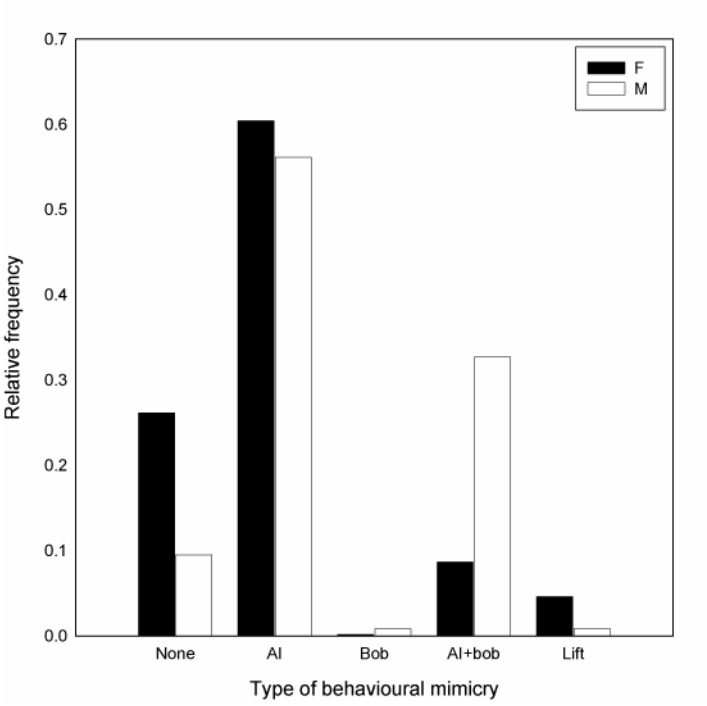


Figure 5.7: Relative frequencies of types of behavioural mimicry for *Myrmarachne* females and males. Females are represented by the black bars and males by the white bars. ($\chi^2_4 = 114.07$, $p < 0.0001$)

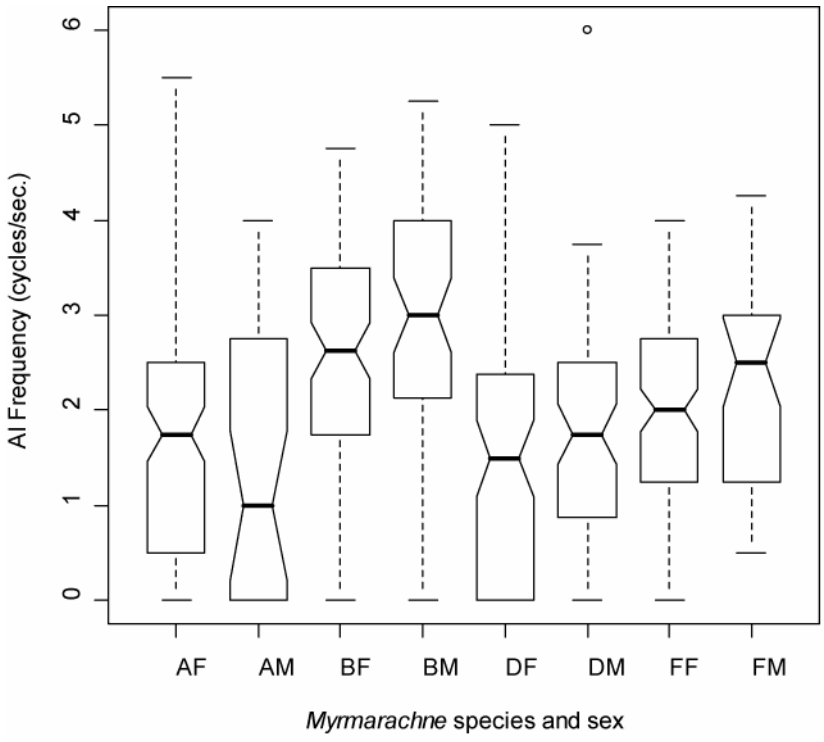


Figure 5.8: Notched boxplot of the frequency of leg I waving for each *Myrmarachne* species and sex. Species and sex are coded as: AF = females of species A, AM = males of species A; BF = females of species B, BM = males of species B; DF = females of species D, DM = males of species D; FF = females of species F, FM = males of species F. (ANOVA for species: $F_{(3,596)} = 23.16$, $p<0.0001$; ANOVA for sex: $F_{(1,598)} = 5.61$, $p = 0.018$; ANOVA for species-sex interaction: $F_{(3,592)} = 1.98$, $p = 0.115$).

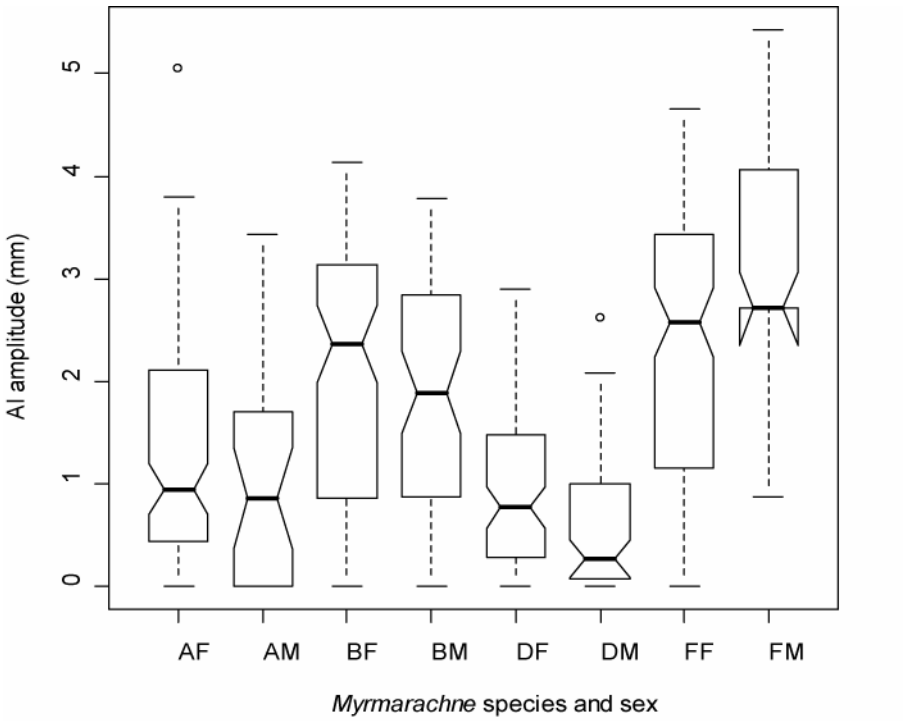


Figure 5.9: Notched boxplot of the amplitude of leg I waving for each *Myrmarachne* species and sex. Species and sex coded as: AF = females of species A, AM = males of species A; BF = females of species B, BM = males of species B; DF = females of species D, DM = males of species D; FF = females of species F, FM = males of species F. (ANOVA for species: $F_{(3,596)} = 62.56$, $p < 0.0001$; ANOVA for sex: $F_{(1,598)} = 0.97$, $p = 0.326$; ANOVA for species-sex interaction: $F_{(3,592)} = 7.44$, $p < 0.0001$)

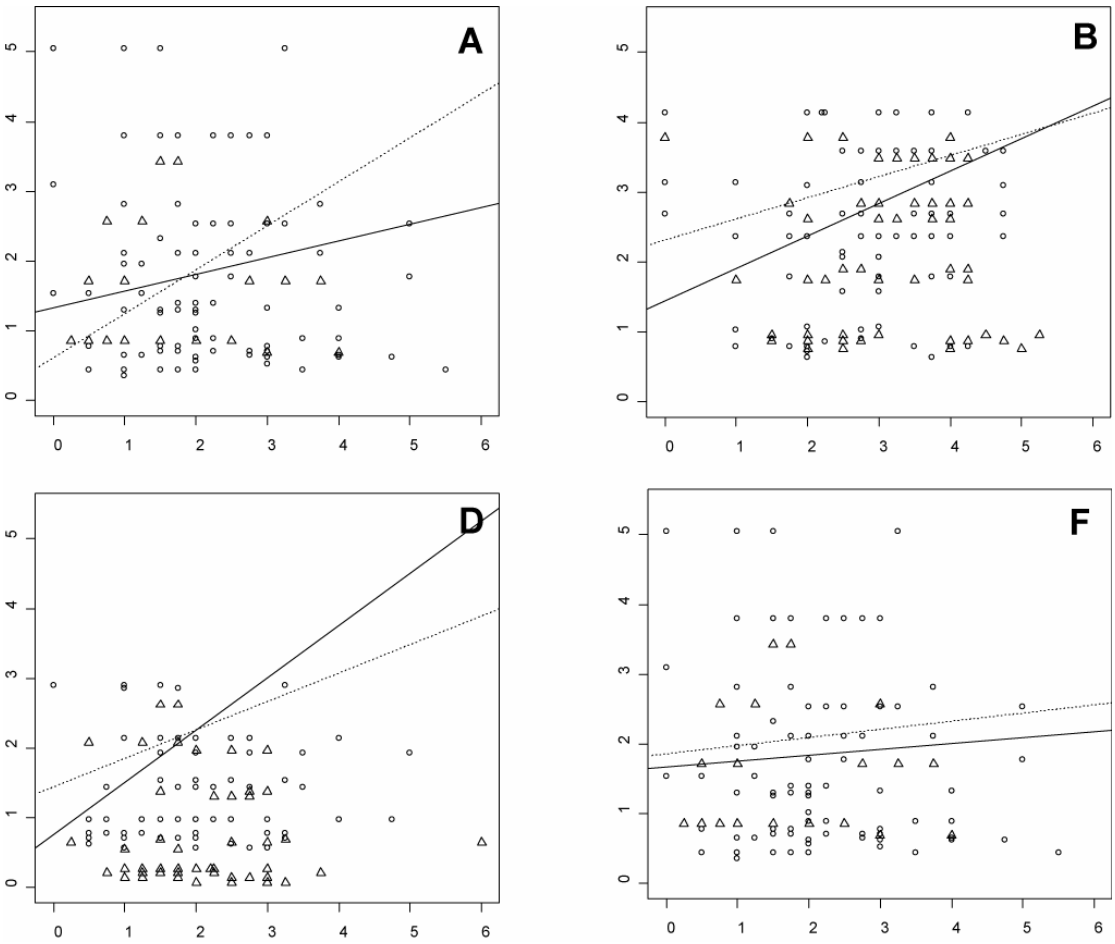


Figure 5.10: Scatterplots of frequency of leg I waving (in cycles per second) on the x-axes, versus the amplitude of leg I waving (in mm) on the y-axes for each *Myrmarachne* species separately
The species are "A", "B", "D" and "F" as shown on the top right-hand corner of each graph. Data for females is represented by the circles, with the solid best-fit line, whereas data for the males is represented by the triangles and the dashed best-fit line.

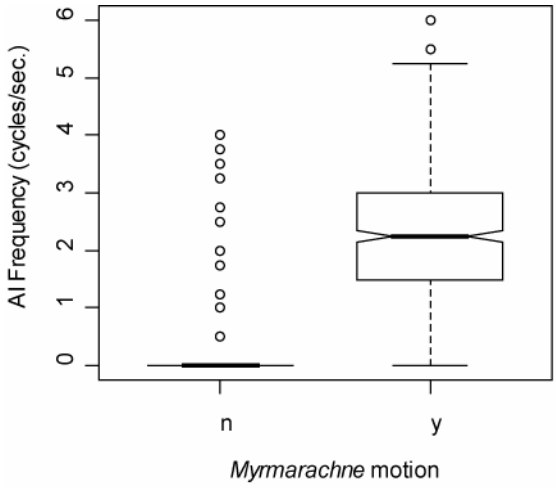


Figure 5.11: Boxplot showing the frequency of leg I waving for both *Myrmarachne* moving and not moving.
Myrmarachne moving is coded as "y" and not moving as "n". (ANOVA: $F_{(1,598)} = 277.32$, $p < 0.0001$)

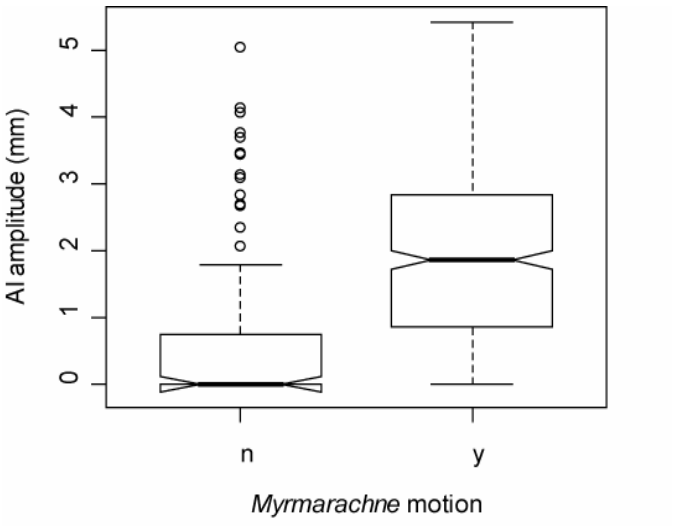


Figure 5.12: Amplitude of leg I waving for *Myrmarachne* moving and not moving.
Myrmarachne moving is coded as "y" and not moving as "n". (ANOVA: $F_{(1,598)} = 79.62$, $p < 0.0001$)

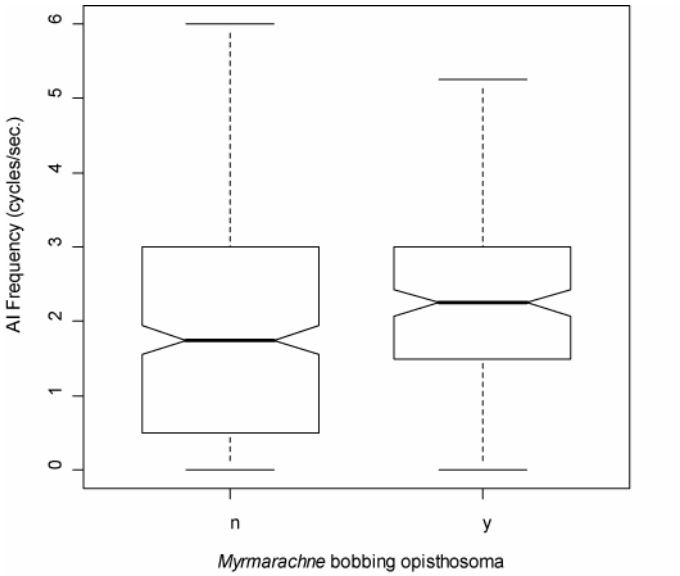


Figure 5.13: Frequency of leg I waving in *Myrmarachne* when the opisthosoma is "bobbing" and when it is still.
Myrmarachne's opisthosoma "bobbing" is coded as "y", and keeping it still as "n". (ANOVA: $F_{(1,598)} = 21.40$, $p < 0.0001$)

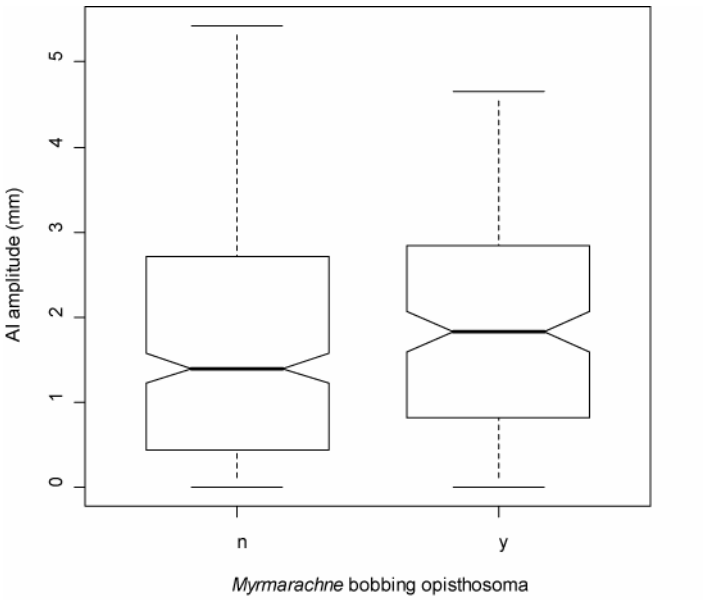


Figure 5.14: Amplitude of leg I waving in *Myrmarachne* when the opisthosoma is "bobbing" and when it is still.
Myrmarachne's opisthosoma "bobbing" is coded as "y", and keeping it still as "n". (ANOVA: $F_{(1,598)} = 2.75$, $p = 0.098$)

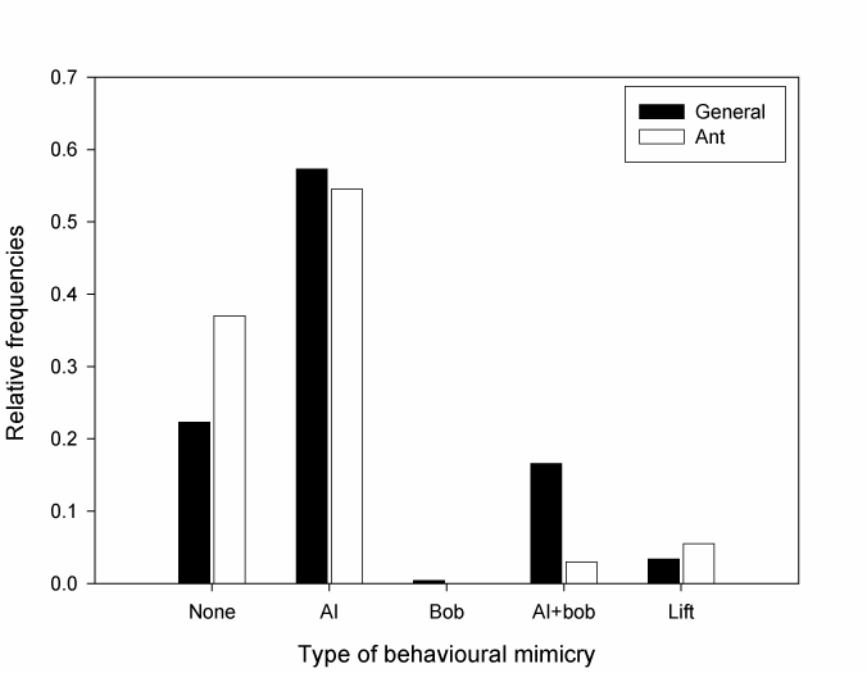


Figure 5.15: Relative frequencies of each type of behavioural mimicry for *Myrmarachne* in general and for *Myrmarachne* reacting to ants.
Myrmarachne in general is represented by the black bars and *Myrmarachne* reacting to ants by the white bars. ($\chi^2_4 = 46.13$, $p < 0.0001$)

The analyses of the data and the graphs show that *Myrmarachne* and *Cosmophasis bitaeniata* vary greatly in the way they carry out their behavioural mimicry ($\chi^2_4 = 46.13$, $p < 0.0001$). For example, *Myrmarachne* waves its legs I more than *C. bitaeniata*, whereas the latter moves its abdomen more frequently. There is also a significant difference in the way the four *Myrmarachne* species carry out the behavioural mimicry ($\chi^2_{12} = 142.69$, $p<0.0001$). *Myrmarachne* species B and D wave their legs I and bob their opisthosoma more frequently than species A and F, whereas the latter lift their legs I without waving at a higher frequency than species B and D. Also, there are interspecific differences in the frequency of the leg I waving, and its amplitude within *Myrmarachne*. There is also a significant difference between *Myrmarachne* males and females in the frequencies at which they carry out each type of behavioural mimicry, with a chi-squared value of 114.07 and 4 degrees of freedom, giving a p-value less than 0.0001. The main difference between males and females is that males bob their opisthosoma more frequently than females, and wave their first pair of legs at a slightly higher frequency than females. Also, males generally carry out any form of behavioural mimicry specified in this study more frequently than female.

The ANOVA for the frequency of leg waving between the *Myrmarachne* species gave an F-value of 23.16 (with 3/596 degrees of freedom), which gives a significance value less than 0.0001, whereas the F-value for the amplitude between species is 62.56 (with 3/596 degrees of freedom), which also gives a significance value less than 0.0001. The frequency of the waving of legs I also showed a slightly significant difference between *Myrmarachne* males and females, with the ANOVA giving an F-value of 5.61 (with dof = 1/598), which gives a p-value of 0.018. The difference lies in the males waving their first pair of legs at a slightly higher frequency than the females. On the other hand, the

ANOVA showed no significant difference in the amplitude of the leg waving between the sexes, with an F-value of 0.97 (with dof = 1/598) and a p-value of 0.336.

The analyses also show a positive correlation (with a coefficient of 0.337 and an R-squared value of 0.114) between the frequency and amplitude of the antennal illusion for all *Myrmarachne* species, the t-value for Pearson's product-moment correlation being 8.76, which makes the p-value lower than 0.0001. The fact that there is a positive correlation between the frequency and the amplitude gives an idea of the intensity of the movement. In other words, the higher up the spider moves its first pair of legs, the quicker it moves them up and down. This trend is consistent throughout all species and both sexes. Analyses of other variables showed that both the frequency and the amplitude of the leg waving increases in general when the spider is moving, and when it bobs its opisthosoma. The frequency of the leg waving when the spider was moving was significantly different to the frequency when it was not moving, as was shown by the ANOVA, which gave an F-value of 277.32 (with dof = 1/598), and a significance value less than 0.0001. Similarly, the ANOVA on the amplitude of the leg waving when the spider was moving or not gave an F-value of 79.62 (with dof = 1/598) and a p-value less than 0.0001. The frequency of the leg I waving is also significantly higher when the spider's opisthosoma is bobbing, with an ANOVA F-value of 21.40 (with dof = 1/598), and a significance value less than 0.0001. On the other hand, the amplitude of the leg waving is only slightly higher when the opisthosoma bobs, with an ANOVA F-value of 2.75 (with 1/598 degrees of freedom), giving a p-value of 0.098.

To see whether or not there is a difference in *Myrmarachne*'s behavioural mimicry when it sees an ant or not, the data set of *Myrmarachne* reactions was compared to the

one where readings were made at set points in time. The latter data set includes very few instances when the *Myrmarachne* reacted to the ant, meaning that it can be used as a comparison to instances when *Myrmarachne* did specifically react to an ant. The chi-squared test of the frequencies of reactions showed that there is a highly significant difference in the frequencies of behavioural mimicry, with a chi-squared value of 46.13, 4 degrees of freedom and a p-value less than 0.0001. The main difference is that *Myrmarachne* bobbed its opisthosoma more when it was not reacting to the ant, but waved, or held up its legs I more frequently when reacting to an ant. When the *Myrmarachne* reacted to the ant, there was also a higher frequency of neither type of behavioural mimicry.

5.4 Discussion

Behavioural mimicry of ants in salticids – as well as other spiders – mainly consists of the "antennal illusion" (e.g.(Reiskind, 1977; Edmunds, 1978; Jackson, 1982, 1986)), and the up-and-down "bobbing" of the opisthosoma (Edmunds, 1978; Jackson, 1982, 1986). These behaviours are variable in the frequency and intensity in which they are carried out by the spiders, even within the space of a minute. This study has shown that the behavioural mimicry also varies depending on factors such as the general movement of *Myrmarachne*. The intensity of the behavioural mimicry increases as *Myrmarachne* move around more, a tactic that may be used by the spider to increase the resemblance to the ant while foraging, which is a time when the spider is at risk from predation (but senses no imminent danger). However, *Myrmarachne* abandons its ant-like gait and behavioural mimicry when escaping potential danger (Edmunds, 1978; Jackson, 1986), such as approaching ants. This observation is supported by the results in this experiment that show that *Myrmarachne* tend to carry out less behavioural mimicry when reacting to an ant, probably because the spider abandons its behavioural mimicry while avoiding ants by jumping or running away from them.

The difference between *Myrmarachne* and *Cosmophasis bitaeniata* in the frequency at which they carry out the different forms of behavioural mimicry can be explained by the fact that their mimicry is targeted at different animals. *Myrmarachne* is a Batesian mimic of ants (Edmunds, 1993), so the resemblance to ants mainly serves as a deterrent to predators, who possibly react to the presence of "antennae" moving around in a manner similar to those of foraging ants. On the other hand, *C. bitaeniata* is an aggressive, chemical mimic of the green ant *Oecophylla smaragdina*, entering the ants' nest to feed on their larvae (Allan, et al., 2002; Elgar & Allan, 2004). Although *C.*

bitaeniata mimics the cuticular hydrocarbons of the ants to enter (and be accepted into) their colony (Elgar & Allan, 2004), there may also be a visual signal the spider produces that the ants interpret as one of their own. That may be why *C. bitaeniata* moves its opisthosoma up and down more frequently than *Myrmarachne*, a movement commonly seen in green ants, usually in the context of recruiting other nestmates to a site for defense (Hölldobler, 1983).

The difference in antennal illusion between sexes has been observed before in salticids, for example in *Sarinda linda*, where the males were observed waving their first pair of legs more often than the females (Jackson & Drummond, 1974). In this study, a difference between the sexes has been found in the frequency at which they carry out leg I waving and opisthosomal bobbing. The males seem to carry out more behavioural mimicry overall, especially bobbing the opisthosoma. This may have physiological explanations i.e. males generally have a more slender opisthosoma (personal observation) that can be moved up and down more easily. This study has also shown that *Myrmarachne* males move their first pair of legs at a higher frequency (cycles per second) than females. One explanation for this intersexual difference could be that due to the males' enlarged chelicerae, females resemble ants more closely than males, so perhaps the latter compensates for their appearance by putting more effort into behavioural mimicry. However, a recent study has found that male's enlarged chelicerae do not compromise their mimicry, but make them compound mimics of ants carrying a "parcel" in their mandibles (Nelson & Jackson, 2006). The other explanation may be that male *Myrmarachne*, when searching for mates, spend relatively more time in the open than the females, who wait inside their retreats for males (Jackson, 1982). This

means that male *Myrmarachne* are more often exposed to predators than females, and must therefore carry out more convincing ant- mimicry.

Behavioural mimicry is likely to be a plastic trait, and in Batesian mimics such as *Myrmarachne*, the selection pressure on resemblance to the model is relatively high (Mappes & Alatalo, 1997). This would probably explain the interspecific differences in *Myrmarachne* regarding the frequencies at which the different types of behavioural mimicry are carried out. Looking at the antennal illusion only, the frequency and amplitude of the waving of the first pair of legs also differs between species. The amplitude could be explained by the size difference between the species. However, the frequency is most likely to be a behavioural mimicry trait that has diverged with the species under Batesian mimicry selection pressure. Further research is needed to find out whether the behavioural mimicry of the *Myrmarachne* species is correlated with the antennal movement of their model ant species. This would show how closely associated the models and mimics are, and whether the selection pressure on antennal illusion has modified it on a small scale to suit each particular model ant species.

Chapter 6 : Reactions of an ant-mimicking spider, *Myrmarachne* species (Araneae: Salticidae), to its model, the green ant *Oecophylla smaragdina*, and to the co-occurring hemipteran ant-mimic *Riptortus serripes* (Hemiptera: Alydiidae)

Abstract

The alydiid bug *Riptortus serripes* and the salticid spider *Myrmarachne* sp. are co-occurring visual mimics of the green tree ant *Oecophylla smaragdina* in North Queensland, Australia. Salticids have been shown to possess remarkable visual abilities, allowing them to distinguish between prey, mates and rivals. The *Myrmarachne* species that mimics *O. smaragdina* lives in close proximity to ant nests and avoids contact with the aggressive workers of the ant colony behaviourally. Because *R. serripes* is a visual mimic of *O. smaragdina*, *Myrmarachne* may react to the bug and the ant in a similar manner. Since *Myrmarachne* and *R. serripes* are sympatric species, it is possible that interactions between the two occur. The question is whether *Myrmarachne* can distinguish between the green ant and the *R. serripes*. Differences in *Myrmarachne* reactions to the ant and the bug indicate that *Myrmarachne* uses visual cues that distinguish the ant and the bug. Potential cues could be the structure of the mouthparts, or the way the two insects move around. Alternatively, chemical cues from both insects may have an effect on *Myrmarachne* behaviour.

6.1 Introduction

Mimicry is a very wide-spread phenomenon, especially amongst tropical arthropods, that has attracted naturalists' attention for centuries. Batesian mimicry is that in which a palatable organism mimics an unpalatable one (known as the "model") to gain protection from predators (Bates, 1862). Ants are considered good models for Batesian mimicry, since predators generally avoid them due to the ants production of formic acid or other noxious volatiles as a chemical defence, and the fact that ants have strong mandibles and stings (Rettenmeyer, 1970). One type of ant-mimicry that involves the behavioural and morphological resemblance to ants (termed Myrmecomorphy) has arisen at least 15 times in spiders and at least 10 times in Hemiptera, to name but two groups of myrmecomorphic arthropods (McIver & Stonedahl, 1993). For example, *Myrmarachne* Mcleay, 1838 (Araneae: Salticidae) is a wide spread, highly speciose genus of ant-mimicking jumping spiders whose species are myrmecomorphs (Mathew, 1944; Edmunds, 1978; Jackson, 1982). Examples of hemipteran families that include several species of myrmecomorphs are Miridae (Jackson & Drummond, 1974) and Alydidae (Mathew, 1935; Kumar, 1966; Oliveira, 1985). Several Batesian mimics undergo transformational mimicry, first described by Mathew (Mathew, 1935), meaning that the mimicry only takes place during certain stages of the mimic's life cycle.

The green tree ant *Oecophylla smaragdina* lives in large colonies and is aggressive, with workers mass recruiting each other to fight against a potential danger to the colony (Hölldobler & Wilson, 1990). These characteristics make *O. smaragdina* a good arthropod model for Batesian mimicry. One species of *Myrmarachne* (*M. plataleoides*) is a known mimic of *O. smaragdina* (Mathew, 1944) and a further, undescribed, *Myrmarachne* species in North Queensland also mimics *O. smaragdina*. The mimicry

includes both accurate dermal coloration, and antennal illusion. The co-occurring alydiid bug *Riptortus serripes*, is another arthropod mimic of *O. smaragdina*. *R. serripes* is an example of a myrmecomorph displaying transformational mimicry, since only the nymphs mimic *O. smaragdina*. There are more similarities between *R. serripes* and *O. smaragdina* since both are insects, and therefore the alydiid bug is an even better visual mimic of *O. smaragdina* than the *Myrmarachne* species.

Myrmarachne are, like all salticids, diurnal hunters relying on their acute vision for prey capture (Forster, 1982; Blest, 1985; Land, 1985; Jackson, 1986; Jackson & Tarsitano, 1993; Jackson & Pollard, 1996). The vision of salticids has also been shown to be important in courtship, and in distinguishing between prey and conspecific rivals (Harland, et al., 1999; Harland & Jackson, 2000). Visual cues salticids use when recognising an item as prey have been investigated in *Salticus scenicus* (Drees, 1952), *Phidippus audax* (Freed, 1984), and *Portia fimbriata* (Harland & Jackson, 2000) . The study on *S. scenicus* concluded that salticids used mainly the characteristics of the arthropod's legs as a discriminatory factor for prey recognition (Drees, 1952), whereas *P. audax* was interpreted to use prey movement as the main visual cue in its predatory tactics (Freed, 1984). Jackson & Tarsitano, (1993) showed that it is not necessary for the prey to be moving for a salticid to recognise prey. For *P. fimbriata* the most important visual cue seemed to be the presence of large anterior median (AM) eyes (characteristic of salticids) which led to the animal beginning the cryptic stalking used to capture salticids (Harland & Jackson, 2000). Because of their visual capability, salticids can see and avoid potential dangers, such as stronger and more aggressive animals. Thus the *Myrmarachne* species that mimics *O. smaragdina* – and as a Batesian mimic lives in the proximity of the ant colonies – usually avoids contact with

the potentially dangerous ants, as do other *Myrmarachne* species (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2005). Since *R. serripes* and the *Myrmarachne* species occur in sympatry, encounters between the two in nature are plausible, meaning that *Myrmarachne* can see the bug with its image-forming eyes. This raises the question of whether *Myrmarachne* behaves differently when it sees the ant-mimic *R. serripes* to when it sees the ant *O. smaragdina*, given the resemblance between the bug and the ant. If *Myrmarachne* reacts similarly to the ant and the bug, the image cues have a great effect on *Myrmarachne*, and it cannot discriminate between different insect classes if they look similar. However, if there is a difference in *Myrmarachne* behaviour when in the presence of the ant and the bug, there might be certain cues (visual or non-visual) that allow *Myrmarachne* to discriminate between the two groups of insects.

6.2 Materials and methods

For the experiment, 30 *Myrmarachne* individuals were used under three treatments. The first one involved ten *Riptortus serripes* nymphs, the second one ten *O. smaragdina* workers and a control treatment. The control group was Hemiptera from the miriid family, and adult *R. serripes*. Each animal was collected from the wild, ensuring that they were similar in size, and used only once.

The area of the experiment was a plastic petri-dish with a diameter of 10 cm. A *Myrmarachne* was placed into the area with either one of the *R. serripes* nymphs, the *O. smaragdina* worker or the control hemipteran. The animals were left for 1 hour inside the area, while being recorded onto video tape using a low light, high resolution video camera connected to a Panasonic NV-L25 HQ PAL video recorder. A new petri-dish was used for each *Myrmarachne* – treatment animal combination, to avoid possible chemical cues from a previous combination influencing the animals in the next one.

The video tapes were analysed, and the following variables were recorded at the point of interaction: 1. Whether either or both of the animals in the petri-dish were moving or stationary. 2. The position the ant/bug was in relative to the *Myrmarachne* when the *Myrmarachne* first reacted to it (coded as "a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant/bug is behind *Myrmarachne*). 3. The distance (measured from the recordings and re-scaled to be like the real-life distance) between the *Myrmarachne* and the ant/bug just before the *Myrmarachne* reacted. 4. The *Myrmarachne* reaction. Pre-determined variables were also included in the analyses; the variables included the *Myrmarachne* sex and the type of treatment animal used (ant, *R. serripes* nymph or control hemipteran).

Figure 6.1: Photographs of a *Riptortus serripes* adult (A), *R. serripes* nymph (B) and a green tree ant *Oecophylla smaragdina* (C).
Scale bars represent 5 mm.

Table 6.1: *Myrmarachne* reactions at the time of an interaction with an ant or a hemipteran

Reaction	*Description*
0	No reaction, just looking
1	Jumps backwards (x1)
2	Jumps backwards (x2)
3	Jumps backwards (x3)
4	Turns around and run away
5	Turns around and walk away
6	Touches ant (non-aggressive)
7	Attacks ant
8	Side-steps
9	Jumps to the side
10	Moves backwards (not jumping)
11	Runs forwards
12	Follows ant visually by turning
13	Jumps forwards
14	Moves to the side and walk/run forwards
15	Opens chelicerae (males only)
16	Follows ant/bug
17	Moves towards ant/bug

A measure of activity, the total motion index (TMI) was calculated to quantify how much movement there was in the petri dish, using the following formula:

(number of interactions/minute) x (1+ Psm) x (1+ Pam)

where Psm and Pam are the proportions of movement of the spider and ant respectively.

The data was analysed using R version 2.1.1 (R_Development_Core_Team, 2005). A Mantel test with Pearson's product-moment correlation was carried out on the distance matrices of each treatment separated, using the VEGAN package (Oksanen, et al., 2005). Regression trees were also constructed for all three treatments using the R package rpart (Therneau, et al., 2005). Classification and Regression Tree (CART) analysis was popularised by Breiman *et. al.* (Breiman, et al., 1984) as a means of partitioning data sets into similar groups, thus predicting the correlation of one or more independent variables to a categorical dependent variable by building decision trees. CART analysis has increased in popularity over a wide range of areas such as medicine (e.g. (Lehmann, et al., 2003)), palaeobotany (e.g.(Lindbladh, et al., 2002)) and ecology (e.g.(Karels, et al., 2004)). The reaction frequencies under each treatment were compared using a chi-squared test, and an ANOVA was used for testing differences in distance and TMI for the *Myrmarachne* under each treatment. Chi-squared tests were also performed for the combination of treatment position-*Myrmarachne* reaction, and the *Myrmarachne* reaction frequencies for moving versus stationary treatment animals.

6.3 Results

The Mantel test of distance matrices performed with Pearson's product-moment correlation shows that the only significant correlation between two distance matrices is the one where *Myrmarachne* is placed with the *R. serripes* nymphs and the control groups (r= 0.09946, significance < 0.001), meaning that the two matrices are closely enough correlated to be considered similar. Comparing the rest of the treatments shows a significant difference (or no linear correlation) between the distance matrices (r= minus 0.04782, significance = 0.985 for the comparison of *Myrmarachne* with *O. smaragdina* and *Myrmarachne* with *R. serripes* nymphs, and r= minus 0.06425, significance = 0.988 for distance matrices of *Myrmarachne* with *O. smaragdina* and the control hemipterans).

Classification and Regression Tree analysis is a method of building trees by which nodes (or splits) are formed at points where independent variables appear most frequently as predictors of an independent variable. The nodes higher up in the tree show the strongest predictor variables. The annotation of a regression tree is such that the most frequent independent variables appear at the end of branches in the tree, with numbers in brackets indicating the frequency at which each independent variable has been placed in that position. The independent variables are shown at the nodes, with a list of the variable's categories that lead to the left-hand split in the node. The right-hand split is formed by the categories not listed. The following trees were constructed using different data sets: the first one used the combined data of the three treatments, and the following three trees were constructed using the data sets of the three separate treatments (*Myrmarachne* with *R. serripes* nymphs, *Myrmarachne* with *O. smaragdina* and *Myrmarachne* with the control hemipterans).

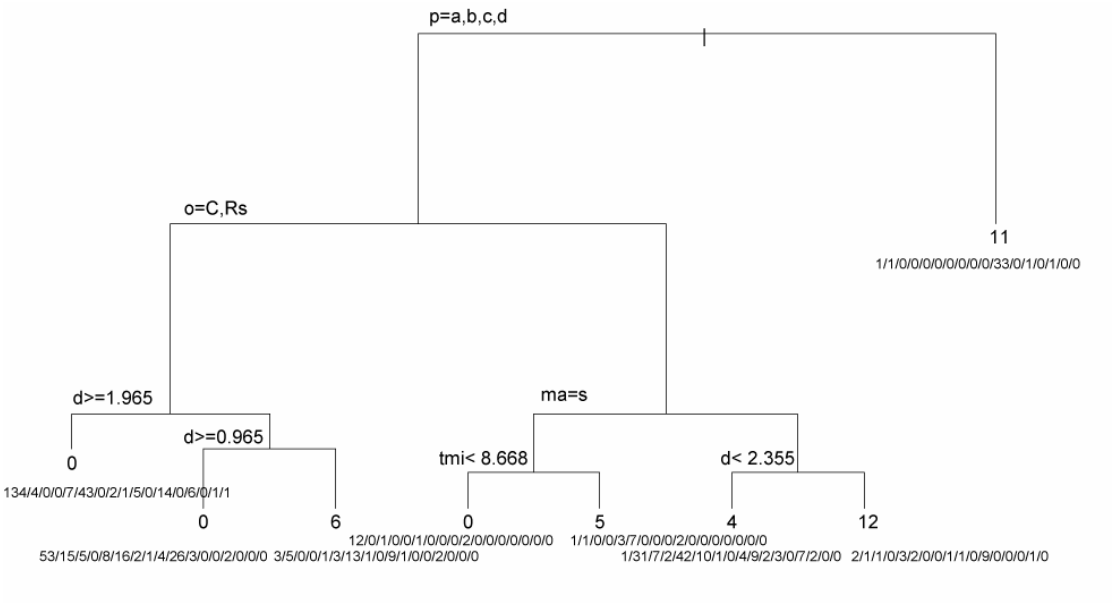


Figure 6.2: Recursive partitioning tree of Myrmarachne reactions to all treatment groups and the correlated variables.
Reactions are shown at the end of the branches, 0= no reaction, 4= turning around and running away, 5= turning around and walking away, 6= making non-aggressive contact, 11= running forwards, 12= following ant/bug visually. p refers to the position the animals are in when *Myrmarachne* sees them ("a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant/bug is behind *Myrmarachne*), o refers to the treatment (Os = *O. smaragdina*, Rs = *R. serripes* nymph, C = control hemiptera), d is the distance (in cm) between the animal and *Myrmarachne* at the time of interaction, ma is the motion of the treatment animal (m= moving, s= stationary) and tmi is the Total Motion Index. The analysis was done by setting the maximum depth of the tree to 4 nodes.

Figure 6.3: Recursive partitioning tree constructed for the *Myrmarachne* reaction data to *R. serripes* nymphs with correlated variables.
The maximum depth of the tree was set to 3 nodes. Reactions are shown at the end of the branches (0= no reaction, 5= turning around and walking away, 6= making non-aggressive contact). rd and rp refer to the distance (in cm) between *Myrmarachne* and the *R. serripes* nymphs and the position the *R. serripes* nymph was in ("a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant/bug is behind *Myrmarachne*) at the time of *Myrmarachne*'s reaction respectively.

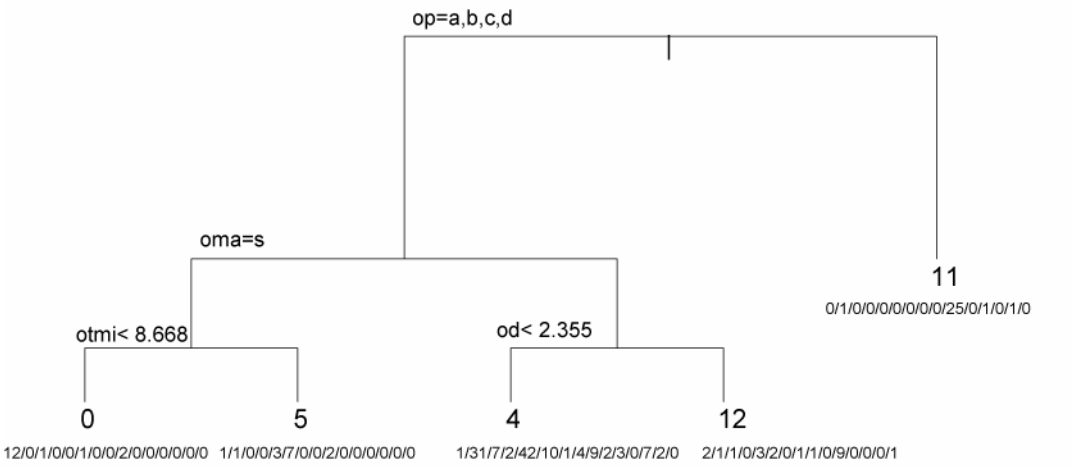


Figure 6.4: Recursive partitioning tree constructed for the *Myrmarachne* reaction data to *O. smaragdina* with correlated variables.
The maximum depth of the tree was set to 3 nodes. Reactions are shown at the end of the branches (0= no reaction, 4= turning around and running away, 5= turning around and walking away, 11= running forwards, 12= following the ant visually). od and op refer to the distance (in cm) between *Myrmarachne* and the *O. smaragdina* and the position the *O. smaragdina* was in ("a"= front of head, "b"= side of head, "c"= side of body, "d"= abdomen, "e"=ant/bug is behind *Myrmarachne*) at the time of *Myrmarachne*'s reaction respectively. oma refers to the instances in which *O. smaragdina* was moving during an interaction (m= moving, s= stationary), and otmi refers to the Total Motion Index of the *Myrmarachne-O. smaragdina* combination.

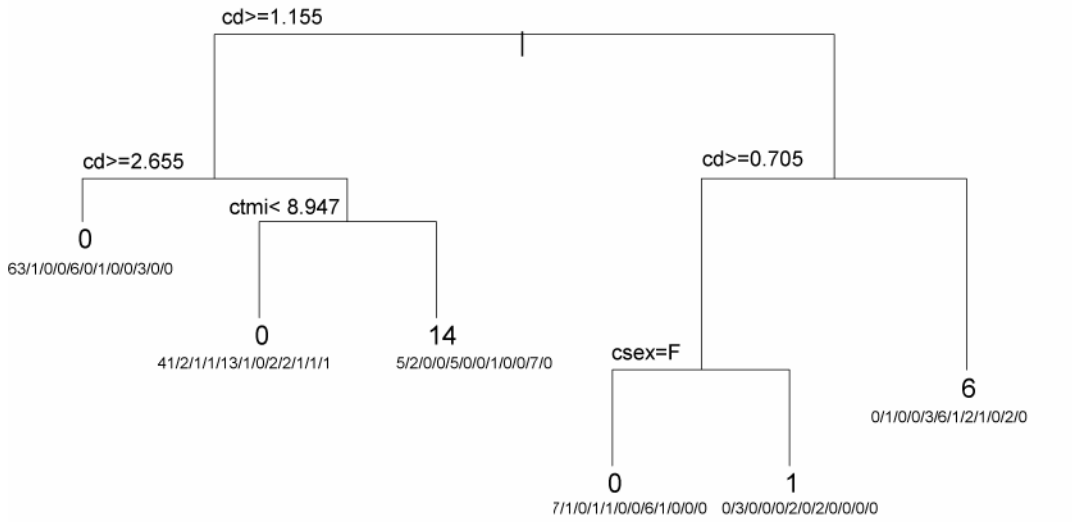


Figure 6.5: Recursive partitioning tree constructed for the *Myrmarachne* reaction data to the control group with correlated variables.
The maximum depth of the tree was set to 3 nodes. Reactions are shown at the end of the branches (0= no reaction, 1= one jump backwards, 6= making non-aggressive contact, 14= moving to the side and walking or running forwards). cd refers to the distance (in cm) between *Myrmarachne* and the control hemiptera at the time of *Myrmarachne*'s reaction. ctmi is the Total Motion Index of the *Myrmarachne*- hemiptera combination, and csex (M= male, F=female) refers to the sex of the *Myrmarachne* present with the control hemiptera.

The regression trees show the independent variables that are most likely to have an effect on the different *Myrmarachne* reactions (listed at the end of the branches). The following explanations apply to the regression tree which includes the treatment animal as a variable. The reaction where *Myrmarachne* runs forwards (reaction 11) seems to be most closely correlated with an animal being behind it (position "e"), whether it is an ant or a bug. The next discriminatory variable found by the recursive partitioning analysis is the type of treatment animal used, where the *R. serripes* nymphs and the control hemipteran group together for further partitioning (Fig 1). The following explanations apply to the regression tree built on the data of *Myrmarachne* with the *R. serripes* nymph. At distances less than 0.98 cm, the most closely associated reaction is making contact (non-aggressively – reaction 6). On the other hand, *Myrmarachne* is most likely to turn around and walk away (reaction 5) when the nymph's body is seen side-on (position c). Next is the regression tree of *Myrmarachne* with *O. smaragdina*. The presence of the ant behind *Myrmarachne* (position "e") is correlated most closely with running forward (reaction 11). No reaction from *Myrmarache* however, is correlated to the ant being stationary and the TMI being less than 8.67. A moving *O. smaragdina* on the other hand is associated closely with *Myrmarachne* turning and running away (reaction "4") when the distance is less than 2.4 cm, and turning to look at the ant (reaction "12") when the distance is greater than 2.4 cm. The last regression tree is of *Myrmarachne* with the control group. At distances greater than 2.7 cm and a TMI lower than 8.9, no reaction is the most commonly correlated *Myrmarachne* response. Under this treatment, a distance of less than 0.7 cm is most commonly associated with *Myrmarachne* making contact with the hemipteran.

Neither of the regression trees showed any similarity when tested for. However, the regression trees for *Riptortus serripes* nymphs and the control group show more resemblance to each other with regards to certain variable splits in the trees. For example, they both have the distance between the *Myrmarachne* and the treatment animal as the first discriminatory variable. A small distance is most closely related to the *Myrmarachne* making contact with the hemiptera. Also, reaction "0" (which is no reaction from the *Myrmarachne*) is more common when the *Myrmarachne* is with the hemiptera than with the ant.

The following Figures show the independent variables separately, and how they differ between the treatments.

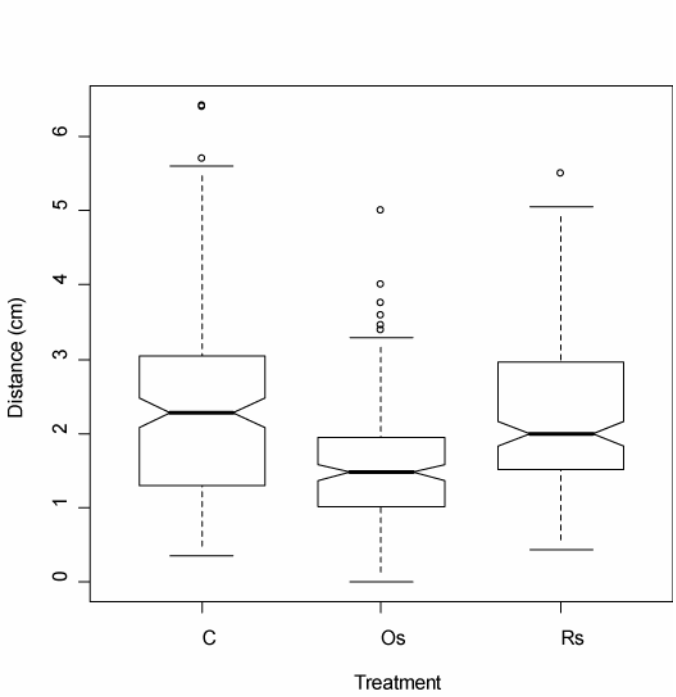


Figure 6.6: Notched boxplots of the mean distances (in cm) between *Myrmarachne* and the treatment animals at the time of Myrmarachne reacting during an interaction.
Treatment animals are coded as: C= control hemipteran, n = 10, Os= *O. smaragdina*, n = 10, Rs= *R. serripes* nymphs, n = 10. (ANOVA: $F_{(2,597)} = 39.02$, $p < 0.001$).

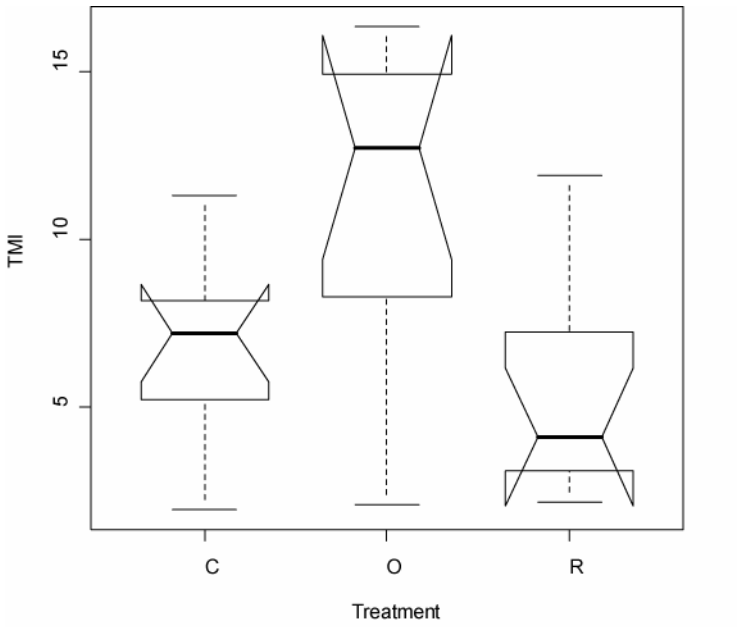


Figure 6.7: Notched boxplot of the Mean Total Motion Index (tmi) for each *Myrmarachne* and the treatment animal.
Treatment animals are coded as: C= control hemipteran, n = 10, O= *O. smaragdina*, n = 10, R= *R. serripes* nymphs, n = 10. (ANOVA: $F_{(2,27)} = 5.085$, $p = 0.013$).

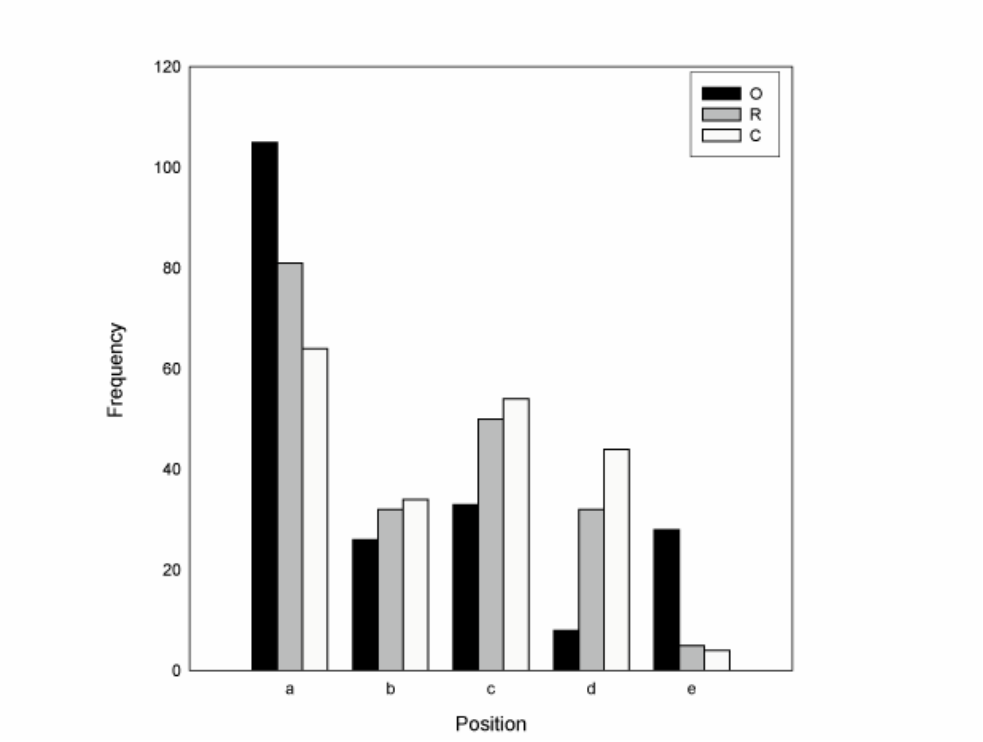


Figure 6.8: Total frequency of each position of treatment group as seen by *Myrmarache* during the interaction.
Positions are coded as: "a"= head-on, "b"= side of head, "c"= side of body, "d"= abdomen, "e"= behind *Myrmarachne.* Treatment groups are: "Os"= *O. smaragdina*, "Rs"= *R. serripes* nymphs, "C"= control hemiptera. ($\chi^2_8 = 70.65$, $p < 0.0001$)

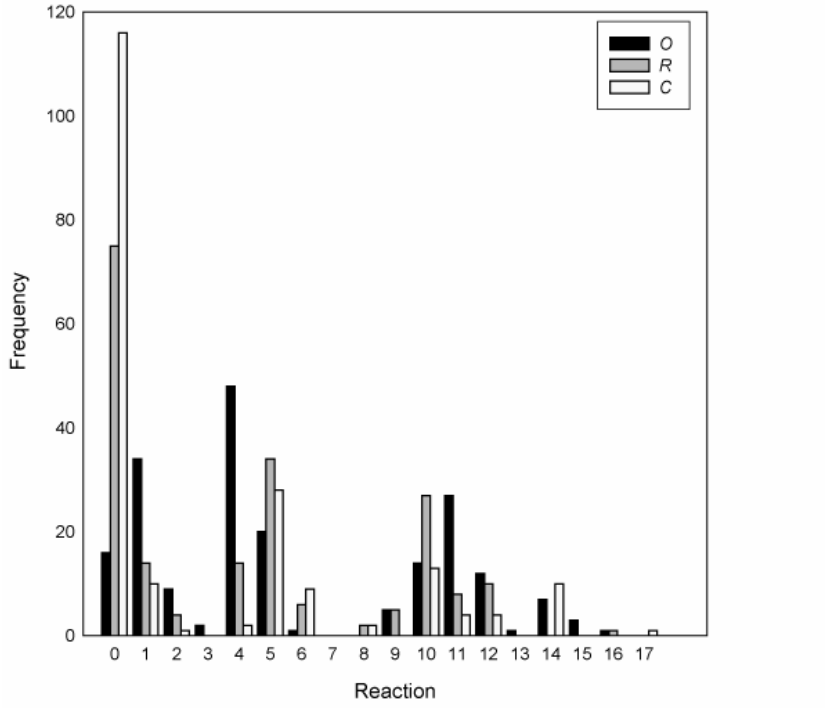


Figure 6.9: Total frequency of each *Myrmarachne* reaction to the three treatment groups during the interactions.
Myrmarachne reactions are as described in Table 6.1. Treatment groups are coded as: "O"= *O. smaragdina*, "R"= *R. serripes* nymphs, "C"= control hemiptera. ($\chi^2_{32} = 225.76$, $p < 0.0001$)

The treatment group has a significant effect on the mean distance at which *Myrmarachne* reacts to the other animal, since the ANOVA resulted in an F value of 39.02 (with 2/597 degrees of freedom), making the significance less than 0.001. There is a significant difference between the mean distance of *R. serripes* and *O. smaragdina*, as shown by the Welch Two Sample t-test, which gave a t value of -8.24, which results in a significance of less than 0.0001, as well as the control group and the *O. smaragdina*, where the Welch Two Sample t-test t value was -7.90, with a significance of less than 0.0001. However, there is no significant difference between the distance of *R. serripes* and the control group, since the Welch Two Sample t-test t value was -0.80, making the significance 0.427.

For the TMI, the ANOVA shows that there is a difference in the overall value between the treatment groups with *Myrmarachne*, with an F-value of 5.085 (with dof = 2/27), and a significance of 0.013. The Welch two Sample t-test gave a t value of 2.77, showing a significant difference in the TMI between the *R. serripes* nymphs and *O. smaragdina*, with a p-value of 0.01463, for the TMI difference between *O. smaragdina* and the control hemipteran, the t value was 2.11, and the significance was 0.05307. However, there was no significant difference between the TMI of *Myrmarachne* and either the *R. serripes* nymphs or the control hemipteran, where the t value was -1.00, and the p-value 0.329.

The next variable to consider is the frequencies of the position the three groups of animals were in when *Myrmarachne* reacted to them. These are significantly different, with a significance of less than 0.0001, and a chi-squared value of 70.65, with 8 degrees of freedom. There was also a significant difference between the frequencies of the positions that *R. serripes* and *O. smaragdina*, as well as *O. smaragdina* and the control group were in, with respective chi-squared values of 37.63 and 59.01, which with 4 degrees of freedom both have a significance level of less than 0.0001. However, the frequency of the positions the control group and *R. serripes* were in when *Myrmarachne* reacted to them is not significantly different, since the chi-squared value of 4.21 with 4 degrees of freedom results in a significance value of 0.38.

There is a significant difference between the frequency if *Myrmarachne* reactions to all three treatment groups, with a chi-squared value of 225.76 and 32 degrees of freedom giving a significance level of less than 0.0001. The same is true when comparing the groups in pairs. For *O. smaragdina* and *R. serripes*, the chi-squared value was 103.97,

which with 15 degrees of freedom has a significance level of less than 0.0001. For *O. smaragdina* and the control group, the chi-squared value was 181.93, and the significance with 16 degrees of freedom is less than 0.0001. The last comparison - *R. serripes* and the control group – gave a chi-squared value of 47.25, 13 degrees of freedom and a significance level less than 0.0001. However, the difference in *Myrmarachne* reaction frequencies between the control group and *R. serripes* is less significant than that between other combinations of the three groups.

Table 6.2: Chi-squared values for the frequencies of *Myrmarachne* reactions when the treatment animals are seen in the 5 positions

Position	*All treatment groups*	*R and O*	*R and C*	*O and C*
	χ^2 (d. f.)	χ^2 (d. f.)	χ^2 (d. f.)	χ^2 (d. f.)
a	$107.3305_{(32)}$ ***	$49.2896_{(16)}$ ***	$26.4664_{(16)}$ *	$86.7192_{(16)}$ ***
b	$56.6174_{(32)}$ **	$22.0558_{(16)}$	$14.3335_{(16)}$	$36.3852_{(16)}$ **
c	$42.7012_{(32)}$.	$14.5381_{(16)}$	$21.0743_{(16)}$	$25.9671_{(16)}$.
d	$11.637_{(32)}$	$2.7083_{(16)}$	$6.0315_{(16)}$	$7.5694_{(16)}$
e	$9.3701_{(32)}$	$0.5893_{(16)}$	$0.0141_{(16)}$	$7.5102_{(16)}$

*** p<0.001, ** p < 0.01, * p<0.05, . p <0.1

Table 6.3: Chi-squared values for the frequencies of *Myrmarachne* reactions under the different movement conditions

Motion	*All treatment groups*	*R and O*	*R and C*	*O and C*
	χ^2 (d. f.)	χ^2 (d. f.)	χ^2 (d. f.)	χ^2 (d. f.)
Treatment group moving	$152.182_{(32)}$ ***	$66.964_{(16)}$ ***	$33.880_{(16)}$ **	$134.357_{(16)}$ ***
Treatment group stationary	$39.540_{(32)}$	$11.234_{(16)}$	$17.250_{(16)}$	$20.513_{(16)}$
Myrmarachne moving	$195.964_{(32)}$ ***	$91.489_{(16)}$ ***	$42.206_{(16)}$ ***	$160.776_{(16)}$ ***
Myrmarachne stationary	$27.793_{(32)}$	$16.926_{(16)}$	$7.467_{(16)}$	$9.310_{(16)}$

*** p<0.001, ** p < 0.01, * p<0.05, . p <0.1

The analysis of *Myrmarachne*'s reaction frequencies to each position the control animals were in showed that there is no significant difference in reaction frequencies for positions "c", "d" and "e" (body side-on, abdomen and ant/bug behind *Myrmarachne* respectively). The difference in reactions was found when *Myrmarachne* saw the ant or the hemipteran head-on (position "a"), where the chi-squared value was 107.33, and with 28 degrees of freedom a p-value less than 0.0001. In that case the *Myrmarachne* carried out more avoidance reactions (such as jumping back or running away) to the ant, and more stationary reactions to the hemipteran.

The motion of the animals (i.e. whether they were moving or stationary at the time of an interaction) also affected *Myrmarachne* reaction frequencies. This is also true for the motion of *Myrmarachne*. When either the *Myrmarachne* or the treatment animal are stationary there is no difference between the reaction frequencies of *Myrmarachne*, with chi-squared values of 27.80 and 39.54 respectively, which give significance values of 0.680 and 0.169 respectively, with 32 degrees of freedom. The differences occur when there is movement from either the *Myrmarachne* or the treatment animal during the interaction, with a chi-squared value of 195.96 for *Myrmarachne*, with 32 degrees of freedom and a significance value of less than 0.001, and for the treatment a chi-squared value of 152.18, 32 degrees of freedom and a significance value less than 0.001.

6.4 Discussion

Oecophylla smaragdina is an aggressive ant species whose workers will attack, and recruit other workers to attack any organism that represents a possible threat to the colony (Hölldobler & Wilson, 1990). This includes responses to its Batesian mimics, such as *Myrmarachne*, that live in the colony's proximity (Nelson, et al., 2005). Therefore, for its safety *Myrmarachne* must avoid close encounters with *O. smaragdina*. *Myrmarachne* have been shown to avoid contact with ants (Mathew, 1944; Edmunds, 1978; Nelson, et al., 2005), and an earlier study classified the reactions of *Myrmarachne* species to sympatric model- and non-model ant species (see Chapter 2). Salticids, such as *Myrmarachne*, rely on the visual abilities of the AM eyes for functions such as predation, (Jackson & Tarsitano, 1993; Jackson & Willey, 1994; Jackson & Pollard, 1996; Harland & Jackson, 2000), courtship (Jackson, 1982) and presumably keeping away from threats (such as ants). If *Myrmarachne* relies on visual cues to recognise ants, it might be expected to react in a similar manner to other arthropods mimicking *O. smaragdina* and the ant itself. A capacity to distinguish ants and mimics of ants would imply some degree of cognitive processing.

In general, the results for the different variables with the treatments show that *Myrmarachne* reaction frequencies vary between the three groups (*O. smaragdina*, *R. serripes* nymphs and the control hemiptera). However, the response patterns for the *R. serripes* nymphs and the control hemiptera are more similar to each other than to the pattern of responses with the green ant *O. smaragdina*; *Myrmarachne* reacts to the *R. serripes* nymphs and the other bugs at a similar distance (an average distance of 2.25 and 2.34 cm respectively), and the reactions when *Myrmarachne* sees the two groups of

bugs head-on are also similar (an average of 2.01 cm for the *R. serripes* nymph and 2.30 cm for the control hemiptera as opposed to an average of 1.44 cm for *O. smaragdina*).

Salticids have been shown to use certain cues to recognise prey items (Drees, 1952; Freed, 1984; Harland & Jackson, 2000), but *Myrmarachne* may not react to *R. serripes* as it would to prey, because it probably does not habitually prey on it. Yet *Myrmarachne* may react differently to *O. smaragdina* and *R. serripes* nymphs due to certain visual cues that are not obvious to all animals. There are certain differences between *R. serripes* nymphs and the ant – such as their mouthparts – that *Myrmarachne* could use as visual cues to distinguish between ants and bugs (i.e. if there are large mandibles it could be an ant).

On the other hand, *Myrmarachne* may just be responding to more general visual cues. Since the size of all insects used was more or less constant, that cannot be considered as a factor. However, ants and bugs have different ways of moving around. The ants are generally faster, and often approach the salticid directly. This means that *Myrmarachne* could react to how fast and in which direction the insect is moving, for example jumping backwards away from a front-on approaching animal moving at a high speed. The two insects also vary slightly in the way they move their antennae – *O. smaragdina* generally moves them more jerkily than *R. serripes*. To remove the effect of movement from the experiment, the reaction of *Myrmarachne* to motionless lures (as in (Jackson & Tarsitano, 1993)) of the ants and the hemipterans could be studied (provided *Myrmarachne* will react to the lures).

Myrmarachne reactions are varied enough to conclude that the *R. serripes* nymphs do not have the same effect on *Myrmarachne* as *O. smaragdina* workers. So to *Myrmarachne*, *R. serripes* nymphs and *O. smaragdina* workers are not indistinguishable, suggesting that the mimicry of the green ant by *R. serripes* does not work on *Myrmarachne*. The mechanism by which *Myrmarachne* develops this discrimination remains a question to be answered. It may be related to minor visual cues, such as the different mouthparts. The different chemical cues given out by *O. smaragdina* and *R. serripes* could also affect the behaviour of *Myrmarachne*, presuming the salticid can detect and respond at a distance to chemical cues from insects. Alternatively, the different methods of locomotion of the insects could play a role in determining *Myrmarachne* reactions. Judging from general *Myrmarachne* behaviour observed during this study, the reactions are presumed to be mainly a result of the way the animals moved, including leg and antennae movement.

Chapter 7 : Dynamics of the evolution of Batesian mimicry: molecular phylogenetic analysis of ant-mimicking *Myrmarachne* (Araneae: Salticidae) species and their ant models

Abstract

Batesian mimicry is seen as an example of evolution by natural selection, with predation as the main driving force. The mimic is under selective pressure to resemble its model, whereas it is disadvantageous for the model to be associated with the palatable mimic. In consequence one might expect there to be an evolutionary arms race, similar to the one involving host-parasite coevolution. In this study the evolutionary dynamics of a Batesian mimicry system of model ants and ant-mimicking salticids is investigated by comparing the phylogenies of the two groups. Although Batesian mimics are expected to co-evolve with their models, the results of this study suggest found the phylogenetic patterns of the models and the mimics to be indicative of adaptive radiation by the mimic rather than co-speciation between the mimic and the model. This shows that there is strong selection pressure on *Myrmarachne*, leading to a high degree of polymorphism. There is also evidence of sympatric speciation in *Myrmarachne*, the reproductive isolation possibly driven by female mate choice in polymorphic species.

7.1 Introduction

Of the different types of mimicry described, Batesian mimicry is that in which a palatable organism gains protection from predators by mimicking a sympatric, unpalatable model (Bates, 1862), whereas in Müllerian mimicry both model and mimic are unpalatable, reinforcing the learning process of avoidance by the predator (Müller, 1879). Mimicry has always been considered evidence for natural selection, so the evolution of mimicry has received much attention from theoretical biologists and geneticists. Several studies have presented population genetics models to explain Batesian mimicry (Matessi & Cori, 1972; Charlesworth & Charlesworth, 1975), finding that polymorphism can be maintained in the mimic population. Different environmental parameters such as the relative population sizes of the model and the mimic, the closeness of the mimic's resemblance to the model (Mappes & Alatalo, 1997) or predator learning (Turner & Speed, 1996; Speed & Turner, 1999) also have an effect on the evolutionary rates of the Batesian mimics.

The predicted fitness costs of both the mimic and the model in a Batesian mimicry system are such that the mimic's fitness increases with increased resemblance to the model, whereas the model's fitness decreases (Nur, 1970; Edmunds, 1974). Mathematical models have shown that an evolutionary chase between the model and the mimic is possible, since the resemblance of the mimic to the model is favourable to the former, but disadvantageous to the latter (Gavrilets & Hastings, 1998; Holmgren & Enquist, 1999). This "chase" between the model and its Batesian mimic is predicted to be such that "*most mimics should be able to evolve towards their models faster than the models would evolve away from them*" (Nur, 1970). In other words, mimics adverge towards (or converge on) the models (Brower & Brower, 1972). In some cases, isolated

populations of the model may undergo speciation and diverge in morphology. This would increase selection pressure on the sympatric population of mimics, who are expected to undergo morphological adaptations matching those of their models. Co-speciation of model and mimic would happen if the population of mimics is also geographically isolated, or if it evolves reproductive isolation. A possible cause of reproductive isolation could be differential female mate choice.

Although the theoretical models support the coevolution (and possible co-speciation) of models and mimics, Batesian mimicry is so widespread amongst animal taxa that generalisations based on models do not always apply. In practice, the question of co-speciation can be resolved by determining the phylogenies of the organisms involved. Phylogenies contain information about the relative evolutionary age of the species to each other, and they can also be useful in revealing patterns of speciation. Co-speciation between two groups of organisms would be supported when their phylogenetic trees show congruence (Page, 1996b). This is traditionally studied in the context of host-parasite co-speciation (Brooks, 1988; Paterson, et al., 1993; Charleston & Robertson, 2002). Müllerian mimicry evolution has also been studied using phylogenetics (Simmons & Weller, 2002; Machado, et al., 2004), but little is known about the evolution of real-life Batesian model-mimic systems.

Ants are considered a suitable model for Batesian mimicry, since they have developed stings and defensive chemicals (McIver & Stonedahl, 1993). Morphological resemblance to ants is known as myrmecomorphy, and occurs in a diverse range of arthropod species. At least 43 spider genera, including 14 within the family Salticidae exhibit myrmecomorphy (Cushing, 1997). *Myrmarachne* Mcleay, 1838 (Araneae:

Salticidae), is one such spider genus, all of whose species bear a striking resemblance to particular model ants. All *Myrmarachne* species are thought to be Batesian mimics of ants (Jackson, 1986; Edmunds, 1993).

Myrmarachne is a speciose genus of mainly tropical salticids, with over 200 named and described species (Platnick, 2005). The *Myrmarachne* species found in North Queensland, Australia, each mimic a different ant species, some of which are outlined in Table 7.1. In spiders, identification to species level is based on the morphology of male and female genitalia. *Myrmarachne* species display little differentiation in the genitalia, especially within species groups (Wanless, 1978; Berry, et al., 1996). Within species however, *Myrmarachne* may be polymorphic, especially in their dermal coloration. *Myrmarachne* species also display transformational mimicry, where the juveniles and adults mimic different ant species (Mathew, 1944; Edmunds, 1978). In addition, many species of *Myrmarachne* are sexually dimorphic (Wanless, 1978). Hence, species polymorphism, sexual dimorphism, transformational mimicry and little difference in genital structures all contribute to the difficulty of grouping *Myrmarachne* by species. Phylogenetic studies using molecular data from nuclear or mitochondrial gene fragments may help in re-enforcing species boundaries determined by slight variations in genital morphology.

Mitochondrial gene sequences have been used for reconstructing spider phylogenies at a range of taxonomic levels, including phylogenies of subfamilies, genera (Hedin & Maddison, 2001b; Vink, et al., 2002), and congeneric species (Hedin, 1997a; Bond, et al., 2001; Croucher, et al., 2004). A morphological phylogeny of the salticid subfamily Ballinae (Benjamin, 2004) led to the inference of independent origins of

myrmecomorphy in this group, and a phylogeny of jumping spiders has shown that ant-mimicry arose at least four times in this spider family (Maddison & Hedin, 2003b).

Here sequences from four mitochondrial genes are used to infer a phylogeny of 12 *Myrmarachne* from North Queensland, and sequences of one mitochondrial and one nuclear gene to study the phylogeny of their ant models. The phylogenies contain information about species homogeneity, as well as the evolutionary dynamics between the different species. In addition, a comparison of the phylogenies of the *Myrmarachne* and their model ant species explain the evolutionary dynamics of the two groups, and serve as an example of Batesian mimicry evolution.

7.2 Materials and methods

7.2.1 Specimen collection

12 individual *Myrmarachne* (11 from Townsville (19˚ 13' S, 146˚ 48' E) and one from Cairns (16˚ 51' S, 145˚ 43' E)) and one *Cosmophasis bitaeniata* were collected. The *Myrmarachne* were from eight morphologically distinct groups. In four cases, two individuals that were morphologically very similar were collected. Since none of the *Myrmarachne* are formally named or described, they are referred to as species A, B, C, D, E, F, G and H (type specimens have been deposited in the Queensland Museum's arachnology collection registration numbers s66648 to s66652, and in the Australian Museum's arachnology collection with registration numbers KS93119 to KS93122). The alphabetical names assigned to species are tentative, and different names do not necessarily imply that the individuals belong to a distinct species. The collecting also included one ant from the following species (only from Townsville): *Odontomachus ruficeps* Smith 1858 (used as the outgroup), *Oecophylla smaragdina* (Fabricius) Smith 1860, *Opisthopsis haddoni* Emery 1893, *Opisthopsis pictus* Emery 1895, *Polyrhachis* near *obtusa* Emery 1897, *Polyrhachis senilis* Forel 1902 and *Tetraponera punctulata* Smith 1877. Voucher specimens of the ants have been deposited in the Queensland Museum's insect collection (registration numbers QM T133692 - QM T133710) where they were identified by Chris Burwell and Rudy Kohout. For the mimic-model association between the *Myrmarachne* and the ants see Table 7.1. Allocation of model ant species to each *Myrmarachne* was done by visual resemblance between sympatric species, mainly based on coloration and overall body shape.

Table 7.1: *Myrmarachne* from North Queensland used in this study and their ant models

***Myrmarachne* species (mimic)**	**Ant species (model)**
Myrmarachne sp. A[1]	*Opisthopsis haddoni*
Myrmarachne sp. B	*Polyrhachis* near *obtusa*
Myrmarachne sp. C	*Polyrhachis senilis*
Myrmarachne sp. D	*Tetraponera punctulata*
Myrmarachne sp. E[1]	*Opisthopsis pictus*
Myrmarachne sp. F	*Oecophylla smaragdina*
Myrmarachne sp. G	*Polyrhachis* sp.
Myrmarachne sp. H[1]	Unknown

[1]*Myrmarachne* with different species names assigned may not be 'species' in the strict sense, but have been labelled differently because of variations in their morphology.

7.2.2 DNA extraction

The spiders and ants were preserved in 100 % ethanol and kept at - 80ºC. DNA extraction was carried out on whole spiders/ants minus their abdomens using the Puregene® DNA purification kit from Gentra Systems. Tissue was ground in 100 µl cell lysis buffer and incubated at 65˚C for 30 minutes. Then 33 µl protein precipitation solution was added to the tube and vortexed for 20 seconds, sat on ice for 5 minutes and spun for 3 minutes. The supernatant was poured into 100 µl isopropanol, inverted 50 times and spun for 15 minutes. The isopropanol was discarded and the pellet was washed with 200 µl cold EtOH (70%), spun for 1 minute, then the EtOH was discarded. When the tubes with the DNA pellet were dry, 50 µl of ddH_2O was added. All spins were performed at 13,000 rpm on a Beckman GS-15R centrifuge. 1:5 aliquots of the DNA were used for polymerase chain reactions (PCR) and sequencing.

7.2.3 PCR and sequencing

All thermal reactions were carried out using a Gene Amp® PCR system 9700 thermal cycler. The primers used for PCR of the *Myrmarachne* and ant DNA are presented in

Table 2. Cytochrome Oxidase I (cox1), 16s rRNA- $tRNA^{Leu(CUN)}$ -nad1 and Cytochrome B (cob) primers were used on the *Myrmarachne* DNA, whereas cox1 and Elongation Factor 1 alpha (EF-1-α) primers were used with the ant DNA. The 25 µl PCR mix consisted of 15 µl ddH_2O, 2.5 µl Promega 10x PCR buffer, 3.5 µl of 25mM $MgCl_2$, 5 mM dNTPs, 5 µM of each primer, 0.1 µl *Taq* DNA polymerase and 1 µl of the DNA. Thermal conditions for amplification of the cox1 and cob fragments were 94˚C for 3 minutes and 35 cycles of 94˚C for 30 seconds denaturing, 40˚C for 30seconds annealing and 72˚C for 1 minute 30 seconds extension, followed by 7 minutes extension at 72˚C. For the other fragments, all the conditions were the same except for the annealing temperature, which was set at 46˚C for the 16s-nad1, and at 50˚C for the EF-1-α fragment.

Sequencing was carried out using the Amersham Biosciences DYEnamic™ Energy Transfer terminator reagents kit. The reaction mix contained 4 µl ET dye, 5 µMol of the primer and 20 µMol PCR product. The thermal conditions consisted of 35 cycles of 95˚C for 20 seconds, 50˚C for 15 seconds and 60˚C for 1 minute. The product was purified through Sephadex G50 columns before being analysed in a 96 capillary MegaBACE.

Table 7.2: Primers used for the PCR of ant and spider DNA

Primer name	Sequence	Fragment amplified	Reference
C1-J-1751	5'GGATCACCTGATATAGCATTCCC 3'	cytochrome oxidase 1 (cox1)	(Simon, et al., 1994)
C1-N-2191	5'CCCGGTAAAATTAAAATATAAACTTC3'	cytochrome oxidase 1 (cox1)	(Simon, et al., 1994)
N1-J-12261	5'TCRTAAGAAATTATTTGAGC 3'	16s rRNA-$tRNA^{Leu(CUN)}$-nad1	(Hedin, 1997b)
Faw16s2	5'GCACCTCGATGTTGGATTAA 3'	16s rRNA-$tRNA^{Leu(CUN)}$-nad1	(Simon, et al., 1994)
CB 1	5'TATGTACTACCATGAGGACAAATATC3'	cytochrome B (cob)	(Jermiin & Crozier, 1994)
CB 2	5'ATTACACCTCCTAATTTATTAGGAT 3'	cytochrome B (cob)	(Jermiin & Crozier, 1994)
Udeg	5'TTGGSGTSAAGCAGYTGAT 3'	Elongation Factor 1 alpha (EF-1-α)	(M. Guzik pers. comm.)
Cho-trs	5'GCTTCRTGGTGCATYTCNAC 3'	Elongation Factor 1 alpha (EF-1-α)	(Cho, et al., 1995)

7.2.4 Data analysis

All DNA sequences were aligned using Sequencher version 4.2 (Gene Codes Corporation). The cox1 and cob sequences were used whole, whereas the 16s rRNA-$tRNA^{Leu(CUN)}$-nad1 fragment was divided into the 16s rRNA, nad1 and $tRNA^{Leu(CUN)}$. The nuclear (EF-1-α) fragment was separated into intron and exon sequences for analysis. All the sequences (apart from the $tRNA^{Leu(CUN)}$) were then passed through Codonsplit (Ingrid Jakobsen, University of Queensland) to separate the sequences into first, second and third codon positions. Because differences between lineages in the substitution rate matrix can interfere with phylogenetic inference, a χ^2 stationarity test was carried out to check for potential variations in base compositions among sequences

in the datasets (Lockhart, et al., 1994). The stationarity test was performed using the program Tree-Puzzle version 5.2 (Schmidt, et al., 2002). The sequence alignments that passed the stationarity test were then analysed using the PAUP v. 4.0b10 add-on Modeltest (Posada & Crandall, 1998) to find the most appropriate model of substitution. The DNA sequences that did not pass the stationarity test were converted into amino acid sequences, and Modelgenerator v0.6 (Keane, et al., 2004) was used to find the most suitable model of substitution.

The phylogenetic trees were constructed by Bayesian inference using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) with a Markov Chain Monte Carlo (MCMC) technique, running two parallel analyses for 4,000,000 generations, with a burn in of 39,000 generations. The consensus trees were constructed using the final 1,000 generations. For both the *Myrmarachne* and the ant phylogenies, trees were constructed from all the combined fragments. To draw phylogenetic trees from the information from the MrBayes analyses, TreeView version X (Page, 1996a) was used.

The program TreeMap version 2.0.2 β has been developed to find evolutionary trends between an independent host - and a dependent parasite tree through cophylogeny mapping (Page & Charleston, 2002). In this case, the model ant tree was taken to be the independent – and the *Myrmarachne* - the dependent tree. Jungle analysis (Charleston, 1998) was performed for reconstructing evolutionary events between the two separate taxonomic groups. The constraints used for the Jungle analysis were set as: a minimum of 30 non-codivergence events, at most ten lineage duplications (at least 0 lineage codivergences), at most five host switches, at most 30 lineage losses, and at most six parasites per host. Any reconstruction that fell within these parameters was selected as a

possible outcome. Randomization tests were then used on each of the possible outcomes to obtain significance values and select the most likely reconstruction. The constraints for the randomization test were specific to each reconstruction tested, and each reconstruction was tested with exact numbers of codivergences/unbound non-codivergence events, as well as exact numbers of non-codivergences/unbound codivergence events.

7.3 Results

7.3.1 DNA sequences

For the *Myrmarachne* cox1 fragment, 389 of the sequenced bases were used for phylogenetic reconstructions. For the 16s-tRNA$^{Leu(CUN)}$-nad1 fragment 391 sequenced bases were used which included the partial nad1, and the partial tRNA$^{Leu(CUN)}$ sequences. For the cob gene, 460 sequenced bases were used. The Genbank accession numbers for the sequences are listed in Table 3. All three codon positions of the cox1 and nad1 fragments passed the stationarity test. The cob fragment, however, did not pass the stationarity test, and as a consequence the sequences were converted from DNA to amino acids to construct phylogenies. This means that the tree constructed from the cob fragment is likely to be the least informative or accurate phylogenetic tree. For the ants, the cox1 sequence was also converted to amino acids, and used together with the partitioned EF-1-α exon and the EF-1-α intron sequences to construct one tree. The substitution models selected by Modelgenerator or Modeltest for the *Myrmarachne* sequence alignments and partitioned codon positions were as follows: cox1 1st codon: TIM+I (transition model), cox1 2nd codon: TrN+I (Tamura & Nei, 1993), cox1 3rd codon: TIM+I (transition model); nad1 1st codon: F81 (Felsenstein, 1981), nad1 2nd codon: HKY+G (Hasegawa, et al., 1985), nad1 3rd codon: TrN+G (Tamura & Nei, 1993); tRNA$^{Leu(CUN)}$ alignment: F81 (Felsenstein, 1981); cob amino acid sequence alignment: CpREV (Adachi, et al., 2000). For the sequence alignments of the ants, the models selected were: wag (Goldman & Whelan, 2000) for the cox1 amino acid sequence, JC (Jukes & Cantor, 1969), K80 (Kimura, 1980) and TrNef (Tamura & Nei, 1993) for the EF-1-α exon (first, second and third codon positions respectively) and HKY (Hasegawa, et al., 1985) for the EF-1-α intron sequences.

Table 7.3: Genbank accession numbers for each DNA fragment sequenced per individual organism

Organism	**cox1 fragment**	**nad1-tRNA$^{Leu(CUN)}$ fragment**	**cob fragment**	**EF-1-α fragment**
Myrmarachne sp. A (1)	DQ372990	DQ373008	DQ373028	-
Myrmarachne sp. A (2)	DQ373000	DQ373009	DQ373029	-
Myrmarachne sp. B (1)	DQ372996	DQ373010	DQ373035	-
Myrmarachne sp. B (2)	DQ372991	DQ373011	n/a	-
Myrmarachne sp. C	DQ372989	DQ373012	DQ373032	-
Myrmarachne sp. D (1)	DQ372994	DQ373013	DQ373038	-
Myrmarachne sp. D (2)	DQ372995	DQ373014	DQ373030	-
Myrmarachne sp. E	DQ372999	DQ373015	DQ373033	-
Myrmarachne sp. F (1)	DQ372992	DQ373016	n/a	-
Myrmarachne sp. F (2)	DQ372993	DQ373017	DQ373031	-
Myrmarachne sp. G	DQ380142	DQ373018	DQ373037	-
Myrmarachne sp. H (B)	DQ372998	DQ373019	DQ373036	-
Cosmophasis bitaeniata	DQ372997	DQ373020	DQ373034	-
Opisthopsis haddoni	DQ373006	-	-	DQ373026
Opisthopsis pictus	DQ373007	-	-	DQ373021
Polyrhachis near *obtusa*	DQ373002	-	-	DQ373025
Polyrhachis senilis	DQ373003	-	-	DQ373027
Tetraponera punctulata	DQ373001	-	-	DQ373022
Oecophylla smaragdina	DQ373004	-	-	DQ373023
Odontomachus ruficeps	DQ373005	-	-	DQ373024

7.3.2 Phylogenetic trees

For the *Myrmarachne*, the phylogram constructed using all four mitochondrial fragments combined is shown. For the ants, the phylogram is shown combining all the information obtained in this study.

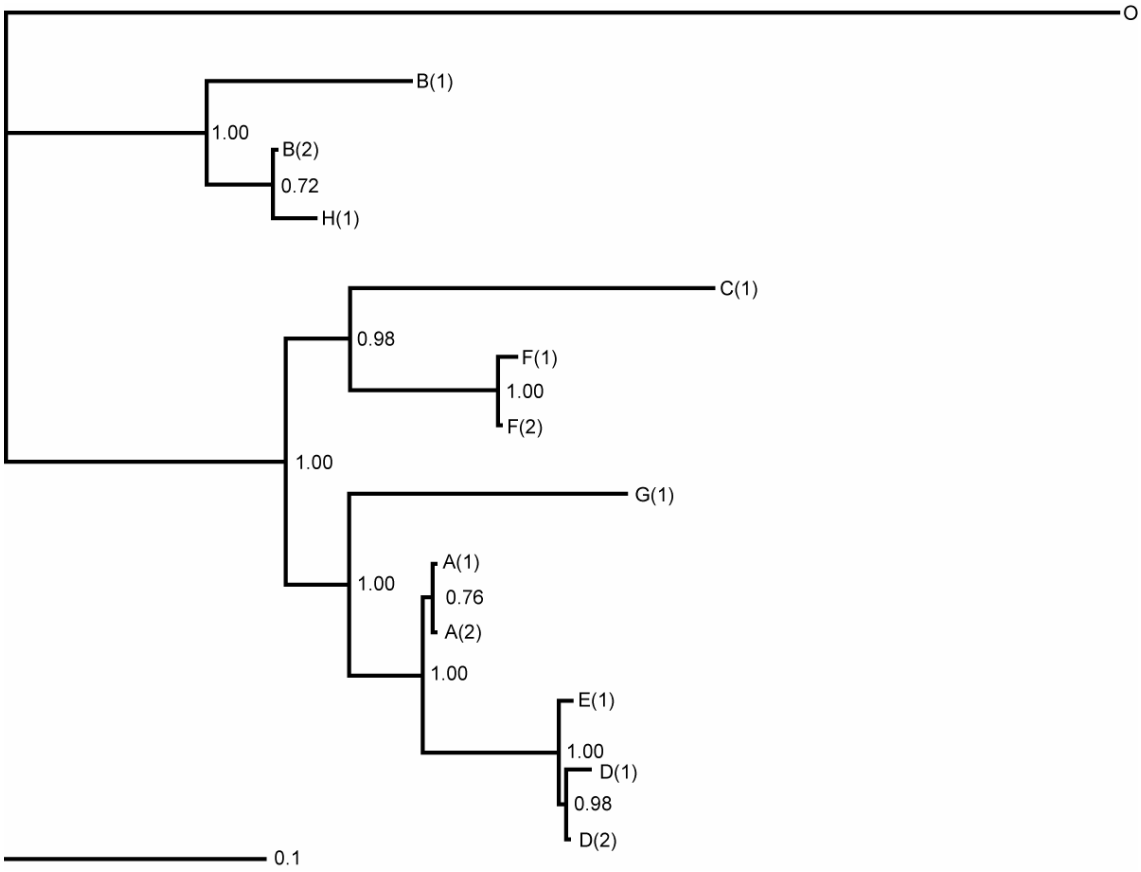


Figure 7.1: *Myrmarachne* species tree constructed by Markov Chain Monte Carlo analysis using the information of all four fragments (the cox1, nad1, $tRNA^{Leu(CUN)}$, and cob fragments) with 4,000,000 generations. Branch lengths and posterior probability values are shown. *Myrmarachne* species names are shown at the end of the branches (with two replicates for some species), and O refers to the outgroup *Cosmophasis bitaeniata*.

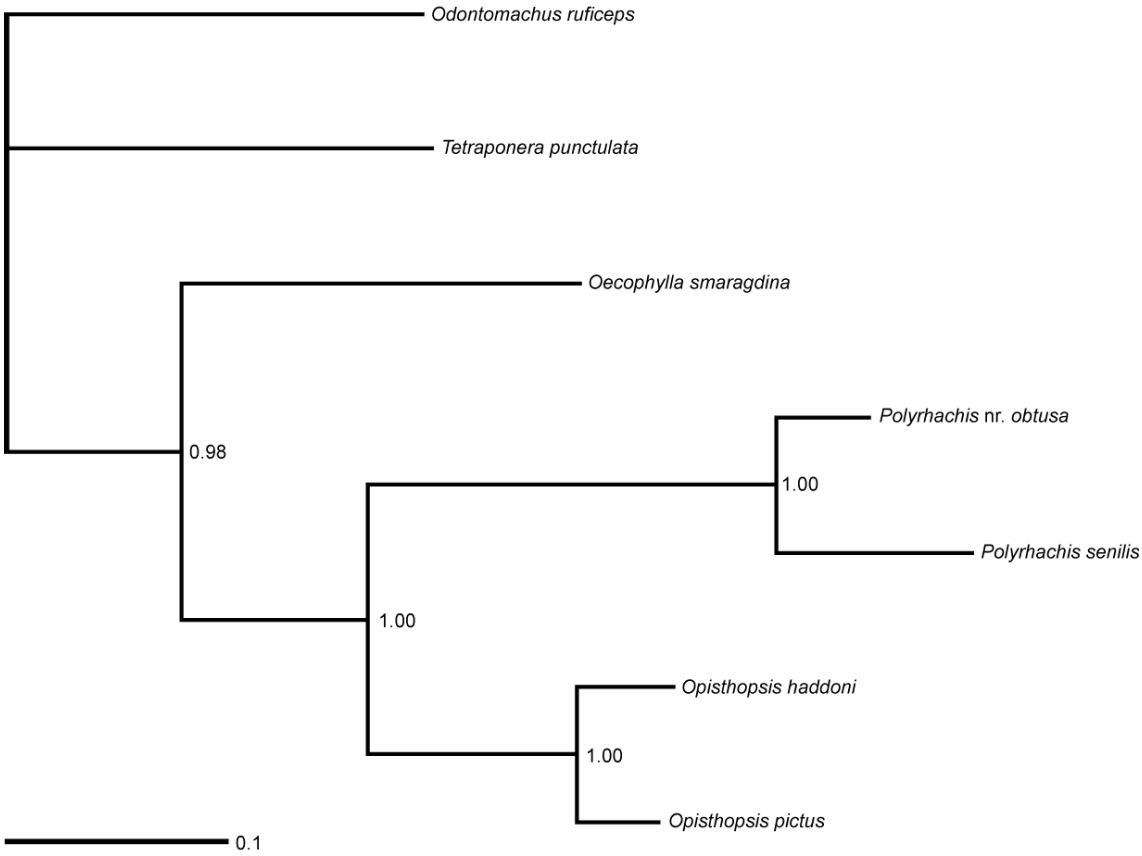


Figure 7.2: Phylogenetic tree of model ant species constructed from the cox1 amino acid -, partitioned EF-1-α exon -, and EF-1-α intron sequences
by Markov Chain Monte Carlo analysis with 4,000,000 generations. The substitution models used were wag (Goldman and Whelan, 2000) for the cox1 amino acid sequences, JC (Jukes and Cantor, 1969), K80 (Kimura, 1980) and TrNef (Tamura and Nei, 1993) for the EF-1-α exon (first, second and third codon positions respectively) and HKY (Hasegawa et al., 1985) for the EF-1-α intron sequences. Branch lengths and bootstrap values are shown. Tree agrees with several previous findings (Baroni Urbani et al., 1992, Johnson et al., 2003, Ward and Brady, 2003).

For the *Myrmarachne* phylogeny all clades were well resolved when the cox1, 16s-nad1, $tRNA^{Leu(CUN)}$ and cob fragments are all used together. The resolution particularly affects the clade containing *Myrmarachne* spp. A, D and E, placing each individual in a separate clade, yet showing little divergence time. Species integrity could only be established between A and D (and not between A and E) through morphological differences in the genitalia. Otherwise, individuals belonging to *Myrmarachne* sp. A display a high degree of colour polymorphism, and can sometimes be mistaken for *Myrmarachne* sp. D, or vice versa.

Initially, *Myrmarachne* sp. H could not be placed into any species group due to undeveloped genitalia (as the individual was in its last juvenile stage), and due to its distinctive dermal coloration it was placed in a species of its own. However, it falls into the same clade as *Myrmarachne* sp. B. The *Myrmarachne* species collected from Cairns (here named *Myrmarachne* sp. G) was placed within the clades of the *Myrmarachne* from Townsville at every tree construction.

The model ant phylogenetic tree in this study contains ants from the subfamilies Ponerinae (*Odontomachus ruficeps*), Pseudomyrmecinae (*Tetraponera punctulata*) and Formicinae (*Oecophylla smaragdina*, *Polyrhachis* near *obtusa* and *P. senilis*, and *Opisthopsis haddoni* and *O. pictus*). Within the Formicinae, *Oecophylla* is separated from *Opisthopsis* and *Polyrhachis*. The placements of the subfamilies and genera here are consistent with previous phylogenetic studies (Baroni Urbani, et al., 1992; Bolton, 2003; Johnson, et al., 2003; Moreau, et al., 2006).

7.3.3 Phylogenetic reconstructions

The Jungle analysis in TreeMap found three likely reconstructions. Based on the randomization tests (used to obtain a significance value for each reconstruction), one reconstruction was chosen to represent the most likely evolutionary scenario in this study.

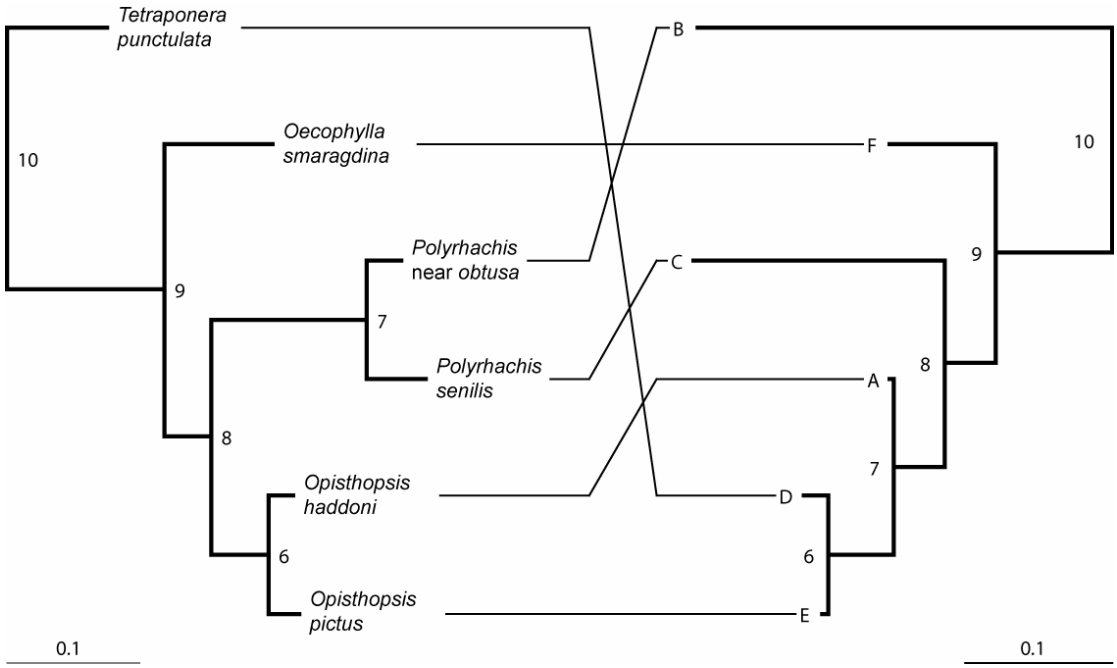


Figure 7.3: Host-parasite Tanglegram showing the associations between model ant species and the mimic *Myrmarachne* species.
Model ant species are shown on the left, and mimic *Myrmarachne* species on the right. The scale bars represent the genetic distance of the branch lengths for each species.

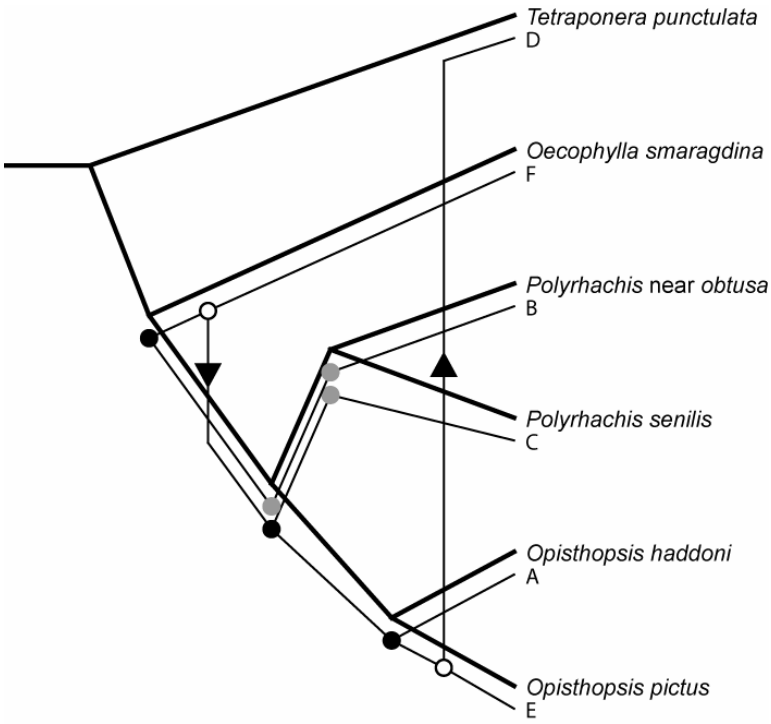


Figure 7.4: Reconstruction of *Myrmarachne* and ant evolutionary events chosen as the most accurate representation of the evolution of *Myrmarachne* species with regards to the model ants.
Myrmarachne species tree is represented by the thinner lines, and the model ants' by the thicker lines. *Myrmarachne* species names are at the end of the branches. The reconstruction shows two host-switches (black triangles), three lineage duplications (black circles) and three lineage losses (grey circles). The randomization of this reconstruction gave a p-value of 0.06 +/- 0.024 (sampling error in estimation of p).

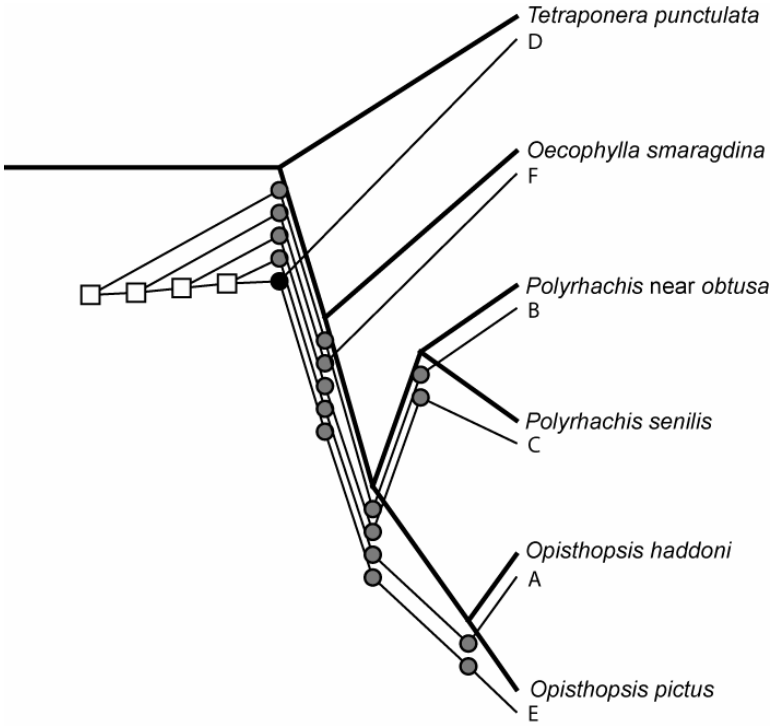


Figure 7.5: Reconstruction of *Myrmarachne* and ant evolutionary events with the lowest p-value
Myrmarachne species tree is represented by the thinner lines, and the model ants' by the thicker lines. *Myrmarachne* species names are at the end of the branches. The reconstruction shows no host-switches, one lineage duplication (black circle) and 17 lineage losses (grey circles). The randomization of this reconstruction with unbound events gave a p-value of 0.04 +/- 0.0196 (sampling error in estimation of p).

Comparing the phylogeny of the *Myrmarachne* species to that of their model ant species shows that there is no relationship between the two groups, as is shown by the tanglegram (see Fig. 7.3). For example, *Tetraponera punctulata* is the outgroup to the other ants, yet its mimic, *Myrmarachne* sp. D falls into the most recently evolved clade of the *Myrmarachne*. On the other hand, the *Polyrhachis* nr. *obtusa* mimic – *Myrmarachne* sp. B - is the most basal of the *Myrmarachne* in this study, whereas the ant species turned out to be the most recent one of the ants used. The two *Polyrhachis* species (*P.* nr. *obtusa* and *P. senilis*) have *Myrmarachne* mimics (*M.* sp. B and *M.* sp. C respectively) which do not group together in the phylogenetic tree. Lastly, *Myrmarachne* spp. A and E appear to be polymorphic forms of the same species, mimicking the two distinct *Opisthopsis* species (*O. haddoni* and *O. pictus*, which are of similar size).

Table 7.4: Parameters of the reconstructions obtained from the Jungle analysis

R	*C*	*D*	*L*	*S*	*E*	$p_1 \pm$ *sampl.err*	$p_2 \pm$ *sampl.err*
1	6	4	4	1	9	0.19 ± 0.039	0.18 ± 0.038
2	6	4	3	2	9	0.06 ± 0.024	0.41 ± 0.049
3	2	8	17	0	25	0.1 ± 0.030	0.04 ± 0.020
4	4	6	0	3	9	0.39 ± 0.049	0.25 ± 0.043

R = Reconstruction number
C = number of codivergence events
D = number of duplications
L = number of losses
S = number of host switches
E = total number of events
p_1 = significance with exact number of non-codivergence events and unbound number of codivergence events
p_2 = significance with unbound number of non-codivergence events and exact number of codivergence events.

Although the third reconstruction (Figure 7.4) from the jungle analysis had the lowest p-value ($p<0.05$), the fact that it contained 25 events made it an unlikely candidate for

explaining the most probably evolutionary events of the *Myrmarachne* species. On the other hand, the second reconstruction (Figure 7.5) shows nine events, namely four lineage duplications, three lineage losses (when there is a split in the model- but not the mimic tree) and two host switches. This reconstruction was chosen to represent the most likely set of evolutionary events based on the $p = 0.06$ significance value. Although there were six codivergence events, none of these appears to be co-speciation.

7.4 Discussion

7.4.1 Myrmarachne phylogeny

This study has shown that dermal coloration and texture are inadequate indicators of what species an individual *Myrmarachne* belongs to. This is particularly true for the spiders in the clade containing *Myrmarachne* spp. A, D and E, where the coloration ranges from red to black with various intermediate colour tones. The different dermal colorations could arise due to differences in diet between individuals (Oxford & Gillespie, 1998), but is more likely to be a form of polymorphism. The maintenance of polymorphism in Batesian mimics is very plausible (Charlesworth & Charlesworth, 1975), so it comes as no surprise that in this study a polymorphic species of *Myrmarachne* was found. The high degree of polymorphism could explain the fine resolution of the clade (containing *Myrmarachne* spp. A, D and E) in the phylogenetic tree constructed here. The clade also shows little divergence in time, and it seems like *Myrmarachne* sp. E could be an intermediate form between A and D in the ongoing speciation process. As a matter of fact, there was no apparent difference in adult genital morphology of *Myrmarachne* spp. A and E, but *Myrmarachne* sp. D did show slight variations in genital morphology (see chapter 8), suggesting that reproductive isolation led to speciation in that case.

In addition to species polymorphism, sexual dimorphism and immature individuals are factors that make it difficult to recognise individuals of the opposite sex - or off different instar stages – as belonging to the same species. As to date there are no morphological structures other than the genitalia that are useful in distinguishing

between species of *Myrmarachne*, one could search for common structures between the different instars of a species as a means of identification for juveniles. The trait where the *Myrmarachne* species change their coloration at every instar stage during their development has been documented before (Mathew, 1935; Edmunds, 1978; Wanless, 1978), is known as transformational mimicry, and can be found in other mimetic species (Mathew, 1935).

This study has also shown that there are at least five distinct *Myrmarachne* species in Townsville, four of which were subsequently named and described (Ceccarelli, ms submitted, also see chapter 8 for unpublished data). In addition, the one species from Cairns that was included showed that geographical distance does not imply phylogenetic distance in this case, since *Myrmarachne* sp. G from Cairns was placed in between the clades from Townsville. Although this study involved a relatively small number of species from a restricted area, it offers the possibility for largely expanding the number of *Myrmarachne* from various other locations throughout Queensland, and possibly even from surrounding countries. This would allow conclusions to be based on a larger scale.

7.4.2 Mimicry evolution

The fact that *Myrmarachne* represents one genus, whereas its model ants belong to different subfamilies means that the evolutionary rates between the two groups are expected to be different, making co-speciation unlikely. The evolutionary patterns found in this study show that rather than co-speciating with their model ants, *Myrmarachne* have converged towards different sympatric ant species. This supports

the findings that Batesian mimics are under strong selection pressure to gain a close resemblance to their models (Mappes & Alatalo, 1997). The model and the mimic do not seem to be locked into a 'coevolutionary chase' (Gavrilets & Hastings, 1998) in a strict sense, since that scenario would have produced congruence in the phylogenies of the two groups, rather than events such as host switching (or in this case model switching) observed in this study.

As Batesian mimics, *Myrmarachne* species are under strong selection pressure to radiate and evolve towards the various ants' phenotypes. The pressure on mimics can be increased by certain events such as the model becoming rare. While the phenotype is at an intermediate stage between models, the mimic may not be as well protected. In the case of *Myrmarachne* an intermediate phenotype occurs during their development. Being transformational mimics, *Myrmarachne* can resemble a different ant species at each instar (Edmunds, 1978), but in some cases certain instars do not particularly resemble any ant species (personal observation). These intermediate phenotypes may also be stages upon which selection pressure can act more significantly, maintaining polymorphisms. In the case of polymorphic species of *Myrmarachne*, differences in female mate preference (based on coloration) could lead to reproductive isolation between the populations, and eventually to speciation. Selection pressure from predation maintaining polymorphisms and sexual selection driving speciation are possible explanations to why in this study a highly polymorphic species was found as well as individuals at intermediate stages of mimicking ant species. These findings point to ongoing speciation as a 'by-product' of events such as host-switching. As Elgar (1993) pointed out, "*there are no clear taxonomic affiliations between the species of spider mimics and the species of ant models*". The explanation for this lack of

taxonomic affiliation is provided by the results of this study that show relatively rapid host-switches (or in this case model-switches) by certain mimic species, due to the strong selection pressure.

The use of phylogenetics in determining evolutionary dynamics between two taxonomically distinct groups has so far only focussed on host-parasite (Brooks, 1988; Paterson, et al., 1993; Charleston & Robertson, 2002) – or Müllerian mimicry evolution (Simmons & Weller, 2002; Machado, et al., 2004). Predictions of Batesian mimicry evolution on the other hand has mainly been based on the construction of mathematical models (Gavrilets & Hastings, 1998; Holmgren & Enquist, 1999). This study has for the first time used a real-life example of Batesian mimicry evolution using phylogenetics. This method can in future be applied to a more global sample of *Myrmarachne* and ants, as well as other taxa of Batesian mimics and their models.

Chapter 8 : New species of ant-mimicking spiders of the genus *Myrmarachne* (Araneae: Salticidae) from North Queensland

Abstract

Four new species of *Myrmarachne* (Araneae: Salticidae) from Townsville, North Queensland are described. The proposed new species are *Myrmarachne rubii*, *Myrmarachne aurea*, *Myrmarachne sawda*, and *Myrmarachne smaragdina*. A key to their identification is presented. The main taxonomic characters used are the male and female genitalia as well as the male chelicerae. Information is also given on the biology and ecology of the species. This is done for each species separately; a comparison between the species is also presented, supported by data collected over two and a half years.

8.1 Introduction

The spider family Salticidae occurs worldwide and contains to date some 5027 named species in 550 genera (Platnick, 2005). Such a large grouping is taxonomically unwieldy and at least for practical purposes requires subdivision. Simon (1901) divided the Salticidae into the Pluridentati, the Unidentati and the Fissidentati, based on their cheliceral dentition. This classification will stand until a more natural system is found (Wanless, 1978). *Myrmarachne* McLeay, 1838, is a genus of specialized ant-mimicking salticids assigned to the pluridentati group, since the chelicerae of *Myrmarachne* contain several teeth. The genus is one of the largest in the Salticidae, with over 200 named species. Most species occur in tropical regions (Proszynski, 2003) and it is likely that there are many that remain undescribed.

Myrmarachne presents taxonomic problems because most species are sexually dimorphic. This difference in the sexes is especially pronounced when looking at the chelicerae, which are thought to be a sexually selected feature in males, often protruding in front of the carapace (Jackson, 1982; Pollard, 1994). *Myrmarachne* also display transformational mimicry (Edmunds, 1978): a species mimicking several different models at different stages of its life (Mathew, 1935). The most important recent revision of the genus *Myrmarachne* has been done for the Ethiopian region by Wanless (Wanless, 1978). Other descriptions of new *Myrmarachne* species include the ones by Bradoo (1980), Logunov and Wesolowska (1992), Berry *et. al.* (1996), Wesolowska and Salm (2002), and Edmunds and Proszynski (2003).

The genus *Myrmarachne* can be sub-divided into species groups based on overall genital morphology such as the form of the tibial apophysis of the male palp, or the

form of the female spermathecae. Here, three of the species groups will be described (the *tristis*-group, the *formicaria*-group and the *volatilis*-group). Males of the *tristis*-group usually have a well developed flange, a hooked tibial apophysis and a depression in the proximal ectal margin of the cymbium, and females are characterized by the presence of lateral pouches in the epigyne. The males of the *formiacaria*-group generally have a more or less sinuous tibial apophysis and a poorly developed flange, and the female epigyne has looped or twisted spermathecae and a median subtriangualr pouch. Males of the *volatilis*-group have a large, marginate seminal reservoir, no flange and a more or less sinuous tibial apophysis; females have a median subtriangular pouch and simple spermathecae, without loops or twists. Species within one species-group can show little variation in the structure of their genitalia. The *tristis* and *formicaria* groups occur in the Palaearctic and Oriental regions, but could possibly be found in Australia, whereas the *volatilis*-group definitely occurs in the Australasian region (Wanless, 1978).

In Australia, 11 species of *Myrmarachne* have been described: *M. bicolor* (Koch, 1879), *M. cognata* (Koch, 1879), *M. cuprea* (Hogg, 1896), *M. erythrocephala* (Koch, 1879), *M. jugularis* (Simon, 1900), *M. luctuosa* (Koch, 1879), *M. lupata* (Koch, 1879), *M. macleayana* (Bradley, 1876), *M. maxillosa* (Koch, 1879), *M. simoni* (Koch, 1879) and *M. striatipes* (Koch, 1879).

This chapter is modeled on a paper that names and describes four new species of *Myrmarachne* found in North Queensland, Australia during the course of a study into model-mimic systems. Since the actual manuscript has not yet been published, elsewhere in this thesis the given names have not been used. Species integrity was

established using DNA data and specimens were then examined for distinguishing morphological features to enable categorization of living material. Comments on biology and ecology are provided as microhabitat use and patterns of association with the ant models provide strong clues to the identity of these *Myrmarachne* species.

8.2 Materials and methods

Specimens were collected in Townsville (19˚ 13’ S, 146˚ 48’ E), and preserved in 100% Ethanol until they were examined. Photographs of the type specimens were taken through the eye piece of a dissecting microscope. For the females, the epigyne was removed under a dissecting microscope and mounted on a cavity slide in Grey and Weiss mounting fluid, to then be examined under a compound microscope. Further specimens from the arachnology collections at the Australian Museum (Sydney) and the Queensland Museum (Brisbane) were examined.

Live *Myrmarachne* were also observed, and notes were taken between February 2003 and September 2005 to find out more about each species’ biology and ecology. Occasionally, egg sacs were brought into the lab for further studies on spiderling instar changes. The description of the species follows the conventions set out by Wanless (1978). Measurements of the male chelicerae and carapaces were also taken, and the data was analysed in R 2.1.1 (R_Development_Core_Team, 2005) performing ANOVAs and divisive cluster analyses (diana) to see whether the chelicera-to-carapace length ratio is significantly different between the *Myrmarachne* species, and whether it contains evolutionary information.

8.3 Key to *Myrmarachne* species from Townsville

1. Males: Chelicerae approximately the same length as the length of the carapace, containing 18 retromarginal teeth and a fang apophysis; hooked tibial apophysis. Females: Spermathecae in a figure-of-eight configuration……....***M. smaragdina***

Males: Chelicerae shorter than the length of the carapace, containing fewer than 10 retromarginal teeth and no fang apophysis; tibial apophysis more or less sinuous. Females: Spermathecae not in a figure-of-eight configuration.…..…….2.

2. Males: Chelicerae not protruding, containing 3 promarginal and 3 retromarginal teeth; palpal bulb diameter about two-thirds of the length of the cymbium, tip of cymbium containing two major setae. Females: Spermathecae round, two separate lateral pouches, but touching……....***M. aurea***

Males: Chelicerae protruding, but shorter than the length of the carapace; palpal bulb larger than two-thirds of the length of the cymbium, tip of cymbium without major setae. Females: Spermathecae oval or D-shaped, continuous median pouch instead of separate lateral pouches…….....3.

3. Males: Chelicerae about two-thirds the length of the carapace, containing four retromarginal teeth at the distal end and one retromarginal tooth at the proximal end of each chelicera; tibial segment of palps longer than wide at proximal end, flange not well developed. Females: Spermathecae D-shaped……..…..…..……..***M. rubii***

Males: Celicerae protruding, but shorter than two-thirds the length of the carapace (about half the length), containing one tooth at the proximal retromarginal edge and two large and one small tooth at the distal retromarginal end; tibial segment of palps wider at proximal end than long, flange well developed. Females: Spermathecae oval rather than D-shaped, ducts not joined for the whole length……..…….……..***M. sawda***

8.4 Description of species

8.4.1 Myrmarachne rubii

new species

Figure 8.1

Holotype in Queensland Museum Arachnology collection with registration number S66648.

Etymology: Named after the red colour that most individuals have on their carapace.

Distribution: Specimens recorded from North Queensland, Australia: Atherton (17° 17' S, 145° 30' E), Dimbulah (17° 08' S, 145° 04' E), Kuranda (16° 48' S, 145° 35' E), Townsville (19° 13' S, 146° 48' E) and Wolfram (17° 05' S, 144° 55' E).

Diagnosis: Male chelicerae usually two-thirds the length of the carapace, with four retromarginal teeth at the distal- and one at the proximal end of each chelicera. The bulb of the male palp is three-quarters of the total length of the cymbium; the tibial segment has a poorly developed flange, and a small, sinuous apophysis, and the whole segment is longer than wide at its base. The female epigyne has D-shaped spermathecae, and a continuous median pouch. *Myrmarachne rubii* can be distinguished by *M. erythrocephala* and *M. striatipes* by the number of spines on the first pair of legs, where *M. rubii* has a pair of spines on the metatarsi, and a single spine and a pair on the tibia, whereas both *M. erythrocephala* and *M. striatipes* have two pairs of spines on the metatarsi of legs I.

Descriptive notes: MALE: *Carapace*: ranging from orange-brown to red-brown or black with sparse white hairs, wedge-shaped depressions behind the anterior lateral eyes. *Eyes*: surrounded by black pigmentation, procurved anteriors surrounded by hairs. *Clypeus*: fringed with white hairs. *Chelicerae*: rugulose and protruding, orange-brown

to red-brown with black pigmentation at distal end, no fang apophysis, retromarginal dentition: one tooth on proximal end and four on distal end. *Maxillae* and *labium*: orange with black hairs and setae. *Sternum*: orange-brown with some black markings. *Opisthosoma*: distal end black, front part red-brown to orange-brown or in some cases black; sparsely covered with fine white hairs, central depression more densely fringed with white hairs. *Legs*: slender. Legs I: tarsus black; metatarsus black and orange; tibia light orange with black markings; patella light orange with black markings; femur orange brown and black; trochanter cream; coxa cream. Legs II: tarsus cream; metatarsus black and orange; tibia light orange with black markings; patella light orange with black markings; femur orange brown and black; trochanter cream; coxa cream. Legs III: tarsus cream; metatarsus cream; tibia orange; patella orange; dark brown and black; femur dark brown and black; trochanter cream; coxa black. Legs IV: tarsus cream; metatarsus cream; tibia orange; patella orange; dark brown and black; femur dark brown and black; trochanter cream; coxa yellow and black. Legs I spination: metatarsus 2, tibia 1-2, patella 0. *Palp*: tibial apophysis relatively small, sinuous and black; flange not very developed; cymbium and proximal depression fringed with setae; embolus coiled once around bulbous tegulum of approximately 210 μm in diameter; seminal reservoir large and marginate. *Dimensions*: total length: 3.7 to 4.5 mm; carapace length: 1.8 to 2.4 mm; ratio of carapace-to-chelicera length: 1.22 to 1.67; Ratios AM:AL:PM:PL: 5.78:1.41:1:2.7.

FEMALE: *Carapace*: same as ♂. *Eyes*: same as ♂. *Clypeus*: fringed with white hairs. *Chelicerae*: rugulose, non-protruding, mainly black with some dark red pigmentation. *Maxillae* and *labium*: same as ♂. *Sternum*: same as ♂. *Opisthosoma*: same as ♂, but often more bulbous. *Legs*: slender. Legs I: tarsus black; metatarsus light orange; tibia light orange and cream; patella light orange and cream; femur light orange; trochanter

cream; coxa cream. Legs II: same as legs I. Legs III and IV: same as legs I and II, but slightly darker. Legs I spination: metatarsus 2, tibia 1-2, patella 0. *Epigyne*: orange and dark-red, continuous median pouch, spermathecae simple (D-shaped), ducts relatively wide. *Dimensions*: total length: 4.0 to 4.7 mm; carapace length: 2.0 to 2.5 mm; Ratios AM:AL:PM:PL: 5.78:1.41:1:2.7.

Materials examined: Australian Museum collection: specimens with registration numbers KS93121 (male, collected by F.S. Ceccarelli in Townsville, 05/11/2005), KS18335 (female, collected by M. Zabka north of Kurranda, 1982), KS81341 (female, collected by M. Zabka in the Atherton area, 18/10/2002), KS5770 (male, collected by N.C. Coleman in Wolfram, 15/06/1970). Queensland Museum collection: specimens with registration numbers S66648 (male, collected by F.S. Ceccarelli in Townsville, 11/03/03) and S73296 (female, collected by B.M. Baehr 1km south of Dimbulah, 05/06/1993). Unregistered specimens: Male, collected by F.S. Ceccarelli in Townsville, 20/03/2003; female, collected by F.S. Ceccarelli in Townsville, 04/07/2003; female, collected by F.S. Ceccarelli in Townsville, 09/07/2003.

Additional comments: Up to this point in the thesis, this species has been referred to as *Myrmarachne* sp. A. *Myrmarachne rubii* belongs to the *volatilis*-group. It is a mimic of the ants *Opisthopsis haddoni* and *O. pictus* (Formicinae). *M. rubii* can be found on tree trunks (mainly eucalyptus trees of the species *Eucalyptus platyphylla*), usually closely associated with their model ant. Males and females usually build retreats under loose pieces of bark, but males' retreats tend to be sheet-like, and males are more likely to be found walking up the tree trunks. Females build more wool-like retreats with thicker strands of silk "anchoring" the retreat to the tree, and lay between 10 and 20 eggs in their retreat, which they stay with until the spiderlings hatch. During instars 3 and 4, *M. rubii* look like small black ants from the genus *Crematogaster*.

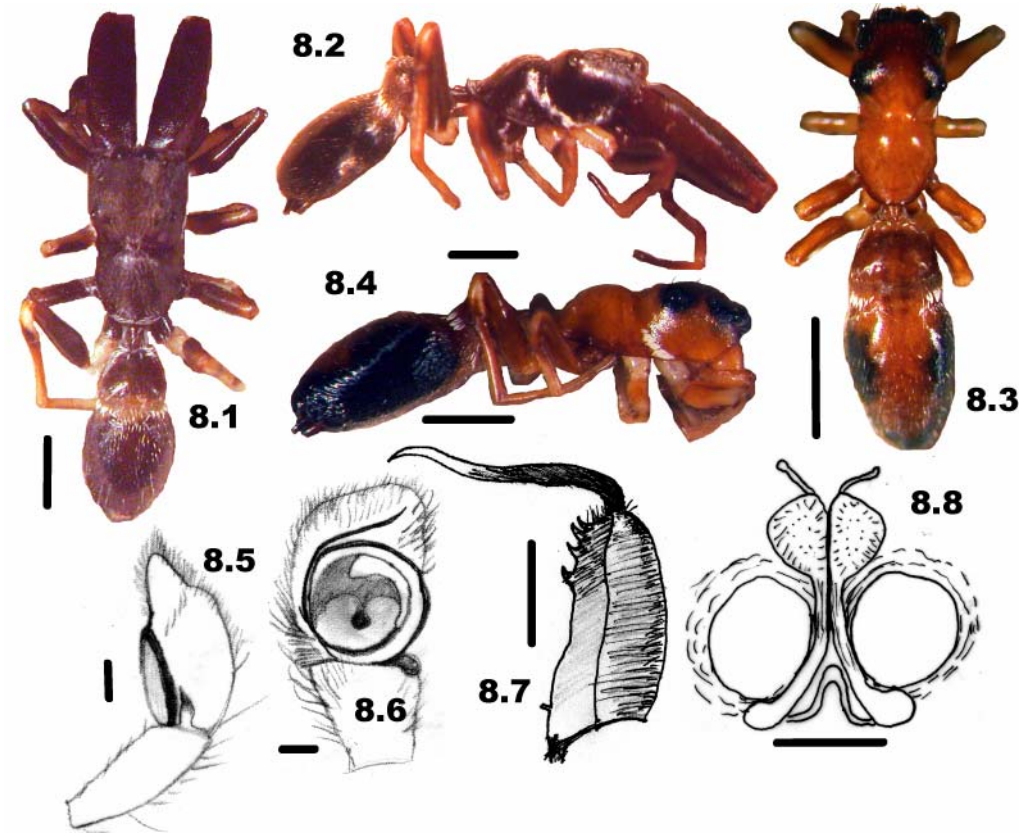


Figure 8.1: *Myrmarachne rubii sp. nov*.
8.1 – 8.8., male (8.1,8.2, 8.5-8.7): 8.1, 8.2, habitus, dorsal (8.1) and lateral (8.2) views; 8.5, 8.6, palpal tibia, cymbium and bulb, retrolateral (8.5), and ventral (8.6) views; 8.7, chelicera medio-lateral view; female (8.3, 8.4, 8.8): 8.3, 8.4, habitus, dorsal (8.3) and lateral (8.4) views; 8.8, epigyne showing internal ducts. Scale lines: 1 mm for Figs 8.1 – 8.4; 100 μm for Figs 8.5, 8.6, 8.8; 500 μm for Fig. 8.7.

8.4.2 Myrmarachne aurea

new species

Figure 8.2

Holotype in Australian Museum with registration number KS93119.

Etymology: Named after the golden look of the opisthosoma.

Distribution: Specimens recorded from Queensland, Australia: Canowindra (33˚ 35' S, 148˚ 38' E), Townsville (19˚ 13' S, 146˚ 48' E) and Kenilworth state forest (27˚ 41' S, 147˚ 46' E); and Northern Territory, Australia: Musgrave (26˚ 05' S, 132˚ 00' E).

Diagnosis: Males' chelicerae are not protruding when viewed from above, and contain three promarginal and three retromarginal teeth. The tibia of the male palps has a poorly developed flange, and a sinuous apophysis; the diameter of the tegulum is approximately two-thirds the length of the cymbium. The cymbium contains two larger setae at the distal tip. Females have small, round spermathecae that are separated from each other; there are also two lateral pouches that are found in contact with each other. *M. aurea* can be distinguished from *M. luctuosa* by the fact that *M. aurea* males' chelicerae do not protrude.

Descriptive notes: MALE: *Carapace*: black with few white hairs, wedge-shaped depression with more dense white hairs. *Eyes*: procurved, surrounded by white hairs. *Clypeus*: fringed with white hairs. *Chelicerae*: not protruding, black with a dark red fang, retromarginal dentition: three teeth, promarginal dentition: three teeth. *Maxillae* and *labium*: black maxillae with a yellow inner edge, labium black. *Sternum*: black. *Opisthosoma*: black and yellow with fairly densely packed golden yellow hairs, giving the opisthosoma a golden sheen, slightly constricted in the middle. *Legs*: slender. Legs I: tarsus black; metatarsus black; tibia yellow with black marks, typically a line running down the length of the leg segment; patella same as tibia; femur same as tibia and

patella; trochanter black and yellow; coxa yellow/cream coloured. Legs II: tarsus yellow with black markings; metatarsus yellow with black markings; tibia yellow with black markings; patella yellow with black markings; femur yellow with black markings; trochanter yellow with black markings; coxa black. Legs III: tarsus yellow; metatarsus black; tibia black; patella yellow; femur black; trochanter black; coxa black. Legs IV: tarsus black; metatarsus black; tibia black; patella black and yellow; femur black; trochanter yellow and black; coxa yellow. Legs I spination: metatarsus 2-1, tibia 2-2-2, patella 0. *Palp*: tibial apophysis with a black sinuous hook, embolus coiled around bulbous tegulum, about 200 μm in diameter (about two-thirds the length of the cymbium), large, marginate seminal reservoir, cymbium and proximal depression fringed with setae with two larger ones at the distal end. *Dimensions*: total length: 4.5 to 5.5 mm; carapace length: 1.8 to 2.5 mm; ratio of carapace-to-chelicera length: 2 to 4; Ratios AM:AL:PM:PL: 4.12:1.61:1:2.

FEMALE: *Carapace*: same as ♂. *Eyes*: same as ♂. *Clypeus*: same as ♂. *Chelicerae*: same as ♂. *Maxillae* and *labium*: same as ♂. *Sternum*: same as ♂. *Opisthosoma*: same as ♂, but occasionally more bulbous. *Legs*: same as ♂. Legs I spination: metatarsus 2-2, tibia 2-2-2, patella 0. *Epigyne*: black and yellow pigmentation, lateral pouches separate, spermathecae simple and round, ducts relatively wide. *Dimensions*: total length: 3.8 to 4.5 mm; carapace length: 1.8 to 2.1 mm; Ratios AM:AL:PM:PL: same as ♂.

Material examined: Australian Museum collection: specimens with registration numbers KS56467 (two females collected by G. Milledge in Kenilworth state forest, 07/05/1998), KS93119 (male collected by F.S. Ceccarelli in Townsville, 16/11/2005), KS93120 (female collected by F.S. Ceccarelli in Townsville, 16/11/2005). Queensland Museum collection: specimens with registration numbers S66649 (female collected by F.S. Ceccarelli in Townsville, 04/06/2003), S41386 (female collected by M.F. Downs in

Townsville, 01/06/1981), S20339 (female collected by M. Shaw near Canowindra, 14/03/1992) and S73293 (male collected by B.M. Baehr at Mary Creek 25 km south of Musgrave, 29/05/1993). Unregistered specimens: Male collected by F.S. Ceccarelli in Townsville, 07/05/2003; female collected by F.S. Ceccarelli in Townsville, 09/07/2003; male collected by F.S. Ceccarelli in Townsville, 07/10/2003.

Additional comments: Up to this point in the thesis, this species has been referred to as *Myrmarachne* sp. B. *Myrmarachne aurea* belongs to the *volatilis*-group, and is widely distributed in Townsville and surrounding areas, and can be found either walking on trees, in particular eucalypts (*Eucalyptus platyphylla* and *Corymbia tesellaris*), or inside retreats under loose bits of bark. It is not uncommon to find more than one retreat under a piece of bark, with several *M. aurea* (typically between 2 and 6, often at different instar stages), each living in a separate retreat. The model ant species for *M. aurea* is *Polyrhachis* near *obtusa* (Formicinae). This species of *Myrmarachne* is not as closely associated to its model ant as the other three *Myrmarachne* are to their model ants. This means that while *M. aurea* and *P.* near *obtusa* are found living in sympatry, they are not always found on the same tree. *Myrmarachne aurea* do not develop the golden-haired opisthosoma until they reach adulthood. Before that, the spiderlings are of a dark brown colour with white markings on both the pro- and opisthosoma.

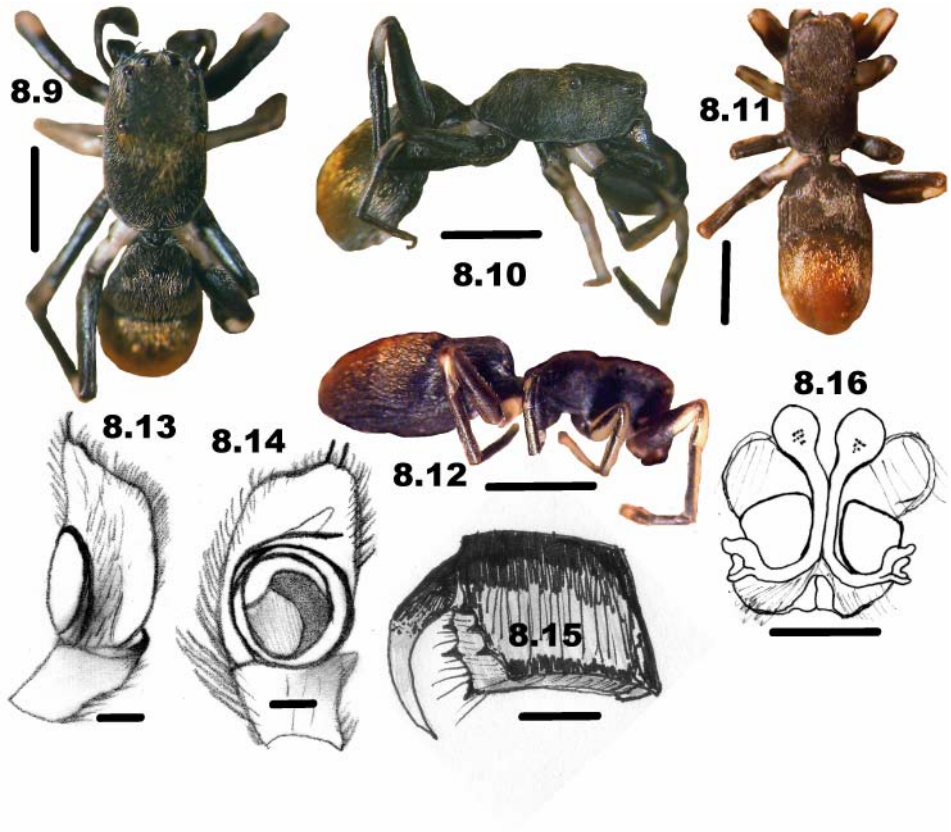


Figure 8.2: *Myrmarachne aurea sp. nov*.
8.9 – 8.16., male (8.9,8.10, 8.13-8.15): 8.9, 8.10, habitus, dorsal (8.9) and lateral (8.10) views; 8.13, 8.14, palpal tibia, cymbium and bulb, retrolateral (8.13), and ventral (8.14) views; 8.15, chelicera medio-lateral view; female (8.11, 8.12, 8.16): 8.11, 8.12, habitus, dorsal (8.11) and lateral (8.12) views; 8.16, epigyne showing internal ducts. Scale lines: 1 mm for Figs 8.9 – 8.12; 100 μm for Figs 8.13, 8.14, 8.16; 200 μm for Fig. 8.15.

8.4.3 Myrmarachne sawda

new species

Figure 8.3

Holotype in Queensland Museum Arachnology collection with registration number S66650

Etymology: Named after the black coloration of the cuticle.

Distribution: Specimens recorded from North Queensland, Australia: Townsville (19˚ 13' S, 146˚ 48' E).

Diagnosis: Male chelicerae protrude, but are only about half the length of the carapace, containing one retromarginal tooth at the proximal-, and three at the distal end. The tibial segment of the palp is shorter than the width at its proximal end; the flange is well developed and the tibial apophysis is black, sinuous and relatively large. The diameter of the tegulum is approximately four-fifths the length of the cymbium. The female epigyne has oval-shaped spermathecae and a continuous median pouch. *M. sawda* can be distinguished from *M. erythrocephala* and *M. striatipes* by the spination of the tibiae of the first pair of legs, where *M. sawda* has one spine (and the females have an additional pair), whereas *M. erythrocephala* has none, and *M. striatipes* has three pairs of spines on their tibiae.

Descriptive notes: MALE: *Carapace*: all black, wedge-shaped depression in centre fringed with white hairs. *Eyes*: anterior medians procurved. *Clypeus*: fringed with white hairs. *Chelicerae*: all black and protruding, but when seen from the side at a slight downward angle, retromarginal dentition: one tooth at proximal end and two large and one small tooth on distal end; no fang apophysis. *Maxillae* and *labium*: black, maxillae with dark red margins. *Sternum*: black. *Opisthosoma*: black, sparsely covered with white hairs, central depression fringed with dense white hairs. *Legs*: slender. Legs I:

tarsus black; metatarsus black; tibia red-brown and dark brown; patella yellow and red-brown; femur yellow and black; trochanter yellow and black; coxa white/cream. Legs II: tarsus dark yellow; metatarsus dark yellow; tibia dark yellow; patella dark yellow; femur dark yellow with black marks; trochanter yellow with black marks; coxa white/cream. Legs III: tarsus yellow; metatarsus yellow; tibia yellow and red-brown; patella dark brown; femur red-brown and black; trochanter red-brown and black; coxa black. Legs IV: tarsus yellow; metatarsus yellow; tibia red-brown and black; patella dark brown; femur brown and black; trochanter white; coxa white and black. Legs I spination: metatarsus 2-2, tibia 1, patella 0. *Palp*: tibial apophysis with a sinuous black hook, flange well developed, embolus coiled once around bulbous tegulum, about 170 μm in diameter (making up four-fifths of the length of the cymbium), seminal reservoir marginate, cymbium and proximal depression fringed with setae. *Dimensions*: total length: 4.0 to 4.8 mm; carapace length: 1.7 to 2.1 mm; ratio of carapace-to-chelicera length: 1.48 to 2.11; Ratios AM:AL:PM:PL: 4:1.8:1:2.5.

FEMALE: *Carapace*: same as ♂. *Eyes*: same as ♂. *Clypeus*: same as ♂. *Chelicerae*: not protruding, black. *Maxillae* and *labium*: same as ♂. *Sternum*: same as ♂. *Opisthosoma*: same as ♂. *Legs*: slender. Legs I: tarsus white with black markings; metatarsus white with black markings; tibia red-brown; patella white with black markings; femur brown with black markings; trochanter white with black markings; coxa white with black markings. Legs II: tarsus cream; metatarsus cream, tibia cream with brown markings; patella cream with brown markings; femur cream with brown markings; trochanter cream; coax cream. Legs III: tarsus white; metatarsus white; tibia red-brown and white; patella red-brown and white; femur red-brown and black; trochanter black and red-brown; coxa black. Legs IV: tarsus white; metatarsus white; tibia red-brown; patella white with brown markings; femur brown and black; trochanter light brown; coxa

white. Legs I spination: metatarsus 2-2, tibia 1-2, patella 0. *Epigyne*: white, surrounded by red-brown pigmentation; continuous median pouches, spermathecae simple and oval-shaped, ducts relatively wide, not touching along the whole length. *Dimensions*: total length: 4.5 to 5.0 mm; carapace length: 2.0 to 2.3 mm; Ratios AM:AL:PM:PL: 3.5:1.8:1:2.2.

Materials examined: Queensland Museum collection: specimens with registration numbers S66650 (male collected by F.S. Ceccarelli in Townsville, 29/04/2003). Unregistered material: Male collected by F.S. Ceccarelli in Townsville, 23/05/2003; female collected by F.S. Ceccarelli in Townsville, 25/07/2003; female collected by F.S. Ceccarelli in Townsville, 25/09/2003; male collected by F.S. Ceccarelli in Townsville, 07/10/2003.

Additional comments: Up to this point in the thesis, this species has been referred to as *Myrmarachne* sp. D. *Myrmarachne sawda* belongs to the *volatilis*-group, and is the mimic of the ant *Tetraponera punctulata* (Pseudomyrmecinae), positively associated with its ant model. This species is the least abundant in Townsville of the four species described here, and can easily be mistaken for *M. rubii*. The spiders can be found walking on trees (mainly eucalyptus trees of the species *Eucalyptus platyphylla*), or inside retreats they build under pieces of bark, or in small indentations of the tree trunk. Males build sheet-like retreats, whereas the female retreats are more wool-like in appearance. Females lay between 10 and 15 eggs. The spiderlings are black during every stage of their life-cycle.

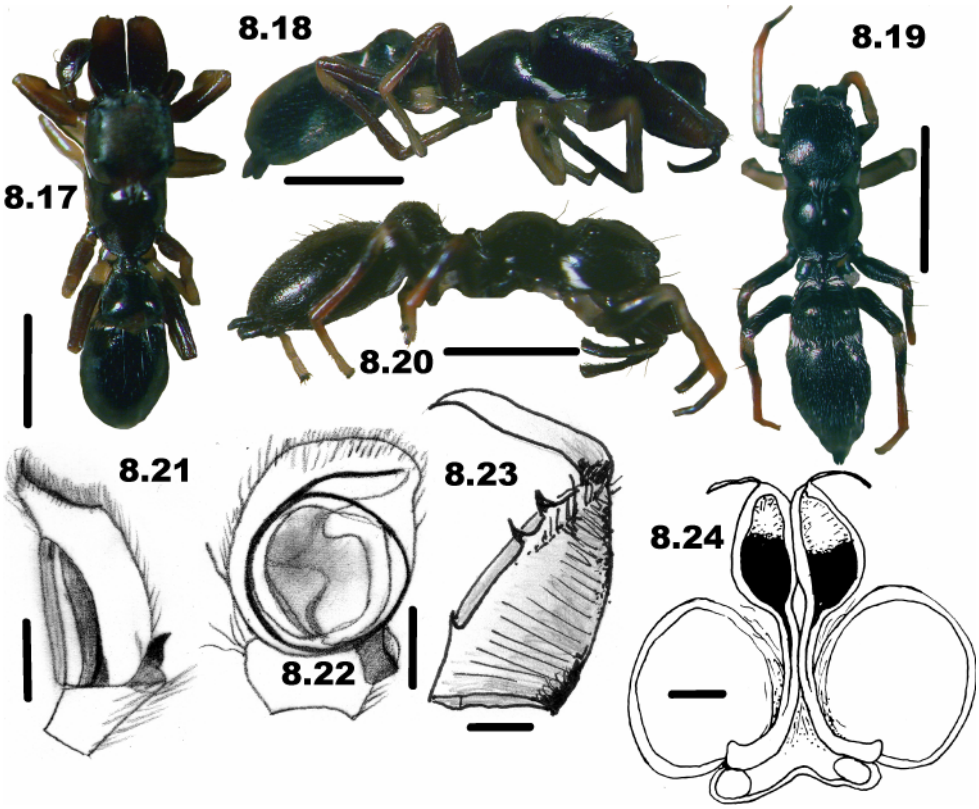


Figure 8.3: *Myrmarachne sawda sp. nov.*
8.17 – 8.24., male (8.17,8.18, 8.21-8.23): 8.17, 8.18, habitus, dorsal (8.17) and lateral (8.18) views; 8.21, 8.22, palpal tibia, cymbium and bulb, retrolateral (8.21), and ventral (8.22) views; 8.23, chelicera medio-lateral view; female (8.19, 8.20, 8.24): 8.19, 8.20, habitus, dorsal (8.19) and lateral (8.20) views; 8.24, epigyne showing internal ducts. Scale lines: 1 mm for Figs 8.17 – 8.20; 100 µm for Figs 8.21, 8.22; 500 µm for Fig. 8.23; 50 µm for Fig. 8.24.

8.4.4 Myrmarachne smaragdina

new species

Figure 8.4

Holotype in Queensland Museum Arachnology collection with registration number S66653

Etymology: Named after its model ant, *Oecophylla smaragdina*.

Distribution: Specimens recorded from Queensland, Australia: Cooktown (15° 30' S, 145° 16' E), Townsville (19° 13' S, 146° 48' E) and Edmonton (17° 02' S, 145° 46' E); Northern Territory, Australia: Angurugu (14° 00' S, 136° 25' E), Berrimah research station (12° 45' S, 130° 95' E), and Mudjinberri (12° 05' S, 132° 88' E).

Diagnosis: Male chelicerae about the same length as the carapace, containing 18 retromarginal teeth, and a fang apophysis. The tibial apophysis of the male palp is black and hooked, and the flange is not developed. The diameter of the tegulum is approximately one-third of the length of the cymbium; the distal end of the cymbium contains one major seta. The female epygine contains looped spermathecae (into a figure-of-eight) and lateral pouches. *M. smaragdina* can be differentiated from *M. lupata* by the relative length of the male chelicerae, by the orientation of the embolus and by the spination of the first pair of legs. The chelicerae of male *M. lupata* are described as being longer than the carapace by a quarter of its length, whereas the chelicerae of *M. smaragdina* are about the same length as the carapace. In the male palps of *M. lupata*, the embolus points straight towards the tip of the cymbium, whereas the depression in the cymbium of *M. smaragdina* forces the embolus to lie at a 45 degree angle relative to the longitudinal line of the cymbium. Finally, *M. lupata* has no spines on its first pair of legs, whereas *M. smaragdina* has two pairs of spines on the metatarsus, and four pairs on the tibia of legs I.

Descriptive notes: MALE: *Carapace*: orange/light brown with black pigmentation around the eyes; sparsely covered with white hairs; wedge-shaped constriction in the middle of the carapace. *Eyes*: procurved AMs, fringed with hairs and black pigment. *Clypeus*: fringed with white hairs. *Chelicerae*: protruding, orange/light brown with black pigmentation ant the distal end; fang apophysis present, retromarginal dentition: 18 teeth (3 large and 15 small ones). *Maxillae* and *labium*: maxillae cream with brown margins. *Sternum*: yellow and orange. *Opisthosoma*: green with sparse white hairs, constriction in the middle fringed with more white hairs. *Legs*: slender. Legs I: tarsus white; metatarsus white; tibia light orange; patella light orange; femur light orange; trochanter white; coxa orange/ light brown. Legs II: tarsus white; metatarsus white; tibia light orange; patella light orange; femur cream; trochanter white; coxa orange/ light brown. Legs III: tarsus white; metatarsus white; tibia light orange; patella light orange; femur light orange; trochanter white; coxa orange/ light brown. Legs IV: tarsus white; metatarsus white; tibia light orange; patella light orange; femur light brown; trochanter white; coxa orange/ light brown. Legs I spination: metatarsus 2-2, tibia 2-2-2-2, patella 0. *Palp*: tibial apophysis black, with a backward-curved distal hook; flange not very developed; cymbium and proximal depression fringed with setae, with one large seta at the distal end of the cymbium; embolus coiled 1 ½ times around bulbous tegulum of approximately 103 μm in diameter (about one-third of the length of the cymbium); seminal reservoir not very large. *Dimensions*: total length: 4.0 to 6.2 mm; carapace length: 2.7 to 3.1 mm; ratio of carapace-to-chelicera length: 0.75 to 1.2; Ratios AM:AL:PM:PL: 3.29:1.13:1:1.62.

FEMALE: *Carapace*: same as ♂. *Eyes*: same as ♂. *Clypeus*: same as ♂. *Chelicerae*: non-protruding, light brown. *Maxillae* and *labium*: same as ♂. *Sternum*: same as ♂. *Opisthosoma*: same as ♂. *Legs*: slender, same as ♂. Legs I spination: same as ♂.

Epigyne: white and orange/brown; lateral pouches separate, spermathecae simple, in a figure-of-eight configuration. *Dimensions*: total length: 5.5 to 6.0 mm; carapace length: 2.6 to 2.9 mm; Ratios AM:AL:PM:PL: 3.31:1.10:1:1.59.

Materials examined: Australian Museum collection: specimens with registration numbers KS18304 (female collected by R. Mascord in Edmonton, 1976), KS19169 (two females collected by D. Levitt in Angurugu via Darwin, 28/05/1969), KS44998 (male, collected by D. Citin at Berrimah research station, 24/03/1988), KS44999 (male, collected by D. Citin at Berrimah research station, 24/03/1988), KS5771 (female, collected by R. Mascord in Edmonton, 27/08/1970), KS93122 (male, collected by F.S. Ceccarelli in Townsville, 04/01/2006). Queensland Museum collection: specimens with registration numbers S66652 (female, collected by F.S. Ceccarelli on Magnetic Island, 28/08/2003), S66653 (male, collected by F.S. Ceccarelli on Magnetic Island, 28/08/2003), S403 (male, collected by G.B. Monteith by the McIver river, 40 miles north of Cooktown, 07/05/1970) and S73295 (female, collected by B.M. Baehr 3 km north of Mudjinberri, 04/11/1984). Unregistered specimens: Male, collected by F.S. Ceccarelli in Townsville, 11/06/2003; female, collected by F.S. Ceccarelli in Townsville, 30/06/2003; male, collected by F.S. Ceccarelli in Townsville, 05/09/2003.

Additional comments: Up to this point in the thesis, this species has been referred to as *Myrmarachne* sp. F. *Myrmarachne smaragdina* belongs to the *tristis*-group, mimics the green tree ant *Oecophylla smaragdina* (Formicinae), and can be found in close association to model ant colonies. As with their model ants *Myrmarachne smaragdina* typically occurs on trees in the proximity of creeks, with no apparent preference for the type of tree they live on. The retreats are usually built on the upper surface of leaves, the males building sheet-like retreats, and the females building woolly-looking ones, often with “anchoring” threads of silk, joining the retreat and the leaf. Retreats have been

found on the outside of *O. smaragdina* nests themselves, although this is a rare occurrence. *Myrmarachne smaragdina* have also been found in leaf-litter, but this is presumably because the leaves on which the retreats were built fell from the tree. The females lay between 15 and 20 eggs, and the spiderlings have a brown carapace and a black opisthosoma during the first two instar stages after leaving the egg sac, bearing a close resemblance to ants of the genus *Crematogaster*. The last instar before adulthood looks like the smaller workers from *O. smaragdina* colonies, with the same colour patterns as the ants, except for more white pigmentation on the opisthosoma.

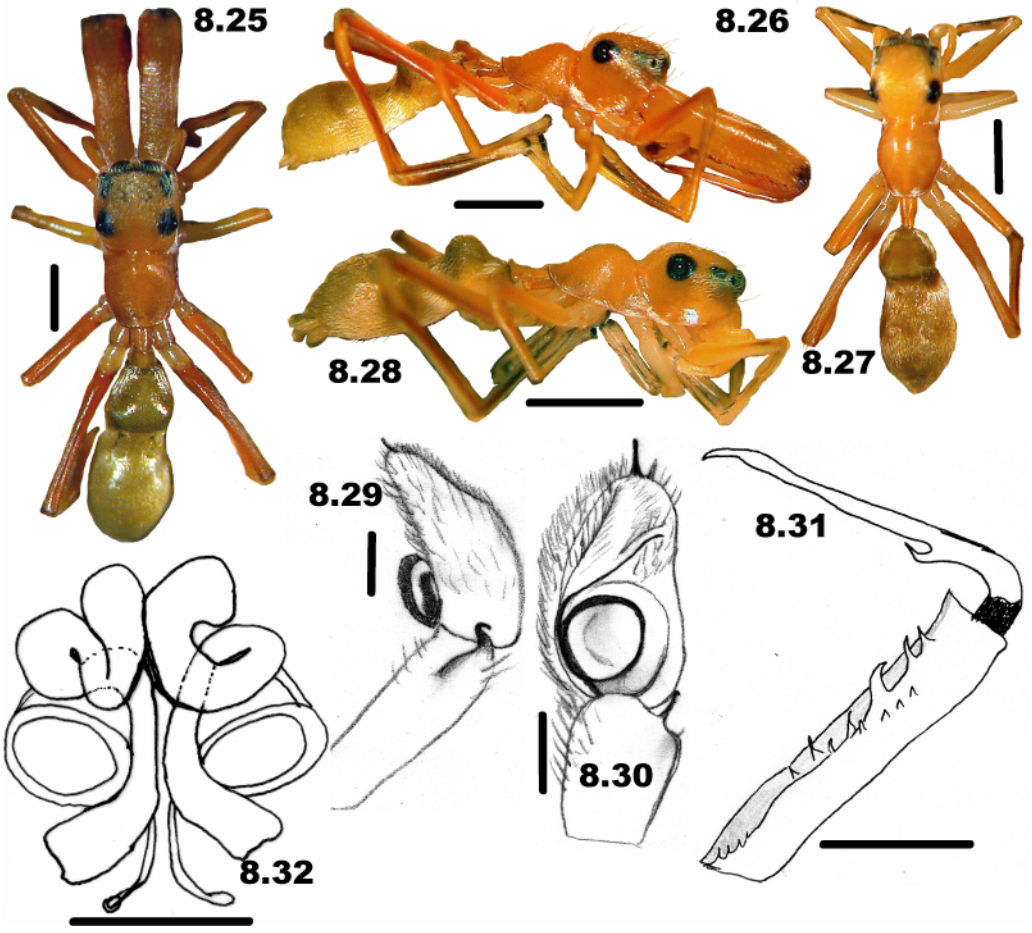


Figure 8.4: *Myrmarachne smaragdina sp. nov.*
8.25 – 8.32., male (8.25,8.26, 8.29-8.31): 8.25, 8.26, habitus, dorsal (8.25) and lateral (8.26) views; 8.29, 8.30, palpal tibia, cymbium and bulb, retrolateral (8.29), and ventral (8.30) views; 8.31, chelicera medio-lateral view; female (8.27, 8.28, 8.32): 8.27, 8.28, habitus, dorsal (8.27) and lateral (8.28) views; 8.32, epigyne showing internal ducts. Scale lines: 1 mm for Figs 8.25 – 8.28, 8.31; 100 µm for Figs 8.29, 8.30, 8.32.

8.5 Comparison of species

8.5.1 Morphological differences

As mentioned previously by several people (Wanless, 1978; Berry, et al., 1996), the genitalia of *Myrmarachne* species are not always differentiated well enough to use them as a discriminating feature. However, in this case the female genitalia do show considerable differentiation. *Myrmarachne rubii* and *Myrmarachne sawda* can be similar-looking due to the degree of colour polymorphism in *M. rubii*, which ranges from bright red to very dark red (almost mistakable for black – the colour of *M. sawda*). A feature that seems to be consistently similar between individuals of the same species is the patterns of the leg coloration (even though the colour hues may vary between individuals). The leg patterns vary slightly between sexes in *M. rubii* and *M. sawda*, which means that both the pattern of males and females need to be known. The males' cheliceral dentition also varied between the species, as well as the proportion of the chelicera-to-carapace length in males. Figures 8.5 and 8.6 show the chelicera-to-carapace length ratio of each *Myrmarachne* species, and its divisive properties.

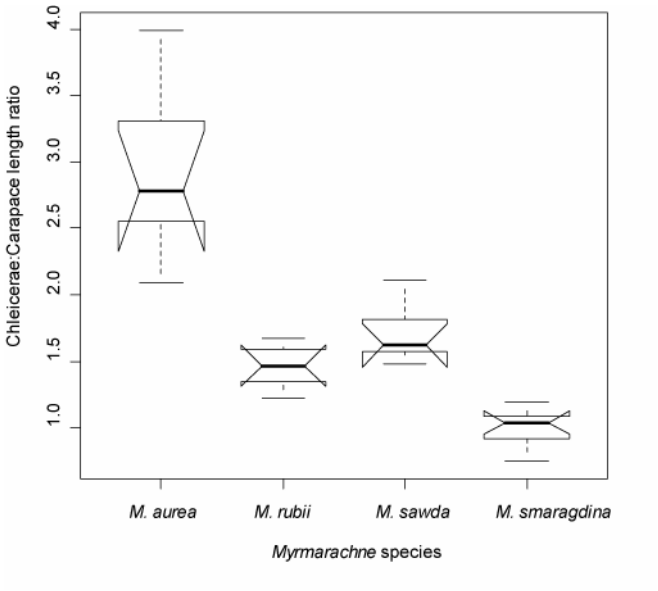


Figure 8.5: Notched boxplot of chelicera-to-carapace length ratio for the males of the four *Myrmarachne* species
(ANOVA: $F_{(3,24)} = 39.25$, $p < 0.0001$)

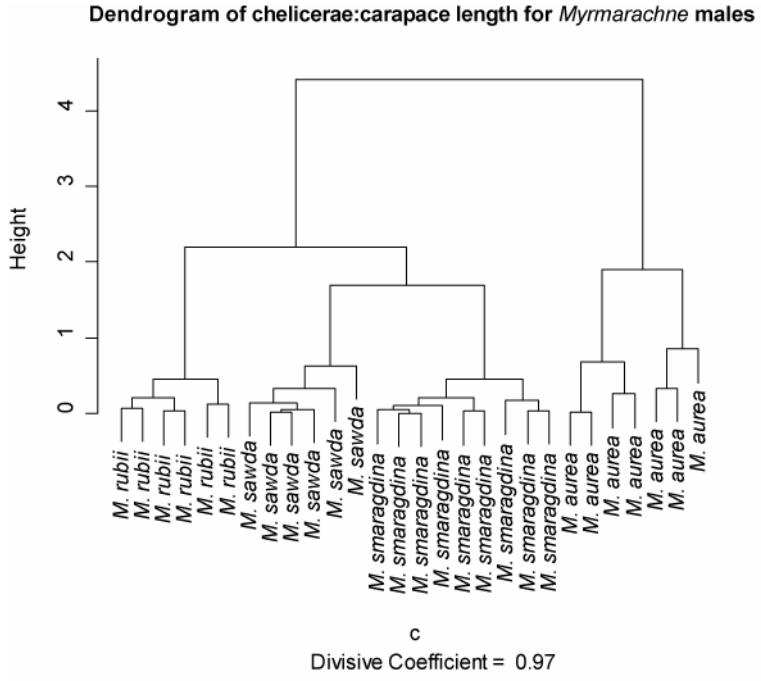


Figure 8.6: Dendrogram showing the divisive properties of the chelicera-to-carapace length ratio of the four *Myrmarachne* species from Townsville
obtained through divisive clustering analysis (diana).

Looking at the data analysis, the chelicera-to-carapace length ratio is different enough between males of different species (ANOVA: $F_{(3,24)} = 39.25$, $p < 0.0001$) to be a basis of differentiation in cases where colour polymorphism and little differentiation of the genitalia make quick identification of the species difficult. The *Myrmarachne* males with enlarged chelicerae are thought to resemble ants with a parcel in their mandibles, making the *Myrmarachne* compound mimics (Nelson & Jackson, 2006). The enlarged chelicerae have also been said to be a sexually selected character, since they complicate feeding (Pollard, 1994). Comparing the molecular phylogeny of the *Myrmarachne* species in this study with the cluster analysis tree shows that the evolution of the *Myrmarachne* species is not reflected in the length of the male chelicerae. This means that the lengthening of the male chelicerae in *Myrmarachne* species is most likely to have evolved independently to the *Myrmarachne* species evolution. The male chelicerae are also useful to tell species apart because for each species described here, the number of teeth is different. For the females, epigynal structure seems to be consistent within species while containing enough differences between species to be able to identify the species.

8.5.2 Ecological differences

Although the four *Myrmarachne* species described here are almost exclusively arboreal, they display variations in their micro-habitats. This can be seen by looking at the different places where the individuals were collected from (see Figure 8.7). For example, more than any other species *Myrmarachne smaragdina* was found inside retreats on the upper surfaces of leaves, whereas *Myrmarachne rubii* was found more frequently inside retreats built in depressions on tree trunks than the other species.

Myrmarachne aurea was found inside retreats built under loose pieces of bark more frequently than the other species. These ecological differences show that the *Myrmarachne* species in Townsville have differentiated not only in morphology and biology, but also in ecology, living in microhabitats preferred by their respective ant models. Even *Myrmarachne* species that preferentially build their retreats on leaves display subtle differences: *M. smaragdina* has - during the course of this study - been observed living in retreats on the upper surface of leaves, whereas *M. lupata* seems to build its retreats on the lower surface of leaves (Jackson, 1982).

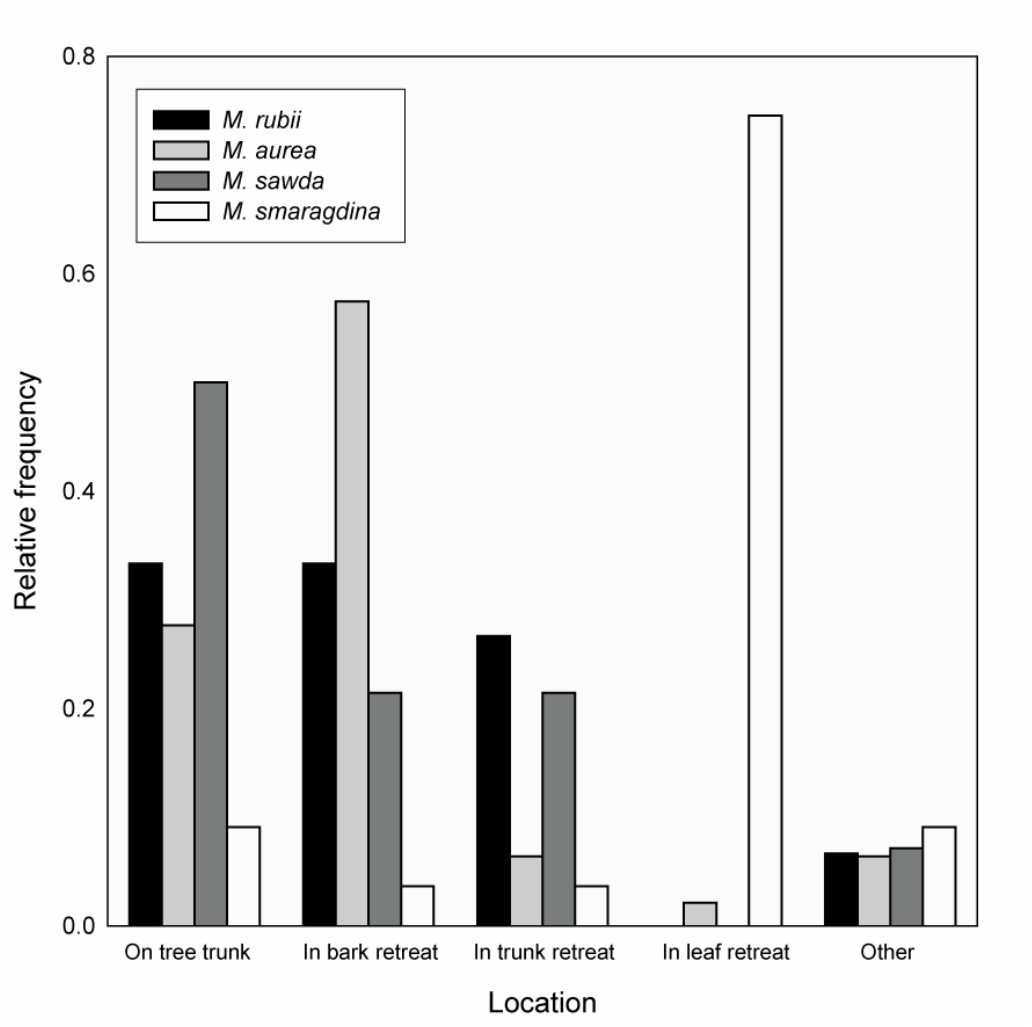


Figure 8.7: Bar chart showing the relative frequency of places where each *Myrmarachne* species was collected from in Townsville
Places are coded as: "On tree trunk" = found walking on the trunk of a tree, "Inside bark retreat" = was found inside a retreat it had built under a loose piece of bark, "Inside trunk retreat" = was found inside a retreat it had built in a depression on the tree trunk, "Inside leaf retreat" = was found inside a retreat built on a leaf, "Other" = was found walking on the ground, on the side of buildings and in other places.

8.6 Conclusion

There remain several Australian *Myrmarachne* species that need describing, and a revision of the genus is desirable. Although the focus here is on a small number of species from a restricted geographical range, it is nevertheless a contribution (together with the papers by Bradley (1876), Hogg (1896), Koch (1879) and Simon (1900)) towards a better understanding of the Australian *Myrmarachne* fauna. The outlined differences in morphology and ecology of the *Myrmarachne* species described here also shows how sympatric species have differentiated. This differentiation is indicative of strong selection pressure, which in the case of Batesian mimics is mainly exerted by predators.

Chapter 9 : General discussion

Mimicry is a topic of great interest, since it is an example of predatory-mediated natural selection. Mimicry occurs in a wide range of taxa, and has been studied particularly intensively in invertebrates. Mimicry in insects has been reviewed by Rettenmeyer (1970), as well as McIver and Stonedahl (1993). Four main categories of mimicry: Wasmannian, aggressive, Müllerian and Batesian are commonly recognised. Batesian mimicry (Bates, 1862) occurs when the mimic gains protection from predators by being associated with an unpalatable model. The salticid spider genus *Myrmarachne* Macleay, 1838 (Araneae: Salticidae), was used as a model organism to study the dynamics of the mimicry. *Myrmarachne* is a fitting model for this study, since its species are Batesian mimics of ants, and it is a highly speciose genus, comprising over 200 species worldwide.

Batesian mimics are under strong selection pressure to closely resemble their models (Mappes & Alatalo, 1997), thus *Myrmarachne* is also expected to be under selection pressure. Mimicry evolution is largely driven by predatory selection pressure (Turner & Speed, 1996; Joron & Mallet, 1998; Speed & Turner, 1999), however, predation is not the only force determining polymorphism dynamics in mimics (Edmunds & Golding, 1999). Regardless of the main driving force, evolutionary pressure has led to a high number of *Myrmarachne* species, many of them polymorphic in colour, and most of them mimicking a different ant species. The close association to each model ant means that *Myrmarachne* species have had to be adaptable and versatile on different levels. Interspecific diversity of *Myrmarachne* with regards to behaviour and appearance, as well as *Myrmarachne*'s behavioural versatility is supported throughout the studies

undertaken. Behavioural, molecular and comparative studies in this thesis all reinforce the hypothesis that the genus *Myrmarachne* is, and has been, under strong selection pressure (possibly from different sources), which has led to high interspecific variability.

During interactions with sympatric ant species, *Myrmarachne* have shown versatile behaviours, mainly in protecting themselves from the ants. Ants are known to be aggressive towards other animals that pose a threat to their colony (Hölldobler & Wilson, 1990), also killing salticids (Halaj, et al., 1997; Nelson, et al., 2004). *Myrmarachne* have not been shown to possess any chemical protection against ants, so avoiding contact with ants is their best method of defense. *Myrmarachne* tend to associate with, but to avoid contact with ants whenever possible (Mathew, 1944; Edmunds, 1978; Jackson, 1986). Despite this conflict in behaviour the behavioural patterns of *Myrmarachne* in the vicinity of ants has not been studied in detail until now. In this study, *Myrmarachne* have shown a range of behavioural reactions to ants, such as jumping backwards and turning around to run away. The type of reaction depended mainly on the distance between the spider and the ant, the position the ant was in when the spider saw it and whether one or both of the animals was moving. This means that *Myrmarachne* react depending on visual cues that give information about the ant's distance and direction. For example, if the ant is at a certain distance, and not moving towards the spider, by not reacting the spider will have conserved energy, as well as not attracted attention to itself. However, if it was in a risky position with regards to the ant, *Myrmarachne* would most commonly react by moving out of the way of the ant. The jumps backwards were the most frequent reactions, as this is a quick move away from the ant, while still being able to keep it in the *Myrmarachne*'s field of vision.

The four *Myrmarachne* species used in the experiment carried out their reactions to the ants at different frequencies. In addition, the ant species encountered also had an effect on the frequencies at which the different reactions were carried out. The main trend was that when a *Myrmarachne* was placed with its model ant species, the combination resulted in an overall lower rate of kinetic energy (measured as the Total Motion Index, or the TMI). This shows that the *Myrmarachne* species have differentiated in their behavioural patterns. These behavioural patterns could include a less agitated state around their own model ant species, since each *Myrmarachne* species is likely to encounter its own model ant most frequently in nature, as there is a positive association between the mimic and its model (Edmunds, 1978).

The fact that *Myrmarachne* lives in close association with its model ants in nature (Mathew, 1944; Edmunds, 1978) means that contact between the two is likely to occur frequently. In this study, the contact between the ant and the *Myrmarachne* was shown to occur mainly between the antennae of the ant and the first pair of legs of the spider. This contact often ended with the *Myrmarachne* running away. Contact also occurred between the *Myrmarachne*'s chelicerae and the ant (body or head), causing the ant to run away. This means that in some instances, *Myrmarachne* is able to defend itself from the ant. The ability to defend itself - as well as being able to escape ants - could be a major reason for the finding that *Myrmarachne* get killed by ants less frequently than other salticids (Nelson, et al., 2004; Nelson, et al., 2005). Through natural selection, *Myrmarachne* have developed this ability enabling them to deal with contact to the ants without getting killed. When each species was considered separately, there were differences both between the *Myrmarachne* as well as the ant species in the frequencies

of the body parts making contact, and the frequencies of the reactions. This means that in addition to having developed strategies for surviving in the vicinity of ants, *Myrmarachne* species have also evolved species-specific contact and reaction responses. This again points to differentiation, as well as possible learning by the *Myrmarachne* species from being confronted with various sympatric ant species.

Myrmarachne is one of the many spiders that associate with ants (for review see (Cushing, 1997)). Another ant mimicking salticid is *Cosmophasis bitaeniata*, which mimics the cuticular hydrocarbons of the green ant *Oecophylla smaragdina* to enter their nest undetected and prey on the ants' larvae (Allan & Elgar, 2001; Allan, et al., 2002). *C. bitaeniata* is therefore an aggressive mimic, unlike *Myrmarachne* species which are Batesian mimics. The comparison of the two green ant-mimicking salticids in this study shows that despite the different purposes of their mimicry, both spiders were equally unlikely to make contact with the ant. *C. bitaeniata* has – like *Myrmarachne* - behaviourally adapted to minimise contact with ants.

The differences in the reactions of *Myrmarachne* and *Cosmophasis bitaeniata* to the ants can be accounted for in terms of their different body shapes. For example, *Myrmarachne* carried out more jumps backwards and turns in the opposite direction to move away from the ant. This could be easier for *Myrmarachne*, which generally have a slender body and comparatively longer legs than for *C. bitaeniata*, which more readily moved sideways. Compared to *C. bitaeniata*, *Myrmarachne* also saw the ants more frequently head-on. On the other hand, *C. bitaeniata* had the ant behind it more frequently than *Myrmarachne* did. This is probably because *Myrmarachne* more readily turns to face an approaching object than *C. bitaeniata* does. Again, the greater agility of

Myrmarachne is implied. In nature, *C. bitaeniata* spends more time inside ants' nests than *Myrmarachne*, who have been found mainly living on tree trunks (personal observation). In ants' nests, the visibility is poorer than on tree trunks, and on trees there is a greater diversity of animals compared to the inside of a nest. So the salticids that spend more time on the tree trunk can rely more on their mobility and vision to detect and respond to various animals in their surroundings. That is probably why *Myrmarachne* have evolved to use mobility and vision more readily than *C. bitaeniata*.

Both *Myrmarachne* and *Cosmophasis bitaeniata* carry out behavioural mimicry in addition to their morphological and chemical mimicry. This behavioural mimicry consists mainly of "antennal illusion" and "opisthosomal bobbing". Antennal illusion means that the spider lifts and waves its first pair of legs making them look like ants' antennae, while walking on their remaining 3 pairs of legs (Reiskind, 1977). The opisthosomal bobbing resembles the ritualised behaviour in ants when they lift their gaster to dissuade each other to attack (Mercier & Dejean, 1996; Mercier, et al., 1997). Selection pressure on these behavioural traits has led to differences between the two salticids. The main difference between the two salticids in their behavioural mimicry is that *Myrmarachne* carries out more antennal illusion (by "waving" its first pair of legs) while *C. bitaeniata* bobs its opisthosoma up and down more frequently. This may be because the main target of *Myrmarachne*'s mimicry is visual predators, whereas for *C. bitaeniata* it is the ants in the colony it is exploiting. Thus *Myrmarachne* could use antennal illusion as a more conspicuous signal that predators can see from different angles, whereas *C. bitaeniata* might bob its opisthosoma as a signal aimed at the ants. The antennal illusion in *Myrmarachne* is also different for each species, which has probably again differentiated due to selection pressure. Batesian mimics are under

pressure to resemble the model's phenotype (Mappes & Alatalo, 1997), which includes behavioural traits. So the evolution of different *Myrmarachne* species has also led to the diversification of behavioural mimicry traits, namely how quickly the legs are waved on average, and how often the opisthosoma is moved up and down.

Mimicry of *Oecophylla smaragdina* is not restricted to salticids, since there are other spiders, as well as insects, that bear a striking resemblance to the green ant. For example, *Riptortus serripes* (Hemiptera: Alydiidae) is a bug that mimics *O. smaragdina* during its nymphal instar stages. Since these bugs occur in sympatry with *Myrmarachne*, an encounter between the two in nature is possible. In this study *Myrmarachne* has shown to be able to differentiate between the *R. serripes* nymphs and the green ants. This was concluded from the fact that *Myrmarachne* reacted to the two animals (and a control group) at different frequencies. If *Myrmarachne* would have shown the same reactions at the same frequencies to the *R. serripes* nymphs and *O. smaragdina*, perhaps the differences between the two insects would not have been apparent to *Myrmarachne*. However, *Myrmarachne* carried out more avoidance reactions towards the ant, and reacted less in general when faced with a *R. serripes* nymph. With the ant, there also was a higher TMI, which was mainly due to the fact that the ants move around more energetically than the bugs. All these differences between *Myrmarachne* faced with *R. serripes* nymphs as opposed to ants lead to the conclusion that *Myrmarachne* does not rely solely on the colour and shape of an organism when reacting to it. Rather, the speed at which the animal moves, or other detailed visual cues may determine how *Myrmarachne* reacts to it. This again points to discriminatory versatility by *Myrmarachne*. The ecological significance of *Myrmarachne*'s discriminatory versatility is that it must avoid ants whenever possible

(for its own survival), but there is no apparent reason for *Myrmarachne* to run away from harmless animals (such as Hemiptera).

The *Myrmarachne* species used in this study all mimic and associate with different ant species that belong to various families. The association was studied in evolutionary terms by comparing the phylogenies of the model ants and the mimicking *Myrmarachne* species. Analyses of the trees showed that there was no coevolution of spiders and ants. The model ants have a longer evolutionary history, independent of the one of *Myrmarachne* species. At one point, one of the *Myrmarachne* species in this study underwent a model switch, breaking away from the usual lineage to mimic a different model. There were also instances duplication, where the mimic speciated along a new lineage of the model ant's tree. The overall trend shows that the rate of differentiation and speciation in *Myrmarachne* is relatively high. This also shows that the strong selection pressure on these Batesian mimics has led to a high rate of speciation.

Some of the *Myrmarachne* species also show polymorphism in colour and patterns, as well as transformational mimicry (juveniles looking different to the adult, possibly mimicking a different species of ant) (Mathew, 1935). This was shown by the grouping of individuals in the phylogenetic trees constructed. In other words, individuals that were thought to belong to separate species turned out to be morphs - or juveniles – of one particular species. The phylogenetic tree also placed a species found in Cairns in between species from Townsville. This means that geographical distance does not imply phylogenetic distance, another characteristic of rapidly speciating taxa such as the genus *Myrmarachne*.

One issue with identifying *Myrmarachne* species is that the dissimilarity of individuals belonging to the same species, as well as the similarity between individuals of different species makes quick identification difficult. Identification is also made difficult by the lack of differentiation of the genitalia, especially the male palps. That is why morphological characters were found to identify the local *Myrmarachne* species. The characters include the number of cheliceral teeth of the males, and the structure of the female epigyne.

Observations in this study also show that the four main *Myrmarachne* species studied have differentiated ecologically. For example, one of the species (*Myrmarachne* sp. F) was found most frequently inside retreats built on the surface of large, waxy leaves. This observation agrees with Jackson's (1982) observations on *Myrmarachne lupata*. The other three species seemed to prefer trunks of trees (mainly eucalypts). When considering their model ant species, the habitat preference makes sense. The model ant of *Myrmarachne* sp. F – *Oecophylla smaragdina* – builds its nests weaving leaves together (usually large, waxy ones) (Hölldobler, 1983), whereas the model ant species of the remaining three *Myrmarachne* species, namely *Opisthopsis haddoni*, *Polyrhachis* near *obtusa* and *Tetraponera punctulata* are more likely to nest in open woodlands, either in the ground or in trees. So the close association of Batesian mimics to their model ant species is supported by *Myrmarachne* living in the habitat preferred by their model ants, as was shown by Edmunds (1978).

The main findings in this study have therefore been the high interspecific diversity and behavioural versatility of *Myrmarachne*, brought about by the strong selection pressure on Batesian mimics. *Myrmarachne* is one of the most speciose genera in one of the

largest spider families (the Salticidae), and considering these facts, relatively little work has been carried out on *Myrmarachne*. The fact that it is such a large and versatile genus means that generalisations based on a few species can be inaccurate when applied to different *Myrmarachne* species. This study has laid the ground works for looking at behavioural interactions between *Myrmarachne* and sympatric ants (as well as other arthropods), and further studies could be constructed to look at discriminatory versatility more in depth. The high selection pressure on *Myrmarachne* means that it is likely to have evolved further interesting behavioural traits to the ones studied here, that would be worth looking at more in depth.

In addition to the behavioural studies, this project has also incorporated a first known attempt to build a *Myrmarachne* molecular phylogeny, and to find out about evolutionary dynamics between the model ant species and the *Myrmarachne* mimics. Since the results of this study showed various evolutionary patterns for *Myrmarachne* species, a study done over a larger geographical scale is likely to produce equally – if not more - interesting results. So to better understand the large-scale evolutionary patterns of this particular mimicry system, a study involving *Myrmarachne* species from more places and their known model ant species would certainly produce rewarding results.

As a model to study the dynamics of salticid-ant mimicry systems, the studies on *Myrmarachne* have shown to be very fruitful. Certainly there are other salticid genera that are ant-mimics, and the comparison of *Cosmophasis* and *Myrmarachne* has shown that *Cosmophasis* behavioural reactions are different enough to those of *Myrmarachne* to be worthy of a new study of their own. However, the large number of *Myrmarachne*

species, and their numerous interspecific differences offer the chance of carrying out interesting and insightful studies on aspects such as their behavioural ecology, biology and evolution.

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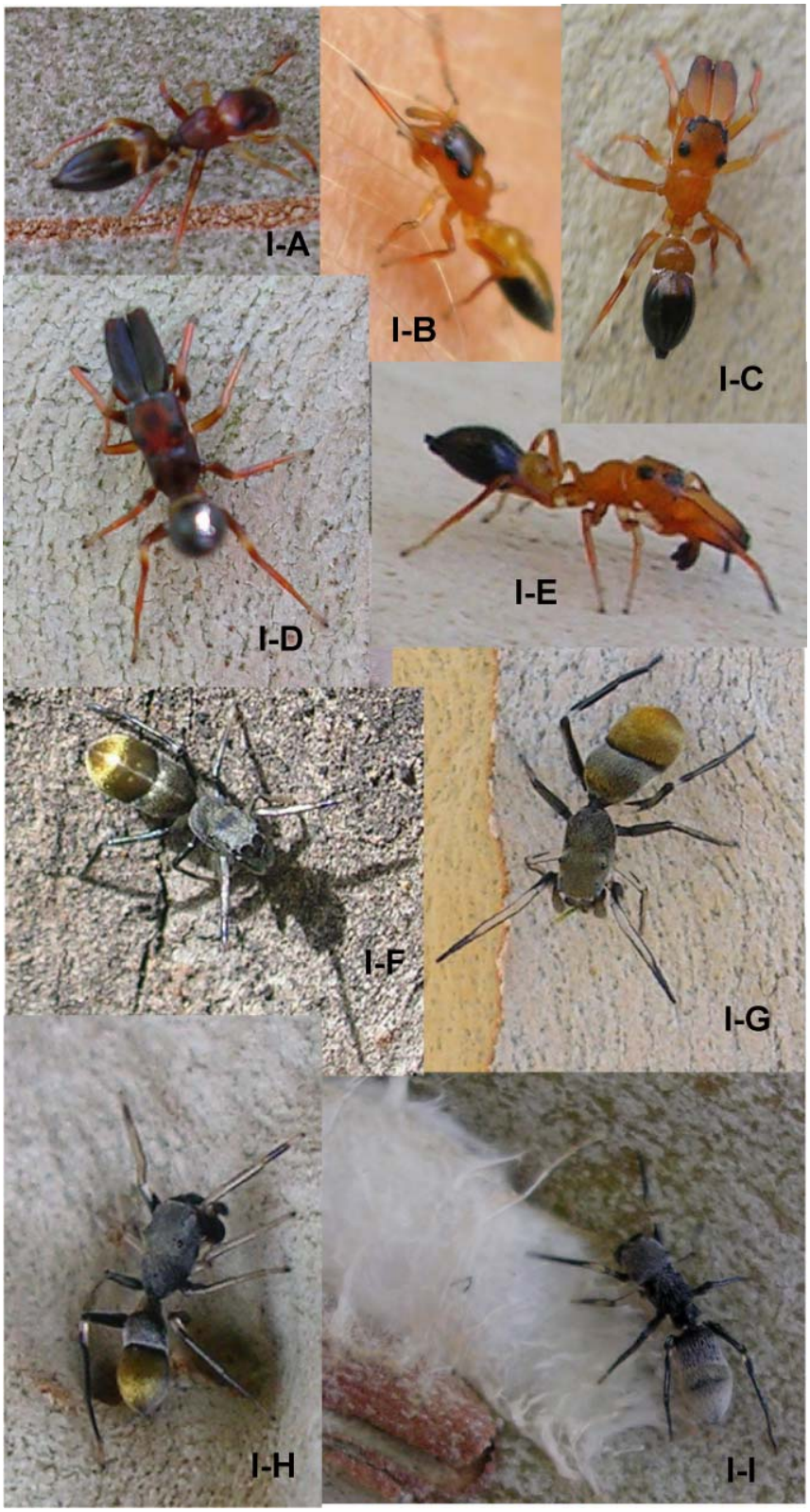
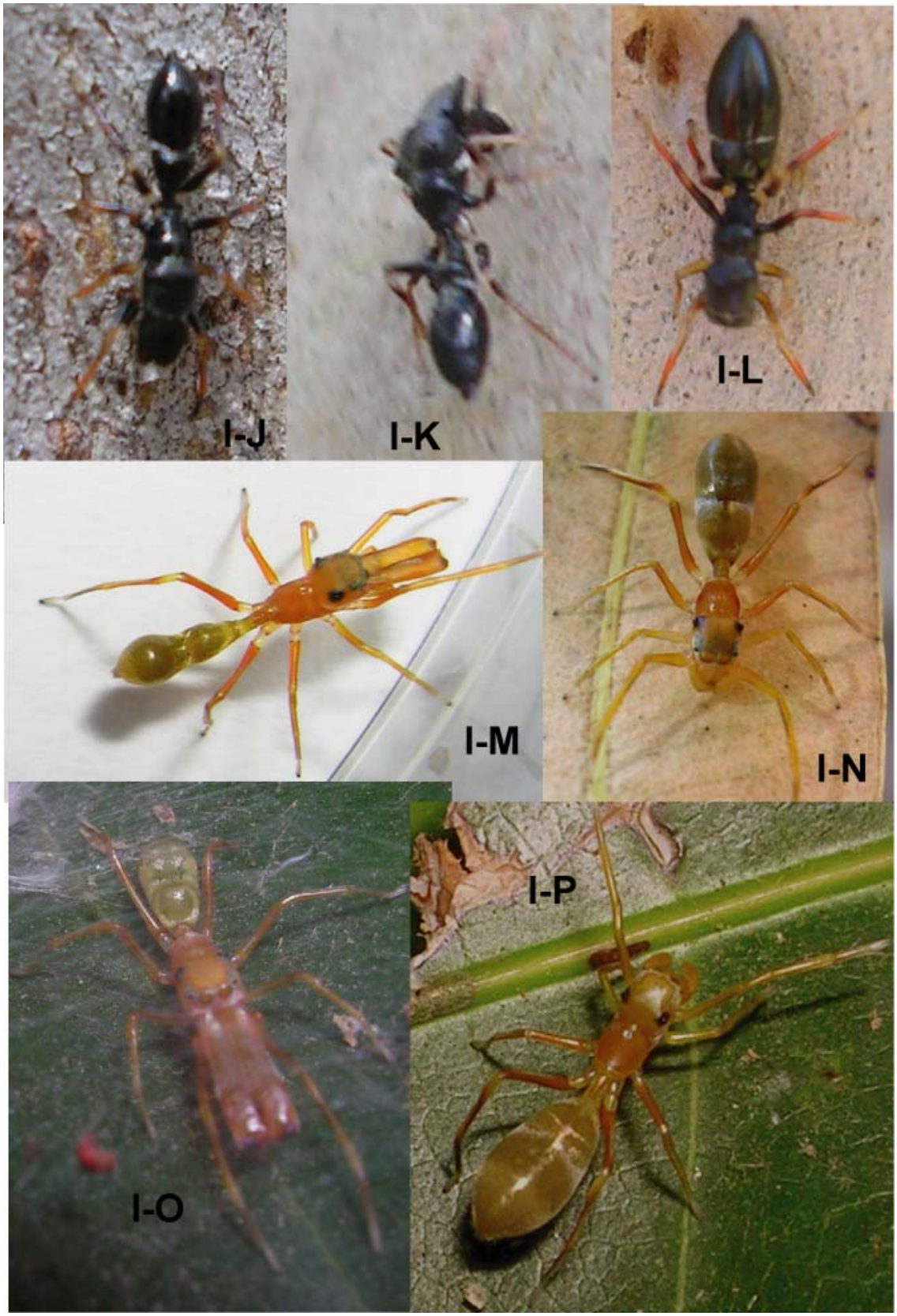
APPENDIX I – **Photographs of *Myrmarachne* species from Townsville**



Appendix I-A – I-I: Photographs of *Myrmarachne* species from Townsville; I-A to I-E: female (I-A and I-B) and male (I-C to I-E) *Myrmarachne rubii* (*M.* sp. A); I-F to I-H: female (I-F and I-G) and male (I-H) *M. aurea* (*M.* sp. B); I-I: female *M.* sp. C. All photographs taken by F.S. Ceccarelli

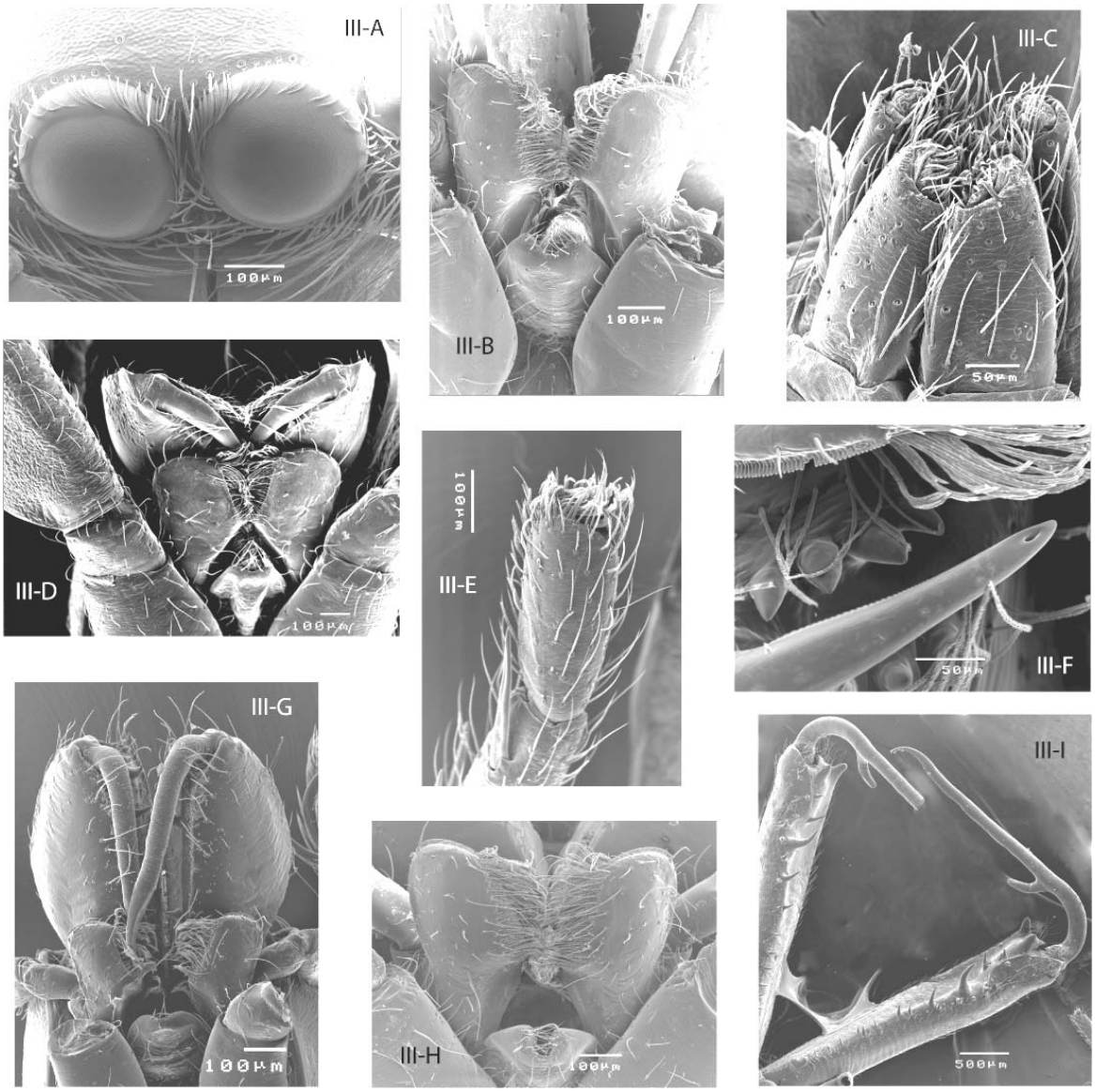


Appendix I-J – I-P: Photographs of *Myrmarachne* species from Townsville; I-J to I-L: male (I-J and I-K) and female (I-L) *Myrmarachne sawda* (*M.* sp. D); I-M to I-P: male (I-M and I-O) and female (I-N and I-P) *M. smaragdina* (*M.* sp. F); male *M. smaragdina* in I-O is inside a sheet-like retreat on a leaf. All photographs taken by F.S. Ceccarelli

APPENDIX II – **Photographs of selected Australian *Myrmarachne* species and their model ants**

Appendix II-A – II-P: *Myrmarachne rubii* (II-A) and model ant *Opisthopsis haddoni* (II-B) , *M.* aurea (II-C) and model ant *Polyrhachis* near *obtusa* (II-D), *M.* sp. C (II-E) and model ant *P. senilis* (II-F), *M. sawda* (II-G) and model ant *Tetraponera punctulata* (II-H), *M. smaragdina* (II-I) and model ant *Oecophylla smaragdina* (II-J), all from Townsville; *M.* sp. E (II-K) and model ant *Podomyrma* sp. (II-L) from Cairns; *M. lupata* (II-M) and model ant *Polyrhachis* sp. (II-N) from lake Tinnaroo (Atherton Tablelands); *M.* sp. (II-O) and model ant *Camponotus* sp. (II-P) from Canberra. All photographs taken by F.S. Ceccarelli.

APPENDIX III – **Scanning Electron Micrographs of *Myrmarachne* features**



Appendix III-A – III-I: Scanning Electron Micrographs of *Myrmarachne rubii* female anterior-median eyes (III-A), *M. rubii* male mouthparts (III-B) showing the maxilla and the labium, *M. rubii* male spinnerets (III-C), *M. aurea* male mouthparts (III-D) showing the chelicerae, maxillae and labium, *M. aurea* male tarsus from leg I (III-E), *M. aurea* female chelicera (III-F) showing some of the cheliceral teeth and the opening of the fang duct, *M. sawda* male mouthparts (III-G) showing the chelicerae, maxillae and labium, *M. smaragdina* female mouthparts (III-H) showing the maxillae and labium, and the chelicerae of *M. smaragdina* male (III-I). Scanning Electron Micrographs taken by Dr. Chris Alexander.