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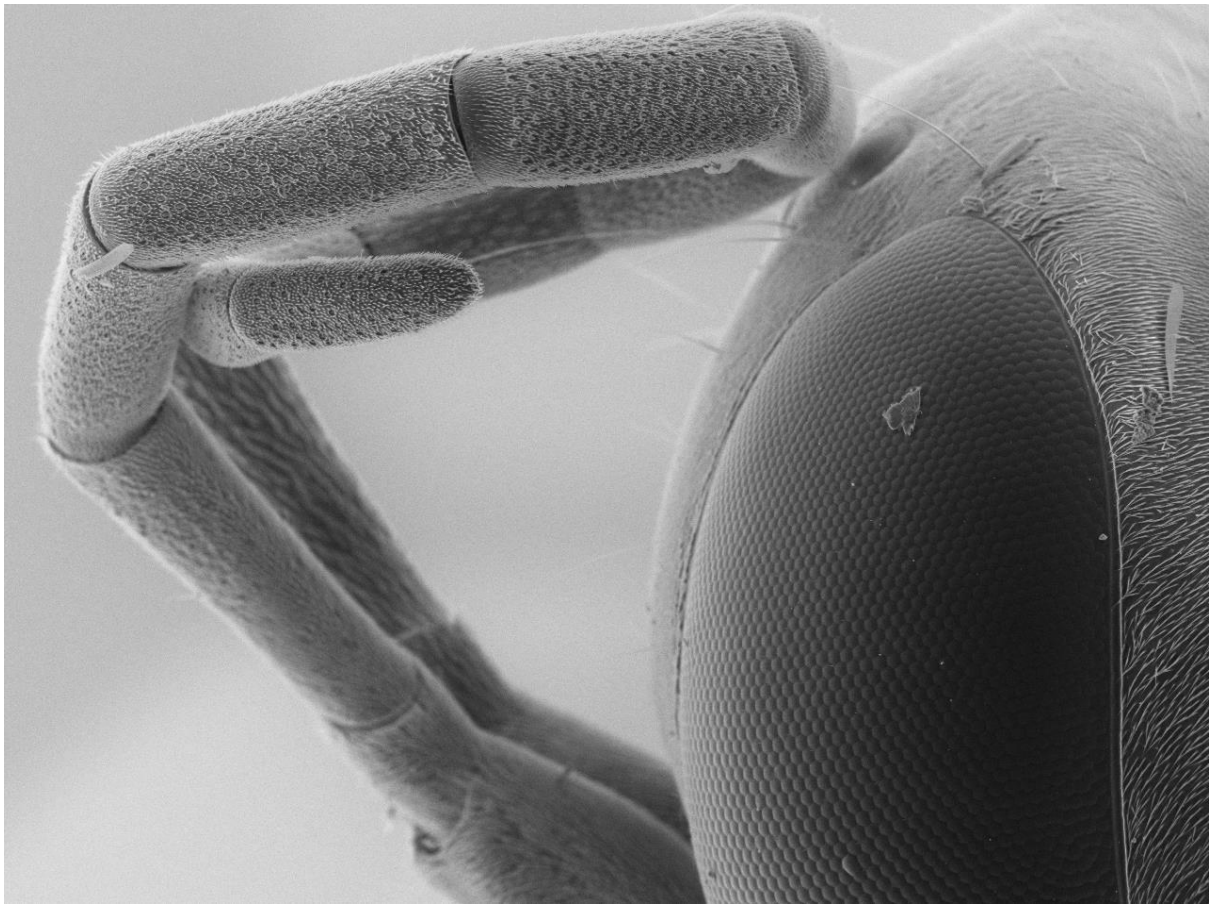
Developed through natural selection: the morphology of insect sensory organs.



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Abstract

All animals must perceive information about their environment, such as the location and nature of potential mates, food sources, shelter, or predators. Detecting this information – which can be in sensory modalities that include vision, olfaction, audition and mechanosensation – requires sensory organs, of which insects have extraordinary morphological diversity. The morphology of sensory organs is shaped by natural selection, being optimised to detect salient signals and cues against background noise in the sensory environment. These selection pressures come from the nature of the signals and cues that must be detected and the complexity of the sensory environment from which information must be perceived.

Obligate interspecific interactions, such as parasitism and mutualism, rely strongly on the detection of signals and/or cues: the interaction can only occur if individuals can identify and locate their parasitic host or mutualistic partner. Despite understanding the signals and cues that underpin many interspecific interactions, little attention has been paid to the sensory organs that the receiver must possess to detect these signals and cues. I explore the influence of parasitism strategy and host ecology on the sensory organ morphology of Pompilid spider wasps, which are formidable parasitoids of spiders. My results show that parasitism strategy and host ecology influence parasite sensory organ morphology in a coevolutionary way: as the host becomes harder to detect, the parasite invests more in sensory organs. In the context of interspecific mutualisms, I explore differences in sensory organ morphology between species of ant-associated (myrmecophilous) and non-ant-associated species of Australian lycaenid butterflies. My comparative analysis reveals that males of obligately myrmecophilous species possess larger eyes but lower densities of olfactory antennal sensilla than non-myrmecophilous species, providing direct evidence that the sensory organs required to detect the cues associated with interspecific mutualism are subject to selection.

The complexity of the sensory environment to which sensory organs are optimised can constrain the development of the sensory organ(s) based upon the availability of information. The availability of light is the most studied of these contexts, although studies of sensory adaptations to

dim-light activity are often taxonomically constrained. By comparing diurnal and dim-light active species across multiple taxonomic insect orders, I find that dim-light activity is consistently associated with larger compound eyes but not with increased or decreased investment in antennae. Beyond the photic environment, captive environments are also likely to influence sensory organ morphology: with a lack of ecological complexity and relaxed selection pressures – such as the absence of predators and abundance of food plants and potential mates – selection is expected to favour reduced investment in sensory organs. I compare sensory organ morphology across wild and captive-bred populations of the critically endangered Lord Howe Island stick insect, finding that individuals from the captive-bred population indeed have smaller compound eyes and a lower density of olfactory antennal receptors.

Together, these four independent studies provide evidence that the signal and cue detection requirements associated with obligate interspecific interactions together with the complexity and constraints of the sensory environment impose strong selection pressures on insect sensory organ morphology.

Declaration

I declare that:

1) This thesis comprises only my original work towards the degree of Doctor of Philosophy except where indicated in the Preface;
2) Due acknowledgement has been made in the text to all other material used; and
3) The thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Christopher B Freelance
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Preface

This thesis was completed under the supervision of Professor Mark Elgar, Professor Bob Wong, and Professor Devi Stuart-Fox.

Chapter 2 of this thesis is unpublished material, and the research was performed as part of a collaboration. I performed 90% of the work, with my and the collaborator's contributions, using the CRediT taxonomy, as follows:

> Christopher B Freelance: Conceptualisation, Methodology, Formal analysis, Investigation, Data Curation, Validation, Writing – Original Draft, Visualisation, Writing – Review & Editing
>
> Juanita Rodriguez: Conceptualisation, Methodology, Resources

Chapter 3 of this thesis is unpublished material.

Chapter 4 of this thesis is the author-accepted version of the manuscript of the article published by *Biological Journal of the Linnean Society* on 12th July 2021:

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> Christopher B Freelance: Conceptualisation, Methodology, Formal analysis, Investigation, Data Curation, Validation, Writing – Original Draft, Visualisation, Writing – Review & Editing
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> Simon M Tierney: Methodology, Resources, Writing – Review & Editing
>
> Juanita Rodriguez: Resources, Writing – Review & Editing
>
> Devi Stuart-Fox: Methodology, Supervision, Writing – Review & Editing
>
> Bob BM Wong: Methodology, Supervision, Writing – Review & Editing
>
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Christopher B Freelance: Conceptualisation, Methodology, Formal analysis, Investigation, Data Curation, Validation, Writing – Original Draft, Visualisation, Funding acquisition, Writing – Review & Editing

Michael JL Magrath: Methodology, Resources, Writing – Review & Editing

Mark A Elgar: Methodology, Writing – Review & Editing, Supervision

Bob BM Wong: Methodology, Writing – Review & Editing, Supervision

Appendix A of this thesis is the Supporting Information for Chapter 2 of this thesis, which is unpublished material.

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Author contributions to the published article are as listed above for Chapter 4 of this thesis, which is the author-accepted version of the manuscript of the article.

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Author contributions to the published article are as listed above for Chapter 5 of this thesis, which is the author-accepted version of the manuscript of the article.

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Chapter 1: Introduction and Synthesis

1.1 The essentiality of sensory organs

An animal's survival depends on their ability to perceive information about their environment. This includes the location and nature of potential mates, food sources, shelter, or predators (Elvidge and Brown, 2012; Prokopy and Owens, 1983; Rosier and Langkilde, 2011; Wyatt, 2014). This information can be in the form of one of multiple sensory modalities including vision, olfaction, audition and mechanoreception. The perception of such information invariably requires organs that can detect information in the given sensory modality. Despite their relatively small size, the insects are unparalleled in the extraordinary morphological diversity of their sensory structures (Elgar et al., 2018).

1.2 Primary sensory organs

The sensory organs of insects are complex and modular. Their primary visual organs are the compound eyes. Each eye comprises multiple ommatidia, with each ommatidium representing a lens that focuses light from a narrow region of the surrounding environment onto photoreceptive cells. These cells pass information into the optic lobes of the brain, where information from adjacent ommatidia is combined to form a visual map with spatial and temporal information (Warrant and Dacke, 2011; Warrant, 2017). The photon capture ability of an ommatidium is directly related to its diameter, with a larger ommatidium having a larger angle of acceptance of light and therefore greater sensitivity to visual information and to changes in luminance in dimmer light (Jander and Jander, 2002; Land, 1997; Tierney et al., 2017). Compound eyes that have fewer but larger ommatidia can detect visual information in lower levels of light but at the expense of visual acuity, whereas compound eyes with more but smaller ommatidia have potentially greater visual acuity but less photon capture ability in lower ambient light (Jander and Jander, 2002; Warrant, 2017). Thus, insects active in low light levels often have eye design and/or neural processing adaptations that increase the visual signal to noise ratio to further increase their ability to perceive visual information (Honkanen et al., 2017; Warrant

and Dacke, 2011; Warrant, 2017). In this way, ommatidia diameter and eye size are useful measures of compound eye morphology that reflect the sensitivity of the eye.

The antennae are the other main sensory organs of insects. Each antenna supports sensilla, which are typically hair- or peg-like in morphology. These either protrude above the cuticle or are housed below the cuticle in a pore or pit. In addition to the physical hair-like or peg-like structure that houses receptors, each sensillum contains one or more sensory neurons that convey information to the antennal lobes of the brain and various supporting cells required to form and support a functional sensillum (Slifer, 1970; Zacharuk, 1980). Some sensilla are olfactory receptors, some gustatory receptors or contact-chemoreceptors, some vibration or stretch receptors, some thermo- and hygro-receptors and some are carbon dioxide receptors. The density (number of sensilla per unit area) of a given type of sensilla influences an individual's behavioural and physiological sensitivity to signals and cues in the respective sensory modality (Elgar et al., 2018; Gill et al., 2013; Spaethe et al., 2007), and is thus an ecologically relevant measure of antennal morphology.

1.3 Natural selection

Like all life history traits, the efficiency of sensory structures – or "organs of sense" – is driven by natural selection in a way that maximises the biological fitness of an organism, as noted by Charles Darwin himself:

> *"As the male has to search for the female, he requires for this purpose organs of sense and locomotion, but if these organs are necessary for the other purposes of life, as is generally the case, they will have been developed through natural selection." (Darwin, 1871)*

Sensory systems are energetically expensive to develop and maintain (Moran et al., 2015; Niven and Laughlin, 2008), and selection will accordingly conserve resources by reducing the size and complexity of sensory organs that are unnecessarily sensitive or that no longer convey information relevant to the fitness of the organism (Elgar et al., 2018). The morphology of, and consequently

energetic investment in, sensory organs is necessarily influenced by both the nature of the signals and cues that must be detected, and the complexity of the background noise in the signalling environment against which those salient signals and cues are perceived (Endler, 1992). Such influences are typically driven by a need for sensitivity to information supporting the sorts of interactions that organisms have with others, or by constraints from the nature of the sensory environment itself (Hansson and Stensmyr, 2011). For example, Halictid bees (Hymenoptera: Halictidae) that have evolved from a social to a solitary lifestyle, and thus no longer need to be sensitive to olfactory signals associated with social behaviour, have a relatively lower density of olfactory antennal sensilla (Wittwer et al., 2017); and the compound eyes of bees that forage in dim light are often composed of larger ommatidia than those of diurnally foraging bees, providing them with greater photon capture ability to allow visual navigation in lower ambient light (Wcislo and Tierney, 2009).

1.4 Interspecific interactions

Almost all animals must interact with conspecific or interspecific individuals during their lives, and so must be sensitive to signals and cues that allow them to detect, identify and respond appropriately. While the use of chemical signals to detect and locate mates is common in many insect species and, accordingly, is often accompanied by a higher density of olfactory antennal sensilla in males than females (Chapman, 1982; Das et al., 2011; Elgar et al., 2018; Fukuda et al., 2016; Gómez and Carrasco, 2008; Handique et al., 2017; Jorge et al., 2019; Ravaiano et al., 2014), signal detection requirements associated with other obligate interactions also influence sensory organ morphology. For example, the link between social behaviour and antennal olfactory sensilla density in Halictid bees (Wittwer et al., 2017) is consistent with previous findings of asymmetry in the distribution of sensilla on the antennae of social but not non-social bees (Anfora et al., 2010; Anfora et al., 2011; Frasnelli et al., 2010) where the antenna with more abundant olfactory sensilla is preferentially used to antennate nestmates. However, the reasons for the aforementioned asymmetry are poorly understood, and factors other than sociality may also drive the evolution of this asymmetry (Freelance et al., 2019). While sociality is an obligate intraspecific interaction, systems of obligate interspecific interaction –

such as parasitism and mutualism – are expected to exhibit similar patterns: for the interaction to occur, at the very least individuals must be sensitive to the signals and/or cues required to identify and locate their parasitic host or mutualistic partner.

1.4.1 Parasitism and parasitoidism

Parasites gain benefit at the expense of a host organism and parasitoids are a specialised type of parasite that complete their larval development on or within a host organism that acts as a food source and eventually dies (Eggleton and Belshaw, 1992; Vinson, 1976). Kleptoparasites are a distinct type of parasite, stealing a resource that has been prepared or acquired by another organism (Iyengar, 2008). The common feature of these relationships is that the parasite/parasitoid benefits at the expense of the host. Importantly, the parasite/parasitoid must be able to locate and assess the suitability of a potential host, which often involves eavesdropping on the host's intraspecific chemical signals (e.g., pheromones) or detecting plant volatile odours induced by the host feeding (Arakaki et al., 1996; Fatouros et al., 2008; Fatouros et al., 2005; Stowe et al., 1995; Vinson, 1976).

Investigations into various parasitic associations have identified the signals and cues enabling these interactions. For example: adults of parasitic lycaenid butterfly species use ant odours as oviposition cues to maximise the chance of their larvae being discovered by workers of an appropriate host ant colony (Fürst and Nash, 2010; Patricelli et al., 2011; Patricelli et al., 2015; van Dyck et al., 2000); female kleptoparasitic bees utilise olfactory signals from social bee colonies to locate and assess the suitability of a nest to parasitise (Cane, 1983; Wcislo, 1995); and parasitoid braconid and trichogrammatid wasps use volatile plant compounds (released when hosts damage the leaves) and the sex pheromones of host species as cues to locate suitable hosts (Chen and Fadamiro, 2007; Cortesero et al., 1997; Reddy et al., 2002; Schöller and Prozell, 2002). Despite the emphasis on the odours that facilitate parasitism, few studies examine the sensory organs that parasitoids must possess to perceive them. Parasitoid braconid wasps with heightened physiological and behavioural sensitivity to plant volatile odours that can indicate the presence of a suitable host (Ngumbi et al.,

2010; Ngumbi et al., 2009) possess a greater density of olfactory antennal sensilla (Bleeker et al., 2004; Das et al., 2011), with host specialists also possessing a higher density of antennal sensilla as predicted by Chapman (1982). Additionally, kleptoparasitic bees have a greater abundance of olfactory sensilla on their antennae than non-parasitic species, reflecting the need to eavesdrop on olfactory signals to locate nests (Galvani et al., 2017). Species of parasitoid chalcid wasps, all of which use olfactory cues to locate hosts, that exhibit host specialisation possess relatively longer antennae (Symonds and Elgar, 2013), but whether these longer antennae possess a higher density of olfactory sensilla is unknown.

Thus, there is empirical support for the prediction that the signal/cue detection requirements associated with parasitism act as selective pressures on the sensory organ morphology of the parasite. However, evidence is predominantly in contexts with little host diversity, which renders of understanding of the association between host ecology and parasite sensory systems superficial. For example, the association between host specialisation and sensory organ morphology in braconid wasps (Das et al., 2011) refers to the number of lepidopteran host species parasitised, with hosts that have little variation in traits such as host plant ecology. Thus, the pattern provides few insights into the influence of cue detection requirements associated with host ecology on parasite sensory systems, despite longstanding recognition of their importance (Chapman, 1982). Similarly, the greater abundance of antennal sensilla in kleptoparasitic bees compared to non-parasitic bees (Galvani et al., 2017) is unsurprising. However, such comparisons provide no insight as to the potential selection pressures associated with kleptoparasitism over other parasitism or parasitoid strategies. This insight would be especially intriguing given that the targets of many kleptoparasitic Hymenopterans are themselves parasites or parasitoids (Evans, 1953). Thus, there are missed opportunities to explore how the signal detection requirements of specific parasitism strategies may be associated with morphological specialisation of the parasite's sensory organs. Such insights have the potential to enhance our understanding of the role of the communicative domain in the evolution and maintenance of parasitic relationships.

Pompilid spider wasps are formidable parasitoids of spiders, using the spider victims as a sole food source for developing larva (Evans, 1953). While all pompilids parasitise spiders, they represent a taxonomic family with considerable diversity in parasitism strategy and host specialisation: in addition to species of 'conventional' parasitoids, some species are kleptoparasites that lay their egg on a spider paralysed by another parasitoid pompilid (Evans, 1953; Nielsen, 1932); some species are host generalists while others specialise on spider with particular ecologies (Evans, 1953; Rodriguez et al., 2016); and there is considerable diversity in the ecological guilds of spider hosts (Cardoso et al., 2011), including web building versus not web building species and diurnal versus dim-light active species. Such differences in strategy and host ecology are likely to translate to diversification in the cues to which parasitoids must be sensitive. Firstly, kleptoparasites need only locate the nest site of another wasp rather than perceive cues revealing the location and suitability of a potential host spider. Secondly, the signal detection requirements for parasitoid species that specialise in spiders from specific ecological guilds (e.g., orb-web building spiders) are likely to depend largely on the sensory ecology that is common to each host spider guild. Examining the relationship between parasite sensory organ morphology and both parasitism strategy and host ecology in this complex study system has the potential to vastly expand our knowledge of the interplay between obligate host-parasite interactions and the morphology of the sensory organs required by a parasite to detect a host.

In Chapter 2 of my thesis, I apply phylogenetic comparative analyses to explore variation in Pompilid wasp sensory organ morphology related to parasitism strategy (kleptoparasitic versus not kleptoparasitic) and host spider ecology. The analysis reveals that kleptoparasites have smaller eyes and lower antennal sensilla density than the other spider wasps, and that the ecology of the host is associated with different investment in the compound eye or antennal sensilla. These patterns not only confirm that parasitism strategy and host ecology exert selective influence on the morphology of a parasite's sensory organs, but also suggest that the relationship between host detection and sensory investment is of a coevolutionary nature: as the host becomes more difficult to detect, the parasitoid requires greater investment in their sensory organs.

1.4.2 Mutualism

Mutualistic interactions are stable relationships between organisms in which each individual reciprocally benefits (Herre et al., 1999). Accordingly, many studies of the mechanisms underlying mutualisms focus on the costs and benefits. For example, in mutualistic associations between ants and aphids, the aphids provide ants with a sugar-rich food source in the form of their honeydew secretions while benefitting by receiving hygiene services to prevent fungal infection and protection from predators (Hölldobler and Wilson, 1990; Way, 1963). A further potential benefit to the aphids is removal of the ants themselves as a probable predation threat in the absence of the mutualism (Stadler and Dixon, 2005). Similarly, in associations between ants and lycaenid butterflies, the butterfly larvae provide ants with sugar-rich honeydew secretions and the butterfly larvae receive critical protection from natural predators (Malicky, 1970; Pierce et al., 2002). Despite these benefits, there is a metabolic cost to honeydew production for both aphids and lycaenid butterfly larvae (Stadler and Dixon, 2005; Stadler et al., 2003). There is also a reduction in dispersal ability for aphids (Yao, 2014) and a cost to lycaenid larvae in the form of specialised organs to modulate the presence of ants (Pierce et al., 2002). The costs to ants come in the form of having to collect and transport honeydew to their colony, and in potentially having to rely on their mutualistic partners as their predominant dietary source of carbohydrates (Stadler and Dixon, 2005).

While net benefit to both organisms is a prerequisite for the evolution of mutualisms, the interaction cannot occur unless the participants can locate and correctly identify each other. While ants are thought to use odours to identify and locate aphids to tend (Müller-Schwarze, 2009), the signals underpinning the widespread symbiosis between myrmecophilous (ant-associated) lycaenid butterflies and their attendant ants are well documented (Pierce et al., 2002). Lycaenid larvae secrete compounds to attract and appease ants via specialised epidermal glands (Malicky, 1970), produce volatile compounds to attract a greater number of attendant ants when distressed (such as in the presence of a natural predator) (Axén et al., 1996), and as pupae produce sounds to recruit more attendant ants when needed (Downey and Allyn, 1973; Hill, 1993; Pierce et al., 2002; Travassos and

Pierce, 2000). Many experiments reveal that this mutualism is largely maintained in ecological time by adult butterflies using their attendant ant species as cues for locating larval host plants and mates (Fraser et al., 2002; Pierce et al., 2002; Pierce and Elgar, 1985).

Despite recognition of the importance of signalling in enabling this symbiosis, less attention has been paid to the sensory organs that adult lycaenids must possess to detect the presence of ants. The necessity for adult myrmecophilous butterflies to detect the presence of an appropriate ant species, in addition to using plant volatile compounds to determine the host plant suitability (Fraser et al., 2002), may result in specialisation of their sensory organs (Fiedler et al., 1996): if ant odours are used as oviposition cues (Casacci et al., 2019; Fraser et al., 2002; Pierce et al., 2002; Pierce and Elgar, 1985; Wagner and Kurina, 1997), myrmecophily is likely to be associated with a higher density of olfactory sensilla on the antennae of myrmecophilous lycaenids. Given the energetically expensive nature of sensory systems (Moran et al., 2015; Niven and Laughlin, 2008), the potential need for sensory organ specialisation to enable myrmecophily may represent an energetic cost to lycaenid butterflies. Consequently, an understanding of the morphology of the sensory organs required to detect the signals enabling such symbioses is necessary to create a more complete picture of the communicative domain associated with systems of interspecies mutualism. Additionally, such an understanding may provide another dimension to the costs – through increased investment in sensory organs – associated with maintaining those relationships.

By applying phylogenetic comparative analyses to examine differences in the sensory organs between myrmecophilous and non-myrmecophilous adult lycaenid butterflies, I discovered that obligate myrmecophily is associated with larger compound eyes but lower olfactory antennal sensilla density in male butterflies, but not in females (Chapter 3). This unexpected pattern is likely related to those males using visual cues rather than ant-derived odours for mate location, and to obligately myrmecophilous females relying primarily on ant odours rather than on both ant-derived and plant-derived odours as oviposition cues and thus not requiring a higher density of antennal sensilla. Whilst

the pattern observed was unexpected, these results provide the first direct evidence that the sensory organs required to detect the signals associated with interspecific mutualism are themselves subject to selection. These findings emphasise the importance of examining both the signals used and the sensory organs that receive them to develop a more complete understanding of the communicative domain that facilitates mutualistic interactions.

1.5 Complexity of the sensory environment

In addition to selection for high sensitivity to the sorts of signals and cues an organism must detect to support appropriate intraspecific and interspecific interactions, the morphological diversity of insect sensory organs likely reflects the developmental constraints posed by their physical environment (Hansson and Stensmyr, 2011). The ecology of the signalling environment can constrain the development of the sensory organ(s) based upon the availability of information in the associated sensory modality. For example, the absence of light in the environment inhibits the development of eyes, as there is no selective advantage to possessing visual organs in the absence of visual information (Fong et al., 1995). Similarly, an increased abundance of volatile chemicals in the environment necessitates possession of antennal sensilla with greater sensitivity to compounds of biological relevance (Hansson and Stensmyr, 2011), as antennae with enhanced sensitivity to salient information have a selective advantage against a background of increased sensory complexity. Regardless of the specific context, any tightening or loosening of the constraints that the physical environment places on the ability of an organism to use information in a given sensory modality is expected to be reflected in differences in the morphology of the relevant sensory organs, with the efficiency of those sensory organs being optimised to the specific signalling environment (Elgar et al., 2018; Endler, 1992).

1.5.1 The photic environment

The availability of light is arguably the most studied context of environmental constraints in relation to the evolution of sensory systems. The Mexican cave fish *Astyanax mexicanus* provides the classic, and arguably most extreme, example of adaptation to the photic environment. Specifically,

populations living in lightless caves no longer have functional eyes, while eyes are retained in populations that remain on the surface (Dowling et al., 2002; Moran et al., 2015). This is consistent with predictions that natural selection will divert resources by eliminating or reducing the size of sensory structures that are inefficient or no longer detect information that contributes to fitness (Endler, 1992). For example, in an environment characterised by the complete absence of light, the inability to detect visual information will favour diversion of energetic resources from functional eyes into other traits that do contribute to the fitness of the individual (Fong et al., 1995).

Adaptations to cave-dwelling extend beyond reduced investment in visual sensory organs. The troglomorphy of cave-dwelling arthropods is typically characterised not only by a reduction in eye size, but also by elaborated antennae and other sensory structures for non-visual information (Christiansen, 2012; Hobbs III, 2012; Jones and Culver, 1989; Peck, 1973, 1977, 1998), reflecting their increased reliance on olfactory, auditory and/or mechanosensory information in the absence of visual information. Similar adaptations have been experimentally produced in the "dark-fly" line of *Drosophila melanogaster* (Diptera: Drosophilidae) which has been raised in constant darkness for over 1,500 generations (Izutsu et al., 2016). Compared to *D. melanogaster* experiencing a natural light/dark cycle, individuals from the dark-fly line demonstrate higher rates of protein-damaging nonsense mutations in genes encoding a type of rhodopsin, a pigment involved in light detection, accompanied by more mutations in a gene related to olfactory detection – corresponding to higher genetic diversity in the trait (Izutsu et al., 2012). These different functional genetic changes suggest adaptation to the altered sensory environment with increased investment in a more useful trait – olfactory sensitivity – and divestment in the less useful trait – light detection (Izutsu et al., 2016; Izutsu et al., 2012). These functional genetic changes are accompanied by changes in the volume of the optic and antennal lobes of the brain, with dark-fly individuals having relatively larger antennal lobes and smaller optic lobes; this pattern was reversed over generations in a population of dark-fly that was returned back to a natural light/dark cycle (Özer and Carle, 2020). While this work provides direct evidence for sensory system adaptation driven by characteristics of the photic environment, it does so only in the context

of an artificially altered environment with a complete absence of light. Additionally, it is not known whether these genetic alterations and brain volume changes are accompanied by changes in morphological investment in, and thus the sensitivity of, the associated sensory organs themselves.

Beyond adaptations to environments devoid of light, sensory organ adaptations to dim-light environments have also been documented. Dim-light (crepuscular and nocturnal) foraging Hymenopteran insects, including bees (Jander and Jander, 2002; Wcislo and Tierney, 2009) and leafcutter ants (Moser et al., 2004), have relatively larger ocelli – photoreceptive organs implicated in flight control (Moser et al., 2004) – compared with their diurnal counterparts. These enlarged organs enhance photon-capture ability (Greiner et al., 2007; Tierney et al., 2017) and are thus considered to have evolved from selective pressures related to low ambient light during peak activity. Similar adaptations of the compound eye have also been observed: compound eye ommatidia diameter is greater in dim-light foraging species compared with diurnal species of wasps (Warrant, 2008), bees (Jander and Jander, 2002; Wcislo and Tierney, 2009) and Myrmecine ants (Greiner et al., 2007; Narendra et al., 2011), and in night-flying species of leafcutter ants (Moser et al., 2004) and Onitine dung beetles (McIntyre and Caveney, 1998).

Similar to that seen in troglomorphic arthropods and dark-fly *Drosophila*, there is also evidence of adaptation of non-visual sensory organs to dim-light activity. The diurnal hawkmoth *Macroglossum stellatarum* (Lepidoptera: Sphingidae) preferentially uses visual cues, whereas the nocturnal species *Deilephila elpenor* preferentially uses olfactory cues and possesses a greater abundance of olfactory sensilla on their antennae (Balkenius et al., 2006). Similarly, nocturnal Myrmecine ants possess a greater abundance of some types of antennal sensilla compared to diurnal species (Ramirez-Esquivel et al., 2014). While cave and dark-fly environments are characterised by an absence of light rather than by the low levels of ambient light experienced by dim-light active insects in nature, insects with compound eye adaptations to dim-light activity often still exhibit lower spatial and temporal visual sensitivity than their diurnal counterparts (Stöckl et al., 2016b; Warrant and

Dacke, 2011; Warrant, 2008, 2017). Such species may similarly benefit from increased sensitivity to non-visual signals, assuming the salient information is available, and so it is possible that the antennal adaptations to dim-light activity seen in hawkmoths and Myrmecine ants are taxonomically more widespread. Contrary to this pattern, nocturnal fireflies (Coleoptera: Lampyridae) possess relatively shorter antennae than diurnal species (Stanger-Hall et al., 2018), but the relationship between antenna length and the density of the antennal sensilla is unknown.

Despite the predicted eye and antennal adaptations being observed in dim-light active insects, such investigations are taxonomically constrained to dim-light active Coleoptera, Hymenoptera and Lepidoptera. Consequently, it is unclear whether these patterns of adaptation hold true across insects more broadly. Moreover, very few studies have investigated antennal morphology in the context of adaptation to dim-light activity, and those that do typically examine the antennae in isolation of the eyes. This not only limits our understanding of the potential generality of non-visual sensory adaptations to dim-light activity but overlooks crucial potential relationships in terms of relative investment between the sensory organs for different sensory modalities.

I address these missing insights by performing a comparative analysis that compares the morphology of both the eyes and antennae of diurnal and dim-light active species across multiple taxonomic insect orders (Freelance et al., 2021b; Chapter 4). This approach reveals that dim-light activity is associated with larger ommatidia and a larger compound eye by surface area, but not with increased (or decreased) investment in antennal sensilla. These findings provide support to predictions that the larger ommatidia and compound eyes typically observed in dim-light active Coleoptera and Hymenoptera would be a hallmark of dim-light activity across all insect taxa, but do not support consistent non-visual adaptations to dim-light activity across taxa. Adaptations of non-visual sensory organs to dim-light activity, such as seen in hawkmoths (Balkenius et al., 2006) and Myrmecine ants (Ramirez-Esquivel et al., 2014), are instead likely to occur only in taxa where there is salient non-visual information available (see Chapter 4).

1.5.2 The captive environment

Captive environments are typically benign compared with natural environments. The lack of ecological complexity and absence of natural selection pressures, such as predation pressure (Mason, 2010), means that populations maintained in these environments frequently exhibit adaptations to captivity that may be maladaptive in natural environments (Lewis and Thomas, 2001; Sutherland, 1998). Studies of insect adaptations to captive environments focus almost exclusively on reproductive (Frankham and Loebel, 1992; Joron and Brakefield, 2003; Lewis and Thomas, 2001; Woodworth et al., 2002) and digestive (Dojnov et al., 2012) traits or wing size (for winged insects) (Lewis and Thomas, 2001). Sensory organ adaptations to such environments are rarely, if ever, investigated.

The relaxed selection pressures associated with captive environments include the reliance on visual and olfactory information. In particular: the absence of predators removes the need to be sensitive to cues indicating the presence of a potential predator; the ready access to food and shelter reduces the need to locate resources; and the reduction in the maximum distance between potential mates reduces the reliance on location-revealing sex pheromones. Additionally, captive environments typically lack stimuli that provide non-salient information – such as odours from non-food plants – that would be present in the wild and thus vastly reduce the background complexity from which salient signals must be discerned. With an easing of selection pressures related to both the diversity of signals to which individuals must be sensitive and the complexity of the sensory environment in which they must detect those signals, selection is expected to favour reduced investment in sensory organs (Endler, 1992; Fong et al., 1995; Hansson and Stensmyr, 2011). Accordingly, one study of laboratory populations of *Drosophila* revealed that long-term captivity is associated with a decrease in eye size due to a decrease in the number of ommatidia (Tan et al., 2005), although the sensory composition of that captive environment is unknown. Consequently, it is unclear whether the observed decrease in eye size reflects adaptation to the sensory environment or may instead have other potential explanations.

Understanding the relationship between captivity and insect sensory organ adaptations has never been more important: as the number of invertebrate species being listed as threatened is increasing (IUCN, 2021), so too is the role of captive breeding programs in invertebrate conservation (Dojnov et al., 2012; Holwell and Andrew, 2015; Honan, 2007; Leather et al., 2008; Pearce-Kelly et al., 1998; Stringer and Chappell, 2008). Founded with individuals from wild populations, conservation captive breeding programs provide a benign environment with the view to establishing insurance populations. Such populations can be used to supplement the numbers of dwindling wild populations of the threatened species, or to reintroduce an extirpated species to the wild following the mitigation of extinction drivers (IUCN/SSC, 2013; Jakob-Hoff et al., 2015; Seddon et al., 2007).

In the context of conservation, the consequences of sensory organ adaptations to captive breeding environments could have dire consequences. Sensory organs that have evolved in captivity are unlikely to be optimised to the sensory complexity of the natural habitat of that species, and so the viability of captive bred populations released into the wild for species reintroduction is likely to be suboptimal. Thus, evaluating whether sensory organ adaptations to captivity occur has the potential to greatly improve the success of species reintroduction programs for threatened species of invertebrates, as sensory ecology has long been ignored in conservation program design (Lim et al., 2008). Furthermore, exploring these potential adaptations presents a valuable opportunity to broaden our understanding of the influence of relaxed selection pressures on the evolution of sensory organ morphology.

In Chapter 5 of my thesis, I compare the morphology of the compound eyes and antennae of individuals from wild and captive-bred populations of the iconic critically endangered Lord Howe Island stick insect *Dryococelus australis* (Phasmatodea: Phasmatidae). These analyses reveal that individuals from the captive-bred population have both smaller compound eyes with smaller ommatidia and a lower density of olfactory antennal sensilla, supporting predictions that relaxed selection pressures in the simplified, sensory depauperate captive environment may lead to reduced

investment in sensory organs (Freelance et al., 2021a; Chapter 5). These data highlight the importance of considering sensory ecology in conservation program design (Lim et al., 2008). More broadly, this chapter provides empirical support for the notion that constraints of the physical environment influence the evolution of sensory organs.

1.6 Eyes versus antennae: inverse resource allocation or complimentary investment?

Predictions around the relationship between morphological investment in sensory organs and selection pressures posed by signals or the sensory environment in a given sensory modality have a strong theoretical and empirical foundation (Elgar et al., 2018; Endler, 1992). In contrast, predictions about the relative investment between the organs for different sensory modalities are less clear. Two models of approaching relative investment in sensory organs prevail: inverse resource allocation, and complementary investment.

There is speculation that relative investment in sensory organs for different sensory modalities can be explained by inverse resource allocation driven by life history trade-offs, whereby the finite resources available to an organism dictates that increased investment in one trait comes at the expense of reduced investment in another, resulting in negatively correlated traits (Stearns, 1989). Indeed, the energetically expensive nature of complex sensory systems is recognised as a potential limiting factor in the evolution of such systems (Niven and Laughlin, 2008), and so it is possible that an insect is unable to increase investment in visual and non-visual sensory organs simultaneously (Emlen, 2001; Keesey et al., 2019). It has been suggested that trade-offs between sensory organ investment may explain the troglomorphy – simultaneous reduction in eye size and elaboration of non-visual sensory organs (e.g., antennae) – of cave-dwelling species (Fong et al., 1995; Jones and Culver, 1989). Similar arguments could be made to explain differential investment between eyes and antennae in dark-fly *Drosophila* (Izutsu et al., 2016; Izutsu et al., 2012).

While trade-offs are consistent with the patterns observed, such interpretations have several caveats. Firstly, there is no *a priori* reason to expect sensory organs to trade-off with each other, as it

is possible that investment in sensory systems as a whole may be traded-off against other somatic traits to develop more complex sensory systems (Moran et al., 2015; Niven and Laughlin, 2008). Secondly, none of these studies specifically assess trade-offs, and an inverse relationship in investment between two structures does not necessarily reflect a trade-off. Thirdly, the patterns observed in the context of troglomorphy are easily explained by natural selection pressures related directly to the relevant sensory modalities: in the absence of light and thus the ability to detect visual information, highly sensitive eyes are not adaptive (Fong et al., 1995). Consequently, selection would favour reduced investment in such eyes and increased investment in antennae, driven by increased reliance upon non-visual information (Fong et al., 1995). Potential trade-offs between eye and antenna size are documented in ants (Jelley and Barden, 2021), and Keesey et al. (2019) document widespread inverse resource allocation between visual and olfactory organs in *Drosophila*, which is thought to represent a trade-off between eyes and antennae driven by restricted resource availability during larval development because both of these sensory organs are derived from a single structure. However, it is noted that simple genetic mutations can mirror inverse allocation between eyes and antennae in *Drosophila* (Keesey et al., 2019), and so these studies do not conclusively demonstrate trade-offs. A broader understanding of the contexts in which apparent inverse investment between sensory organs occurs may be a more useful approach to understanding how inverse resource allocation, and potentially life history trade-offs, may influence the morphology of sensory organs.

As opposed to inverse resource allocation, complementary investment is also thought to be a driver of sensory organ morphology. While signals and cues are typically characterised by the primary sensory modality and can be truly unimodal, animals commonly use information in multiple sensory modalities simultaneously when participating in any given interaction or exploring an aspect of their environment (Partan and Marler, 1999; Partan, 2013); although this necessarily assumes that salient information is available in more than one sensory modality. Support for the complementarity view of investment in sensory organs is provided by studies of Myrmecine ants, where dim-light active species exhibit both larger compound eyes and a higher density of antennal sensilla (Greiner et al., 2007;

Narendra et al., 2011; Ramirez-Esquivel et al., 2014). Here, the investment complementarity approach posits that the increased eye size increases visual sensitivity in response to reduced levels of ambient light, and that this is accompanied by increased investment in antennae, as dim-light active species may rely more than their diurnal counterparts on non-visual cues to compensate for the reduced (but not incomplete) ability to use visual information in lower light. The relatively higher density of antennal sensilla and preferential use of olfactory rather than visual cues in nocturnal hawkmoths (Balkenius et al., 2006) can also be interpreted as supporting evidence. Potential complementary investment has also been observed in contexts other than the photic environment: Shiel et al. (2015) investigated potential trade-offs between eye diameter and antenna length in the painted apple moth *Teia anartoides* (Lepidoptera: Lymantriidae), but instead found that male moths with relatively larger eyes also possessed relatively larger antennae. While this finding is consistent with complementary investment, it could also reflect the reliance of these male moths on both visual and olfactory cues for mate location (Shiel et al., 2015), in which case the larger eyes and antennae would be directly related to selection pressures from the signal detection requirements of this species.

While the patterns observed in the above examples are consistent with complementarity between sensory organs, there are caveats. Firstly, a positive correlation does not depend on complementary investment, with increased investment in both eyes and antennae potentially reflecting an unrelated increase in the availability of salient information in both visual and olfactory modalities. Secondly, positive correlations between structures could reflect mutual development from the same imaginal structure (Emlen and Cerisse, 2003): insect eyes and antennae are derived from the same imaginal disc (Keesey et al., 2019). Identifying additional contexts in which positive correlations between morphological investment between eyes and antennae occur, along with any potential signal detection requirements that may explain the increased investment, is essential for understanding how complementary investment may influence the morphology of insect sensory organs.

The availability of evidence to support both the inverse resource allocation and the complementarity approaches to investment in sensory organs suggests that neither approach is likely to be generalisable across all sensory ecologies. Furthermore, the contexts in which each may influence sensory organ morphology is likely to be limited by the signal detection requirements of the organism. In the case of complementarity, selection is unlikely to favour increased investment in a sensory organ in the absence of salient information in the given sensory modality, as an unnecessarily sensitive organ is not optimised to the sensory environment. Similarly, in the case of inverse resource allocation, selection is also unlikely to favour decreased investment in a sensory organ if information crucial to fitness is present in that sensory modality, as reduced sensitivity to such information would be detrimental to an organism's fitness. Nonetheless, inverse resource allocation and complementarity represent potential drivers of investment in sensory organs and understanding more thoroughly in which circumstances they may influence sensory organ evolution is important for broadening our knowledge of the relationship between selective pressures and sensory ecology. Simultaneous examination of the morphology of sensory organs for multiple sensory modalities, which is typically lacking from most studies to date, in a broader range of contexts and in a greater variety of insect taxa is necessary to generate this insight.

My thesis research, which examined the variation in both compound eye and antennal morphology across different contexts and insect taxa, demonstrates no consistent pattern of increased investment in one sensory organ (e.g., compound eye) being accompanied by decreased investment in another (e.g., antennae). This suggests that inverse resource allocation is not a prominent driver of sensory organ investment. On the other hand, I also do not observe the consistent, positive correlations in investment in olfactory and visual sensory organs across different contexts required to support the complementary investment model. In the contexts where I observe increased investment in one sensory organ and decreased investment in another (Chapters 2 and 3) and either increased or decreased investment in both the compound eyes and antennae (Chapters 2 and 5), these patterns are readily explained by natural selection pressures related directly to the

salient information available in the relevant sensory modalities. This indicates that the availability of salient information in a sensory modality, rather than inverse resource allocation or complementary investment, is the predominant driver of morphological investment in insect sensory organs.

1.7 Summary of thesis organisation and niche

Our limited understanding of how sensory organ morphology is shaped by key obligate interspecific interactions or by prominent environmental characteristics presented an opportunity to investigate how selection pressures associated with signal detection shape the relative investment in the receptor organs for various sensory modalities. Accordingly, my thesis used a combination of population sampling and phylogenetic comparative analyses to explore:

i. The association between sensory organ morphology and both parasitism/parasitoidism strategy and host ecology in Pompilid spider wasps.
ii. The association between sensory organ morphology and myrmecophily amongst Australian Lycaenid butterflies.
iii. The association between sensory organ morphology and the photic environment (diurnal foraging vs dim-light foraging) across multiple taxonomic orders of insects.
iv. The association between sensory organ morphology and long-term captive breeding, using the conservation captive breeding program for the critically endangered Lord Howe Island stick insect *Dryococelus australis* as a study system.

These independent studies adopt a similar theme of exploring the associations between signal detection requirements, the complexity of the sensory environment, and the morphology of the sensory organs required to detect signals. Additionally, the findings of all of these studies suggest that it is predominantly signal detection requirements and constraints of the physical sensory environment, rather than life history trade-offs or complementary investment, that impose strong and pervasive selection pressures on sensory organ morphology.

1.8 Potential practical applications

Beyond providing insights to the specific study contexts and to more broad theories of sensory organ evolution, my approach has value for predicting whether, and how, sensory organ investment, signal perception ability, and thus communication and other associated behaviours, may be altered in insect populations experiencing environmental disruption. Insect communities rely upon effective signalling and communication for the efficient performance of the activities that underpin ecosystem services (Dyer, 2002; Elizalde et al., 2020), and the insights from my research may also contribute to our management of the ecosystem services provided by our planet's abundant insect communities (Dunn, 2005; Elizalde et al., 2020). Specifically, my chapters exploring the relationship between sensory organ morphology and engagement in parasitism (Chapter 2) and in mutualisms (Chapter 3) improve our understanding of the communicative domains that facilitate obligate interspecific interactions. As parasites and parasitoids are used as forms of biological pest control (Waage and Hassell, 2009; Wang et al., 2019), those insights may have implications for such control programs by identifying favourable sensory modalities that could be exploited to artificially increase recruitment of parasitoids. Understanding the influence of the photic environment on sensory organ morphology (Freelance et al., 2021b; Chapter 4) may inform predictions of how insect communities will respond to the penetration of artificial light at night into the night-time environment, which is an anthropogenic disruption of growing concern with the potential to exert a selective influence on communication and sensory systems (Hopkins et al., 2018; Tierney et al., 2017). Beyond answering long-neglected calls to consider sensory ecology in the design and assessment of conservation strategies (Lim et al., 2008) the results of Chapter 5 exploring associations between sensory organ morphology and captive breeding will contribute to developing practical protocols for managing the growing number of invertebrate conservation breeding programs (Freelance et al., 2021a). In this time of pervasive global ecological change, it is perhaps these applications that provide the most meaning to the insights my thesis research contributes to understanding how, as Darwin (1871) eloquently expressed, "organs of sense…have been developed through natural selection".

Chapter 2: The association between sensory organ morphology, parasitism strategy, and host ecology in Pompilid spider wasps

2.1 Abstract

Parasitoids often rely on chemical cues to locate and identify potential hosts, and antennal morphology in parasitoids can play a key role in host location. Pompilid spider wasps are remarkably diverse in both parasitoid strategy and host ecology: some species are kleptoparasites of other spider wasps, some specialise as parasitoids of spiders with particular ecological characteristics, while yet others show little host specificity. Pompilid wasps provide an excellent opportunity to explore how kleptoparasitism and parasitoid host specialisation are associated with sensory organ morphology that is optimised to detect the signals required to identify hosts. My results reveal that kleptoparasites have smaller eyes and a lower density of some antennal sensilla, and that being a parasitoid of web-building spiders only or of nocturnal spiders only is associated with different densities of antennal sensilla. Taken together, these findings suggest that the cue detection requirements associated with parasitism act as a selection pressure on the morphology of the sensory organs that detect those cues. This emphasises the importance of considering the sensory ecology of the host when making predictions about the direction of morphological specialisation that might be observed in relation to different parasitism or host choice strategies.

2.2 Introduction

The ability to locate a suitable host is essential for parasitoids, as the development of their offspring wholly depends on the host as a food resource (Eggleton and Belshaw, 1992). Thus, adult parasitoids must accurately and reliably detect signals and/or cues that allow them to locate potential hosts, either by directly detecting the host or by using indirect cues, such as plant volatiles, that indicate the potential presence of a host. The nature of these signals and cues creates selection pressures on the sensory organs of the receiver, and the morphology of these organs is predicted to be optimised to

detect salient information from amongst the background noise in the receiver's signalling environment (Endler, 1992).

Evidence suggests a critical link between parasitism and sensory organ morphology in Hymenopteran parasitoids. Kleptoparasitic bees (Hymenoptera: Apidae) utilise olfactory cues to detect nests for oviposition and possess a higher abundance of olfactory antennal sensilla than non-parasitic bees (Galvani et al., 2017). Species of braconid wasps (Hymenoptera: Braconidae) that exhibit host specialisation have a higher density of olfactory sensilla on their antennae compared with closely related generalist parasitoids (Bleeker et al., 2004; Das et al., 2011). The density of olfactory sensilla in two species of these wasps is positively associated with the ability to detect and respond to plant volatile odours (Ngumbi et al., 2010; Ngumbi et al., 2009) which may be induced by the presence of hosts on a plant, thus suggesting a link between olfactory sensilla density and use of olfactory cues for host detection. Antenna length in chalcid wasps (Hymenoptera: Chalcidoidea), which use chemical signals to detect hosts, is greater for species that parasitise Hemiptera (Symonds and Elgar, 2013), but whether these larger antennae possess a higher number of olfactory sensilla is unknown.

The diverse family of pompilid spider wasps (Hymenoptera: Pompilidae), with some 5,000 species, are formidable parasitoids of spiders. Female spider wasps typically paralyse a single spider by venom then transport it to the site of a nest that can be built before or after capturing the spider (Evans, 1953; Iwata, 1942; Nielsen, 1932). A single egg is laid on the spider, which is enclosed in the nest, and the larva obtains all of its nourishment from the spider (Evans, 1953). Many spider wasps are generalists and parasitise a diversity of spiders, while other species specialise on spiders with particular ecological characteristics (Evans, 1953; Rodriguez et al., 2016). Intriguingly, some species are kleptoparasites of other pompilids: rather than capture spiders, they oviposit on spiders that have already been paralysed by other species of spider wasp (Evans, 1953; Iwata, 1942).

These different parasitism strategies likely create a diversity of signals and cues used by spider wasps to locate suitable hosts, and thus may influence the morphology of the wasps' sensory organs.

For example, kleptoparasitic chrysid wasps visually orient to the site of parasitoid wasps digging nests that could be parasitised once provisioned with a spider (Rosenheim, 1987). Field observations of a kleptoparasitic pompilid spider wasp suggest the use of visual cues to orient to and remember the location of potential host nests (Wcislo et al., 1988) amongst these brood parasites. Thus, the eyes of kleptoparasites, which visually orient to potential host nests, may be larger and/or consist of a higher number of ommatidia compared with non-kleptoparasitic spider wasps, resulting in comparatively higher visual acuity (Jander and Jander, 2002; Warrant, 2017). The ecology of host spiders parasitised by spider wasps may also act as a selection pressure on spider wasp sensory morphology. For example, while many Hymenopteran predators and parasites use visual cues to locate web-building spiders (Elgar and Jebb, 1999; Powell and Taylor, 2017; Richter, 2000), wasps parasitising cursorial spiders may additionally use non-visual cues, such as odours, to locate hosts that are concealed in the vegetation. Indeed, many predatory wasps visually localise an area in which to search, and then utilise olfactory information to zero in on the prey item (Richter, 2000). Like other parasitoid wasp species (Bleeker et al., 2004; Das et al., 2011), the signal detection requirements associated with specialisation in host ecology may favour sensory organ morphology that is optimised to differentiating those cues within the sensory environment. Pompilid wasps provide a novel opportunity to explore, within a single taxonomic family, how the signal detection requirements of various parasitism and host choice strategies influence the morphology of the sensory organs required to detect the signals and cues used for host location and identification.

Studies that explore associations between host specialisation and Hymenopteran parasitoid sensory organ morphology focus on systems with little diversity in host ecology. In contrast, the hosts of spider wasps vary in both ecological and life history traits, which may influence host-locating cues. I expect three traits of the spider ecological guilds noted by Cardoso et al. (2011) to be particularly important in this context: (i) whether host spiders are active during the day, night, or both; (ii) whether the host spiders build webs; and (iii) whether spiders are located on the ground or in vegetation. These traits that differ across ecological guilds reflect differences in the difficulty of visually locating spiders,

which is likely to influence spider wasp sensory organ morphology: (i) insects active at night tend to have larger compound eyes (Freelance et al., 2021b) to increase visual sensitivity in low ambient light, but the nocturnal prey may be more difficult to detect visually; (ii) the silk used to construct spider webs can provide olfactory cues (Henneken et al., 2017) and the web itself may provide both visual (silk decorations) and/or olfactory (prey remains) cues (Bjorkman-Chiswell et al., 2004; Herberstein et al., 2000; Walter and Elgar, 2012) in addition to the spider also potentially having conspicuous body coloration (Oxford and Gillespie, 1998); and (iii) vegetation may offer different levels of camouflage to the spiders than the ground, thereby influencing the host search image in a way that may require increased visual sensitivity or greater reliance on non-visual cues to locate a host (Richter, 2000).

In the present study, I ask whether parasitism strategy or host spider specialisation is associated with morphological specialisation of the sensory organs in pompilid spider wasps, capitalising on their remarkable diversity in both parasitism strategy and host ecology. I expect that selection will favour increased perceptual acuity, through larger organs, or the use of multiple sensory modalities, when the host is more difficult to find. Ecological circumstances may alter the difficulty of locating the host, and kleptoparasitism may create an antagonistic coevolutionary process in terms of the ability of the wasp to detect nests and the host to conceal their nests. Consequently, I predict that kleptoparasitic spider wasps possess a comparatively larger compound eye and/or higher density of antennal sensilla than species that parasitise a spider directly. Similarly, three predictions arise from host spider specialisation: (i) wasps that are parasitoids of nocturnal spiders only will have larger eyes and/or compound eye ommatidia to enable night flying, and a higher density of antennal sensilla reflecting the challenges of relying on visual cues for host detection in lower light levels; and that a higher density of antennal sensilla and potentially larger compound eyes will be observed in (ii) wasps parasitising spiders that do not build webs and (iii) wasps parasitising only vegetation-dwelling spiders, reflecting increased difficulty of using visual cues to locate a host.

2.3 Methods

2.3.1 Study species selection

I obtained female pinned specimens for 26 species of spider wasps (*N* = 3 per species), for which the parasitism/parasitoid strategy is known, from the Utah State University Entomology Collection. Based on what types of spiders are parasitised by each study species of wasp (J. Rodriguez, personal communication), I used the spider ecological guilds detailed in Cardoso et al. (2011) to classify the host spider ecology of each spider wasp species for the three traits identified in the introduction: (i) where in the light cycle the host spiders are active; (ii) whether the host spiders build webs; and (iii) whether host spiders are located on the ground, in vegetation, or both on the ground and in vegetation. Spider wasps were also categorised according to their host specialisation: wasp species that are parasitoids of only one ecological guild of spider (e.g., orb weaving spiders) are classified as guild specialists while species that parasitise more than one guild of spider are classified as generalists. The above characteristics for each species of spider wasp are listed in Table 2.1.

2.3.2 Sensory organ morphology metrics

Specimens were mounted on an electron microscopy stub and imaged via scanning electron microscopy using a Hitachi TM3030 Plus tabletop scanning electron microscope (15kV acceleration voltage, charge reduction mode) at the Australian National Insect Collection (Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia). From the images, I determined for each specimen the following metrics of sensory organ morphology: surface area of the compound eye (calculated as the area of half a spheroid with semi-axes equivalent to the greatest length and width of the compound eye; mm^2); average diameter of the ommatidia composing the compound eye (diameter of three ommatidium averaged; µm); and the density of each type of antennal sensilla on the dorsal surface of the apical (distal) antennomer (number of sensilla per mm^2 of antenna). Eye size and ommatidia diameter provide information about sensitivity to visual information (reviewed by Warrant (2017)) and the density of antennal sensilla is a behaviourally relevant indicator of sensitivity

to signals in cues in the modality (e.g. olfaction) for which that type of sensilla is a receptor (Elgar et al., 2018; Gill et al., 2013; Spaethe et al., 2007). To account for potential differences in ommatidia diameter between different regions of the compound eye (Perl & Niven, 2016), I measured the ommatidia from the anteromedial aspect (i.e. facing directly in front of the insect) of each compound eye. Both the left and right antennae for each individual were analysed; eyes or antennae that were damaged or had debris obscuring large areas of them were not included. Head width posterior to the eyes was also determined from the micrographs as a proxy measure of body size in Hymenoptera (Freelance et al., 2019). Image analysis was performed using FIJI (Schindelin et al., 2012).

2.3.3 Statistical analysis

To account for non-independence, I used phylogenetic comparative analysis methods to examine the impact of host and parasitoid ecology on spider wasp sensory organ morphology. First, I obtained a phylogenetic tree of my study species by pruning the maximum likelihood phylogenetic tree of Pitts et al. (unpublished) (Figure 2.1) using the APE (version 5.5) (Paradis and Schliep, 2018) package in R version 4.1.0 for Windows (R Core Team, 2021). I assessed the influence of parasitism strategy on sensory organ morphology for each sensory morphology metric by fitting a phylogenetic least squares model with parasitism strategy (kleptoparasitic, non-kleptoparasitic) and head width as fixed effects, with variance partitioned using maximum likelihood.

I assessed the influence of host spider ecology on spider wasp sensory organ morphology by fitting a phylogenetic least squares model with host spider guild specialisation (generalist, specialist), host spider active time (diurnal and nocturnal, nocturnal), host spider web status (webs, no webs), host spider vertical stratification (ground, ground and vegetation, vegetation), and head width as fixed effects, with variance partitioned using maximum likelihood.

Statistical analyses were performed using the PHYTOOLS (version 0.7-80) (Revell, 2012), PICANTE (version 1.8.2) (Kembel et al., 2010) and GEIGER (version 2.0.7) (Pennell et al., 2014) packages in R

version 4.1.0. I discuss all effects with $p < 0.1$ with effect sizes presented, because these effects may still be biologically relevant. Species averages for each sensory morphology are listed in Table 2.2.

Table 2.1. Parasitism strategy and host spider ecological characteristics for each study species of Pompilid wasp. Kleptoparasitic species are not coded with regard to host spider ecology as they do not themselves directly target spiders.

Species	Parasitism	Host spider ecological guilds	Host spider specialisation	Host spider web status	Host spider vertical stratification	Host spider active time
Agenioideus humilis	Non-kleptoparasite	Other hunters, sensing web weavers, ambush hunters, orb web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Ammosphex anomalus	Non-kleptoparasite	Ground hunters, other hunters, ambush hunters	Generalist	No webs	Ground and vegetation	Diurnal and nocturnal
Anoplius fulgidus	Non-kleptoparasite	Ground hunters, sheet web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Arachnophroctonus apiculatus	Non-kleptoparasite	Ground hunters, sheet web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Aporinellus taeniatus	Non-kleptoparasite	Other hunters, ambush hunters	Generalist	No webs	Ground and vegetation	Diurnal and nocturnal
Aporus hirsutus	Non-kleptoparasite	Sensing web weavers	Specialist	Webs	Ground and vegetation	Nocturnal
Arachnophroctonus americanus	Non-kleptoparasite	Ground hunters, sheet web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Anoplochares apiculatus	Non-kleptoparasite	Ground hunters	Specialist	No webs	Ground	Diurnal and nocturnal
Arachnospila scelestus	Non-kleptoparasite	Ground hunters, other hunters	Generalist	No webs	Ground and vegetation	Diurnal and nocturnal
Aridestus bergi	Kleptoparasite	Kleptoparasites	N/A	N/A	N/A	N/A
Episyron conterminus	Non-kleptoparasite	Orb web weavers	Specialist	Webs	Vegetation	Diurnal and nocturnal
Evagetes padrinus	Kleptoparasite	Kleptoparasites	N/A	N/A	N/A	N/A
Gymnochares birkmanni	Non-kleptoparasite	Ground hunters, other hunters	Generalist	No webs	Ground and vegetation	Diurnal and nocturnal
Lophopompilus cleora	Non-kleptoparasite	Ground hunters	Specialist	No webs	Ground	Diurnal and nocturnal

Notiochares amethystinus	Non-kleptoparasite	Ground hunters, other hunters	Generalist	No webs	Ground and vegetation	Diurnal and nocturnal
Paracyphononyx funereus	Non-kleptoparasite	Ground hunters, other hunters	Generalist	No webs	Ground and vegetation	Diurnal and nocturnal
Perissopompilus phoenix	Non-kleptoparasite	Ambush hunters	Specialist	No webs	Ground and vegetation	Diurnal and nocturnal
Poecilopompilus algidus	Non-kleptoparasite	Orb web weavers	Specialist	Webs	Vegetation	Diurnal and nocturnal
Pompilinus marginatus	Non-kleptoparasite	Ground hunters, sheet web weavers, other hunters, ambush hunters, orb web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Pompilus cinerus	Non-kleptoparasite	Ground hunters, other hunters, sheet web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Priochilus regius	Non-kleptoparasite	Other hunters, orb web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Psorthaspis variegata	Non-kleptoparasite	Sensing web weavers	Specialist	Webs	Ground and vegetation	Nocturnal
Ridestus biedermani	Non-kleptoparasite	Space web weavers, sheet web weavers	Generalist	Webs	Ground	Nocturnal
Sericopompilus neotropicalis	Non-kleptoparasite	Ambush hunters	Specialist	No webs	Ground and vegetation	Diurnal and nocturnal
Tachypompil ferrugineus	Non-kleptoparasite	Ground hunters, other hunters, sheet web weavers	Generalist	Webs	Ground and vegetation	Diurnal and nocturnal
Tastiotenia festiva	Non-kleptoparasite	Space web weavers	Specialist	Webs	Ground and vegetation	Diurnal and nocturnal

Table 2.2. Sensory organ morphology metrics for each study species of Pompilid wasp. Head width is measured in mm, ommatidia diameter in µm, eye surface area in mm^2, and antennal sensilla densities as the number of sensilla per mm^2 of antenna. Head width and sensory morphology metrics are species averages.

Species	Head width	Sensory organ morphology metrics							
		Ommatidia diameter	Compound eye surface area	Sensilla trichodea density	Sensilla basiconica density	Sensilla chaetica density	Sensilla placodea density	Sensilla coeloconica density	Sensilla ampullacea density
Agenioideus humilis	1.7	18.5	1.5	21785	703	508	2581	134	75
Ammosphex anomalus	1.7	15.6	0.9	29769	1179	1024	2069	30	30
Anoplius fulgidus	1.9	22.6	2.6	16341	453	402	1844	101	84
Arachnophroctonus apiculatus	2.0	16.0	1.2	19166	283	420	2354	75	131
Aporinellus taeniatus	1.2	12.5	0.6	23993	957	912	1724	259	1076
Aporus hirsutus	1.4	14.9	0.8	23041	1687	1288	3837	140	297
Arachnophroctonus americanus	2.2	18.3	2.5	28622	829	691	2469	47	47
Anoplochares apiculatus	1.6	16.5	1.6	16780	1054	890	1580	36	869
Arachnospila scelestus	2.9	17.7	2.8	19014	1280	776	1352	70	661
Aridestus bergi	1.8	15.9	1.6	23048	1095	765	2336	74	571
Episyron conterminus	2.3	17.2	1.4	15573	407	339	2449	205	135
Evagetes padrinus	1.6	15.7	1.1	19172	430	430	1836	90	62
Gymnochares birkmanni	1.6	18.6	1.7	26699	956	2138	3917	457	1814
Lophopompilus cleora	3.3	23.0	5.3	16183	238	238	1613	70	419
Notiochares amethystinus	3.3	19.1	4.2	18058	173	219	1096	23	139
Paracyphononyx funereus	3.1	26.6	4.3	19926	619	727	1670	57	450
Perissopompilus phoenix	1.8	20.1	1.9	83501	2469	1273	3251	193	420
Poecilopompilus algidus	3.9	22.3	6.1	32358	549	487	3537	0	0
Pompilinus marginatus	1.9	18.1	2.0	23439	950	985	3054	112	485
Pompilus cinerus	1.9	21.0	2.0	20272	365	424	2422	67	67
Priochilus regius	2.7	28.2	4.6	17468	288	317	2529	68	72

Psorthaspis variegata	2.1	22.4	2.5	27868	881	570	1956	57	287
Ridestus biedermani	2.0	18.8	1.9	45422	569	879	2051	86	172
Sericopompilus neotropicalis	2.7	18.2	2.3	19557	556	624	1981	52	468
Tachypompilus ferrugineus	2.7	20.7	3.9	26701	421	414	2309	29	465
Tastiotenia festiva	1.3	15.4	0.5	20549	320	462	2690	244	224

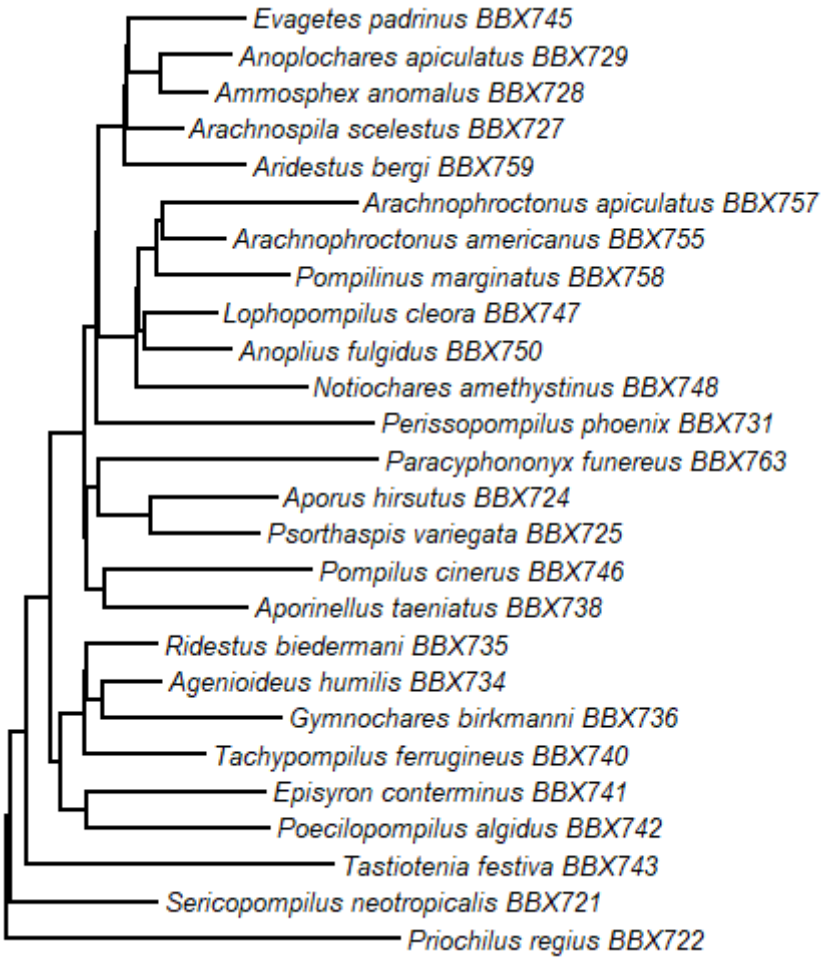

Figure 2.1. Phylogenetic tree of species of Pompilid wasps included in the study. The tree was pruned from the maximum likelihood phylogenetic tree of Pitts et al. (unpublished).

2.4 Results

2.4.1 Antennal sensilla types

I identified six types of antennal sensilla following Wang et al. (2018) and Yang et al. (2018): trichodea (olfactory), basiconica (gustatory/contact chemosensory; possibly olfactory), chaetica (mechanosensory), placodea (olfactory), coeloconica (thermo-/hygro-receptors; possibly olfactory) and ampullacea (CO_2 detectors) (Figure 2.2). Descriptions of the morphological characteristics of each sensillum type identified are in Appendix A.

2.4.2 Parasitism strategy

Kleptoparasitic spider wasps have a significantly smaller eye surface area than their non-kleptoparasite counterparts ($F_{1,23}$ = 4.883, p = 0.037, Cohen's d_s = 0.72; Figure 2.3A; Table 2.3B), but parasitism strategy does not influence ommatidia diameter (Table 2.3A). Head width is positively correlated with both eye surface area (β = 1.922, $F_{1,23}$ = 122.9, p < 0.0001; Table 2.3B) and ommatidia diameter (β = 2.462, $F_{1,23}$ = 10.99, p = 0.003; Table 2.3A).

Kleptoparasites show a trend towards a lower density of sensilla coeloconica on their antennae ($F_{1,23}$ = 3.770, p = 0.065, d_s = 0.27; Figure 2.3B; Table 2.3G); the density of these sensilla also significantly increases as head width decreases (β = –83.61, $F_{1,23}$ = 14.24, p = 0.001; Table 2.3G). There is no influence of parasitism strategy on the antennal densities of any other type of antennal sensilla (Table 2.3C–F, H).

2.4.3 Host spider ecology

Host spider guild specialisation has no effect on the morphology of any sensory organs examined (Table 2.4).

Spider wasps that are parasitoids of web-building spiders have a statistically significantly lower density of CO_2-detecting antennal sensilla ampullacea ($F_{1,17}$ = 9.772, p = 0.006, d_s = 1.29; Figure 2.4A; Table 2.4H) and a trend towards a lower density of mechanosensory sensilla chaetica ($F_{1,17}$ = 3.683, p

= 0.072, d_s = 0.72; Figure 2.4B; Table 2.4E), but a trend towards a higher density of olfactory sensilla placodea ($F_{1,17}$ = 3.650, p = 0.073, d_s = 0.77; Figure 2.4C; Table 2.4F). Variation in spider wasp eye morphology or the density of any other types of antennal sensilla (Table 2.4A–D, G) was not explained by whether the host spiders build webs or not.

Spider wasps that parasitise nocturnal spiders only have a significantly smaller compound eye surface area than their counterparts that parasitise both diurnal and nocturnal spiders ($F_{1,17}$ = 5.268, p = 0.035, d_s = 0.56; Figure 2.4D; Table 2.4B) and trend towards possessing a higher density of antennal sensilla chaetica ($F_{1,17}$ = 3.622, p = 0.074, d_s = 0.54; Figure 2.4E; Table 2.4E). The activity time of host spiders does not influence spider wasp eye ommatidia diameter (Table 2.4A) or the antennal densities of any other types of antennal sensilla (Table 2.4C–D, F–H).

The vertical stratification of host spiders significantly influences the compound eye surface area of spider wasps ($F_{2,17}$ = 8.365, p = 0.003, d_s (ground and vegetation vs. ground-only) = 0.50, d_s (ground-only vs. vegetation-only) = 0.33, d_s (ground and vegetation vs. vegetation-only) = 1.04; Figure 2.4F; Table 2.4B): the mean eye surface area is higher for wasps who parasitise only vegetation-dwelling spiders. Host spider vertical stratification does not influence any other sensory morphology traits examined (Table 2.4).

Eye surface area (β = 2.101, $F_{1,17}$ = 84.54, p < 0.0001; Table 2.4B) and ommatidia diameter (β = 3.808, $F_{1,17}$ = 15.44, p = 0.001; Table 2.4A) increase significantly with head width, while the densities of sensilla chaetica (β = –329.1, $F_{1,17}$ = 7.337, p = 0.015; Table 2.4E) and sensilla coeloconica (β = –116.3, $F_{1,17}$ = 16.94, p = 0.0007; Table 2.4G) decrease significantly as head width increases. Head width does not significantly influence the densities of sensilla trichodea, basiconica, placodea or ampullacea (Table 2.4C–D, F, H).

Table 2.3. Phylogenetic least squares models explaining variation in the ommatidia diameter, compound eye surface area, and antennal sensilla densities of spider wasps in relation to parasitism strategy (kleptoparasite, non-kleptoparasite) and head width (mm). For parameter estimates, lower and upper values represent 95% confidence intervals. *N* = 26 species.

Model/parameter	**Statistics**		
A. Ommatidia diameter			
Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	6.261	11.65	17.04
Parasitism strategy [non-kleptoparasite]	-1.922	2.258	6.439
Head width (mm)	0.926	2.462	3.998
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	2.888	0.103
Head width	1,23	11.00	0.003
B. Eye surface area			
Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	-3.185	-1.926	-0.668
Parasitism strategy [non-kleptoparasite]	-0.828	0.148	1.124
Head width (mm)	1.564	1.922	2.281
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	4.883	0.037
Head width	1,23	123.0	<0.0001
C. Sensilla trichodea density			
Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	-4091	26945	57981
Parasitism strategy [non-kleptoparasite]	-17070	6999	31068
Head width (mm)	-15231	-6388	2455
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	0.126	0.726
Head width	1,23	2.233	0.149
D. Sensilla basiconica density			
Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	153.3	1217	2280
Parasitism strategy [non-kleptoparasite]	-693.3	131.4	956.1
Head width (mm)	-617.1	-314.2	-11.17
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	0.001	0.975

Head width	1,23	4.601	0.043

E. Sensilla chaetica density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	-77.60	715.8	1509
Parasitism strategy [non-kleptoparasite]	-83.94	531.4	1147
Head width (mm)	-515.8	-289.7	-63.68
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	1.850	0.187
Head width	1,23	7.030	0.014

F. Sensilla placodea density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	615.5	2108	3600
Parasitism strategy [non-kleptoparasite]	-430.5	726.7	1884
Head width (mm)	-713.8	-288.6	136.5
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	1.163	0.292
Head width	1,23	1.973	0.174

G. Sensilla coeloconica density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	-29.31	131.5	292.4
Parasitism strategy [non-kleptoparasite]	28.93	153.7	278.4
Head width (mm)	-129.4	-83.60	-37.78
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	3.770	0.065
Head width	1,23	14.24	0.001

H. Sensilla ampullacea density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	-715.7	107.6	931.0
Parasitism strategy [non-kleptoparasite]	-176.0	462.6	1101
Head width (mm)	-375.5	-140.9	93.69
Fixed effects	***df***	***F ratio***	***p > F***
Parasitism strategy	1,23	1.712	0.204
Head width	1,23	1.544	0.227

Table 2.4. Phylogenetic least squares models explaining variation in the ommatidia diameter, compound eye surface area, and antennal sensilla densities of spider wasps in relation to host specialisation (specialist, generalist), host active time (diurnal and nocturnal, nocturnal), host web status (webs, no webs), host vertical stratification (ground, vegetation, ground and vegetation), and head width (mm). For parameter estimates, lower and upper values represent 95% confidence intervals. *N* = 24 species.

Model/parameter	**Statistics**		
A. Ommatidia diameter			
Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	3.936	10.50	17.07
Host specialisation [specialist]	-2.008	0.895	3.799
Host spider web status [web]	-0.872	2.310	5.493
Host spider active time [nocturnal]	-5.584	-1.443	2.699
Host spider vertical stratification [ground and vegetation]	-3.868	-0.344	3.180
Host spider vertical stratification [vegetation]	-12.61	-5.919	0.772
Head width (mm)	1.764	3.808	5.852
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	1.008	0.329
Host spider web status	1,17	0.008	0.928
Host spider active time	1,17	0.497	0.490
Host spider vertical stratification	2,17	0.058	0.944
Head width	1,17	15.44	0.001
B. Eye surface area			
Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	-3.446	-1.897	-0.349
Host specialisation [specialist]	-0.610	0.075	0.760
Host spider web status [web]	-0.219	0.532	1.282
Host spider active time [nocturnal]	-1.420	-0.443	0.533
Host spider vertical stratification [ground and vegetation]	-1.329	-0.498	0.333
Host spider vertical stratification [vegetation]	-2.911	-1.334	0.244
Head width (mm)	1.619	2.101	2.583
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	2.912	0.106
Host spider web status	1,17	0.816	0.379
Host spider active time	1,17	5.268	0.035
Host spider vertical stratification	2,17	8.365	0.003
Head width	1,17	84.54	<0.0001

C. Sensilla trichodea density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	1212	35752	70291
Host specialisation [specialist]	-17269	-1998	13274
Host spider web status [web]	-24013	-7276	9461
Host spider active time [nocturnal]	-11009	10771	32551
Host spider vertical stratification [ground and vegetation]	-18204	329.8	18864
Host spider vertical stratification [vegetation]	-27791	7397	42585
Head width (mm)	-13307	-2555	8197
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	0.152	0.702
Host spider web status	1,17	0.242	0.629
Host spider active time	1,17	1.032	0.324
Host spider vertical stratification	2,17	0.035	0.966
Head width	1,17	0.251	0.623

D. Sensilla basiconica density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	71.85	1107	2142
Host specialisation [specialist]	-355.1	102.5	560.1
Host spider web status [web]	-998.8	-497.4	4.081
Host spider active time [nocturnal]	-189.3	463.2	1116
Host spider vertical stratification [ground and vegetation]	-167.9	387.5	942.8
Host spider vertical stratification [vegetation]	-564.9	489.4	1544
Head width (mm)	-519.2	-197.1	125.1
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	1.600	0.223
Host spider web status	1,17	2.134	0.162
Host spider active time	1,17	2.492	0.133
Host spider vertical stratification	2,17	1.213	0.322
Head width	1,17	1.666	0.214

E. Sensilla chaetica density

Parameter estimates	***Lower***	***Estimate***	***Upper***
Intercept	801.8	1625	2449
Host specialisation [specialist]	-604.9	-240.8	123.4
Host spider web status [web]	-945.7	-546.7	-147.6
Host spider active time [nocturnal]	-86.29	433.0	952.3
Host spider vertical stratification [ground and vegetation]	-335.1	106.8	548.7
Host spider vertical stratification [vegetation]	-332.9	506.0	1345
Head width (mm)	-585.5	-329.1	-72.78

Fixed effects	*df*	*F ratio*	*p > F*
Host specialisation	1,17	0.022	0.885
Host spider web status	1,17	3.683	0.072
Host spider active time	1,17	3.622	0.074
Host spider vertical stratification	2,17	0.208	0.814
Head width	1,17	7.337	0.015

F. Sensilla placodea density

Parameter estimates	*Lower*	*Estimate*	*Upper*
Intercept	672.7	2381	4089
Host specialisation [specialist]	-584.8	170.5	925.8
Host spider web status [web]	-436.0	391.8	1220
Host spider active time [nocturnal]	-1153	-75.49	1002
Host spider vertical stratification [ground and vegetation]	-497.7	418.9	1336
Host spider vertical stratification [vegetation]	-740.1	1000	2741
Head width (mm)	-861.3	-329.5	202.3
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	0.649	0.432
Host spider web status	1,17	3.650	0.073
Host spider active time	1,17	0.158	0.696
Host spider vertical stratification	2,17	0.537	0.594
Head width	1,17	1.709	0.209

G. Sensilla coeloconica density

Parameter estimates	*Lower*	*Estimate*	*Upper*
Intercept	160.3	351.8	543.3
Host specialisation [specialist]	-103.0	-18.30	66.38
Host spider web status [web]	-156.5	-63.66	29.15
Host spider active time [nocturnal]	-126.0	-5.225	115.6
Host spider vertical stratification [ground and vegetation]	-65.07	37.71	140.5
Host spider vertical stratification [vegetation]	-40.68	154.4	349.6
Head width (mm)	-175.9	-116.3	-56.68
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	0.313	0.583
Host spider web status	1,17	0.114	0.740
Host spider active time	1,17	0.0005	0.983
Host spider vertical stratification	2,17	0.656	0.532
Head width	1,17	16.94	0.0007

H. Sensilla ampullacea density

Parameter estimates	*Lower*	*Estimate*	*Upper*
Intercept	307.3	1111	1915

Host specialisation [specialist]	-555.1	-199.7	155.6
Host spider web status [web]	-960.4	-570.9	-181.5
Host spider active time [nocturnal]	-390.4	116.4	623.2
Host spider vertical stratification [ground and vegetation]	-508.8	-77.51	353.8
Host spider vertical stratification [vegetation]	-682.1	136.7	955.5
Head width (mm)	-461.3	-211.1	39.07
Fixed effects	***df***	***F ratio***	***p > F***
Host specialisation	1,17	0.211	0.652
Host spider web status	1,17	9.773	0.006
Host spider active time	1,17	0.474	0.500
Host spider vertical stratification	2,17	0.113	0.894
Head width	1,17	3.170	0.093

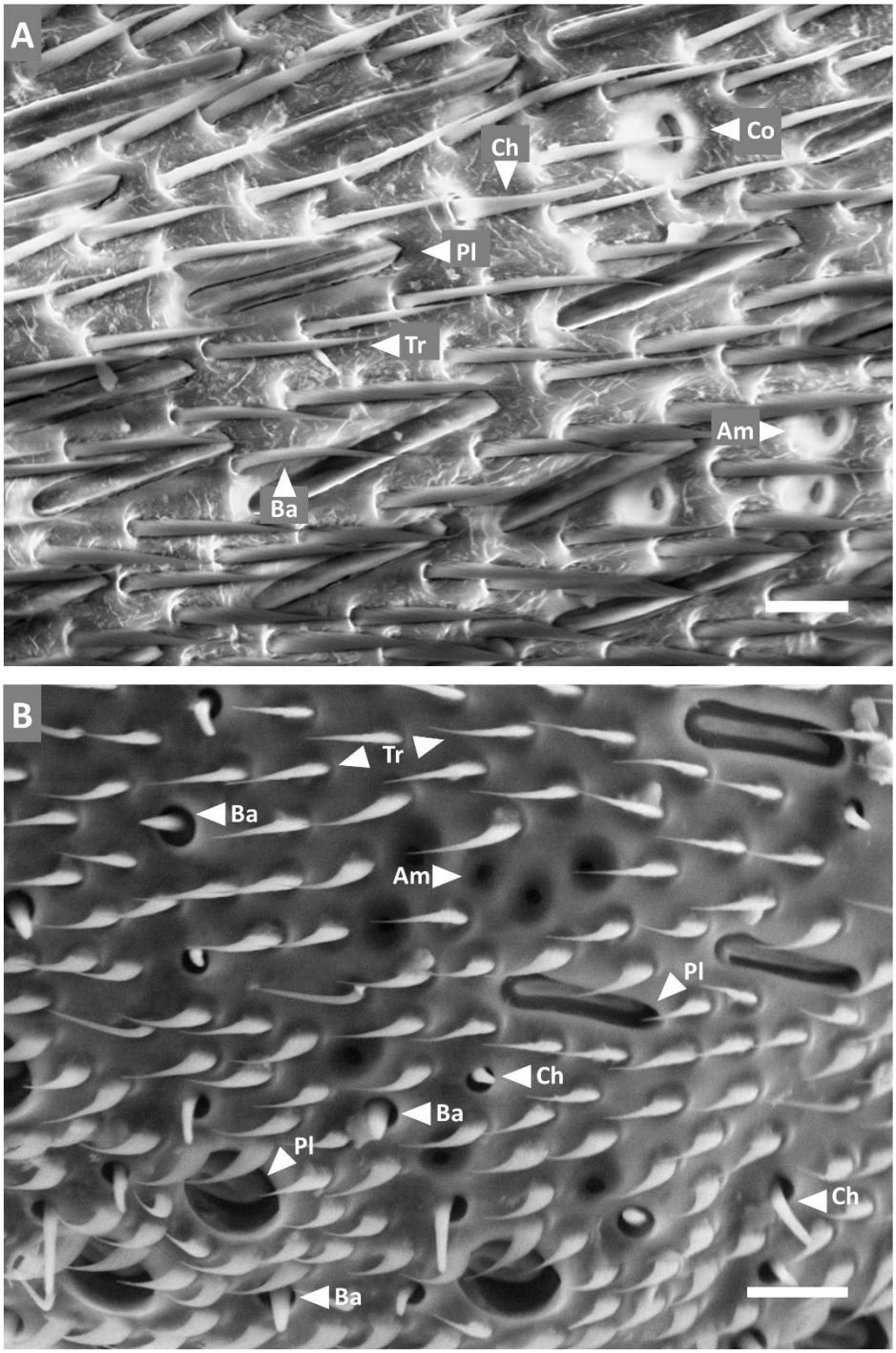

Figure 2.2. Scanning electron micrographs of antennae of **A.** *Priochilus* regius and **B.** *Pompilinus marginatus* displaying the types of antennal sensilla identified for spider wasps. Scale bars = 10 µm. Am = sensilla ampullacea (CO_2 detectors); Ba = sensilla basiconica (gustatory sensilla/contact chemoreceptors); Co = sensilla coeloconica (thermo-/hygro-receptors; possibly olfactory sensilla); Ch = sensilla chaetica (mechanoreceptors/tactile hairs; possible gustatory sensilla); Pl = sensilla placodea (olfactory sensilla); Tr = sensilla trichodea (olfactory sensilla).

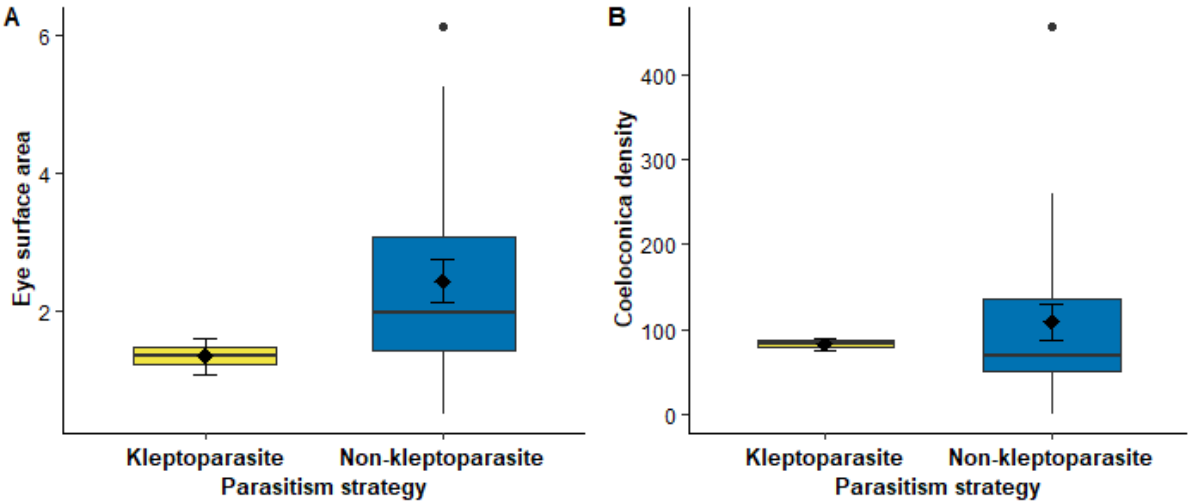

Figure 2.3. The influence of parasitism strategy on eye and antennal morphology amongst spider wasps. Tails indicate the range; box indicates interquartile range; horizontal line within the box is the median; black diamonds are the mean; black capped error bars indicated standard error of the mean; black circles indicate outliers. Compared to non-kleptoparasites, kleptoparasites have **A.** a significantly smaller compound eye surface area (mm^2) ($F_{1,23} = 4.883$, $p = 0.037$) and **B.** a lower density of antennal sensilla coeloconica (no. of sensilla per mm^2 of antenna) ($F_{1,23} = 3.770$, $p = 0.065$).

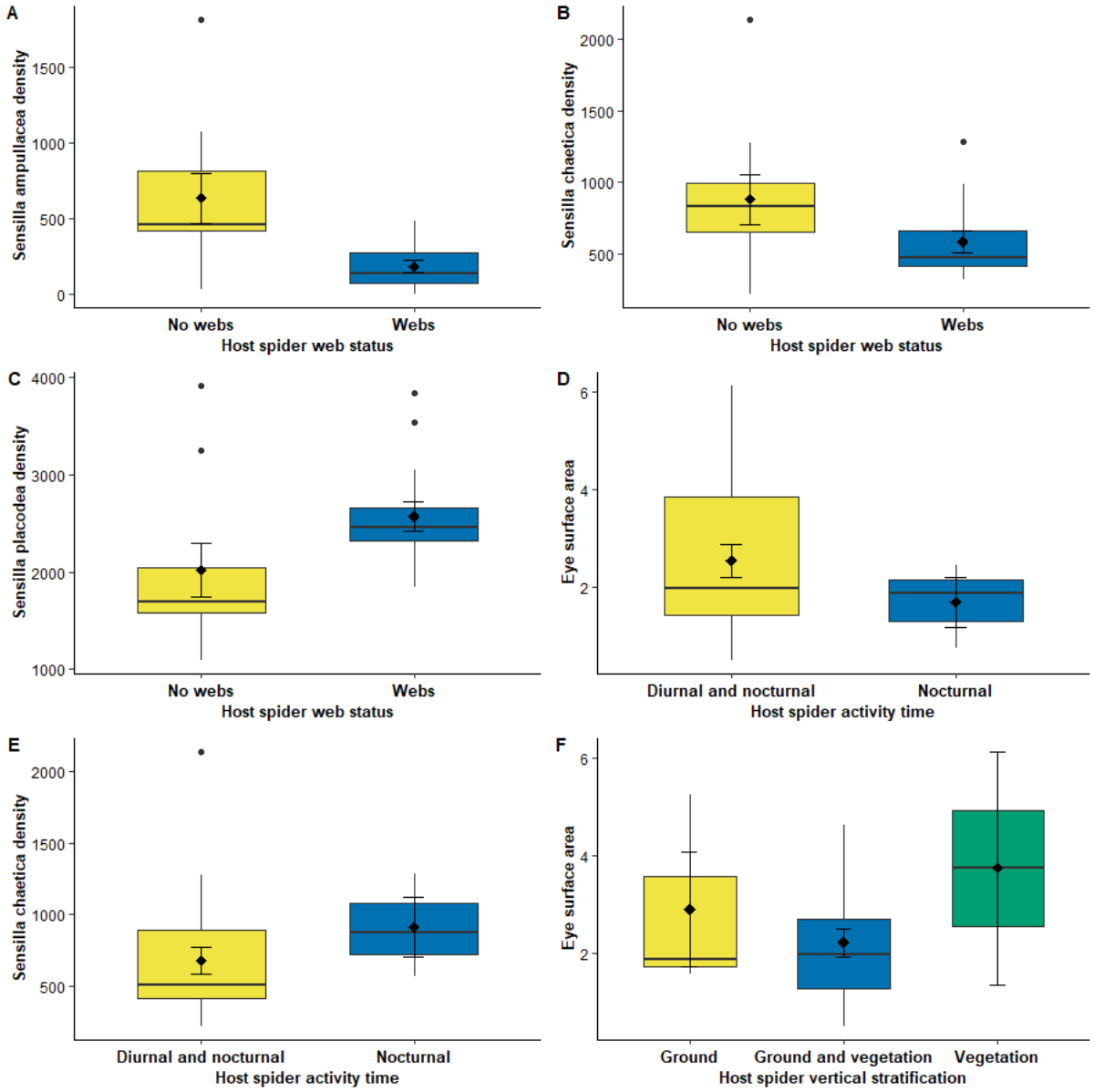

Figure 2.4. The influence of host spider ecology on eye and antennal morphology amongst spider wasps. Tails indicate the range; box indicates interquartile range; horizontal line within the box is the median; black diamonds are the mean; black capped error bars indicated standard error of the mean; black circles indicate outliers. Spider wasps who parasitise web-building spiders have **A.** a statistically significantly lower density of antennal sensilla ampullacea ($F_{1,17}$ = 9.772, p = 0.006) and **B.** a lower density of sensilla chaetica ($F_{1,17}$ = 3.683, p = 0.072), but **C.** a higher density of sensilla placodea ($F_{1,17}$ = 3.650, p = 0.073). Spider wasps parasitising only nocturnal spiders have **D.** a significantly smaller compound eye surface area ($F_{1,17}$ = 5.268, p = 0.035) and **E.** a higher density of antennal sensilla chaetica ($F_{1,17}$ = 3.622, p = 0.074). **F.** Compound eye surface area is smaller for wasps who parasitise ground-dwelling and vegetation-dwelling spiders than for wasps for parasitise only ground-dwelling spiders or only vegetation-dwelling spiders ($F_{2,17}$ = 8.365, p = 0.003).

2.5 Discussion

These comparative analyses reveal that kleptoparasitic spider wasps invest less in eye and antennal morphology than non-kleptoparasitic spider wasps. They also provide evidence that wasps with more discrete hosts invest more in sensory morphology.

Key results of these analyses can be readily interpreted in a coevolutionary framework: as the host becomes more difficult to detect, the parasitoids will experience selection to increase sensitivity to signals and/or cues that reveal the location of potential hosts (Vinson, 1998) by investing more in their sensory organs. My finding that spider wasps that are parasitoids of nocturnal spiders only have relatively smaller compound eyes is surprising because nocturnal insects typically possess larger eyes than their day-active close relatives (Freelance et al., 2021b). Perhaps these wasps are not active at night but instead may hunt for the prey during the day when they are cryptically hidden against vegetation or in silk retreats (Chuang et al., 2007; Yin et al., 1997), with a smaller compound eye being consistent with day flying and relying on non-visual cues to detect these visually inconspicuous hosts. However, if the wasps parasitising nocturnal spiders only are hunting at night, the smaller eye size may still be related to the difficulty of detecting hosts visually: many nocturnal spiders are inconspicuously coloured (Oxford and Gillespie, 1998), and nocturnal orb-weaving spiders that display prey-attracting yellow colouration tend to do so in a mosaic pattern which increases visibility to prey (Peng et al., 2020) but makes them less conspicuous to nocturnal Hymenopteran predators and parasitoids (Fan et al., 2009). The increased antennal sensilla chaetica density I observe in wasps parasitising nocturnal spiders likely reflects the increased difficulty of detecting those spiders visually, and thus favouring greater reliance on non-visual cues. Sensilla chaetica are thought to be vibration- and mechano-receptors (Yang et al., 2018), and may be sensitive to movements generated by spiders, but may also have a potential gustatory/chemosensory function (Nowińska and Brożek, 2017). While gustation is a mechanism by which parasitoid wasps identify if a host is suitable or has already been parasitised (van Baaren et al., 2007), such functionality is unlikely to be beneficial in locating a host in the first instance.

The association between host spider vertical stratification and spider wasp eye surface area is intriguing in this coevolutionary framework. The mean eye surface area is greatest for spider wasps whose host spiders are located in vegetation only, which is consistent with visually detecting these spiders: a larger eye accompanied by no difference in ommatidia diameter suggests the eye is composed of a larger number of ommatidia, which typically results in greater visual sensitivity (Jander and Jander, 2002). Web-building spiders are predominantly located in vegetation rather than on the ground, and diurnal genera are often located near the centre of their webs when active (Chuang et al., 2007; Powell and Taylor, 2017), which may make them more conspicuous to a parasitoid with high visual acuity. Consequently, the relationship between spider wasp eye surface area and host spider vertical stratification may be at least partly associated with host web-building. My observation of higher densities of some antennal sensilla amongst wasps parasitising non-web-building spiders is consistent with this interpretation, as ground-dwelling spiders may be relatively better camouflaged against the substrate and thus unlikely to be easily detected visually by a parasitoid.

Differences in spider wasp antennal sensilla density related to the web status of host spiders further suggests the use of non-visual cues to detect spiders. Sensilla placodea are olfactory sensilla that detect volatile chemicals including pheromones and plant volatiles (Wang et al., 2018), and are present in a higher density on the antennae of spider wasps who are parasitoids of web-building spiders. This pattern is consistent with spider wasps locating hosts with silk-based odours, such as putrescine, that also act as insect prey-attracting allomones for web-building spiders (Henneken et al., 2017). In contrast, parasitoids of non-web building spiders have a higher density of vibration-sensitive sensilla chaetica, suggesting they potentially locate hosts through the movement or vibratory courtship signals that are commonly used by cursorial spiders (Elias et al., 2005; Elias et al., 2010; Gibson and Uetz, 2008; Girard et al., 2011; Jackson and Hallas, 1986; Rivero et al., 2000).

Contrary to my prediction, kleptoparasitic spider wasps have a lower density of antennal sensilla coeloconica and significantly smaller compound eye than their non-kleptoparasitic

counterparts. The relatively larger eye in non-kleptoparasitic wasps may be related to their need to provision a nest: Hymenoptera, including pompilids, often perform orientation flights to utilise visual cues and landmarks to help them remember the location of their nest (Andrietti et al., 2008; Zeil, 2012), whereas kleptoparasites do not need to remember the location of a nest to provision – instead, they need only locate the host nest once to lay an egg in it. Kleptoparasites may thus rely less on visual cues and consequently need less sensitive eyes, instead relying on non-visual cues to detect host nests which are often visually concealed; this would be consistent with field observations that kleptoparasitic pompilids often approach host nests from downwind (Wcislo et al., 1988). Furthermore, parasitoid spider wasps must locate a suitable nesting site and locate and subdue a spider to parasitise, whereas kleptoparasitic spider wasps need only locate an existing nest to parasitise and thus, arguably, use comparatively fewer olfactory cues, which may explain the lower density of antennal sensilla coeloconica among kleptoparasites. Given the energetically expensive nature of sensory systems (Niven and Laughlin, 2008), selection pressures associated with a less complex sensory environment are likely to favour sensory organs that are comparatively less complex (Endler, 1992) in kleptoparasitic pompilids.

Chapman (1982) predicts that insects with specialised signal detection requirements require greater sensitivity and will thus possess a greater density of antennal sensilla compared with generalist species. I find little support for this prediction in spider wasps, despite support from other parasitoid systems: endoparasitic braconid wasps (Hymenoptera: Braconidae) that are host specialists have a higher abundance of olfactory antennal sensilla compared with generalist species (Bleeker et al., 2004; Das et al., 2011). However, in such systems specialisation refers to the number of host species, with little ecological variation across the hosts of generalists. Spider wasps, in contrast, specialise on particular ecotypes of host (Evans, 1953; Rodriguez et al., 2016), with each spider guild differing considerably in the cues available to parasitoids to locate host spiders. Perhaps the predictions around host specialisation advanced by Chapman (1982) are relevant only in systems with little diversity in host ecology.

While consistent with the notion that parasitism strategy and host spider ecology influence the sensory organ morphology of spider wasps, it must be noted my sample sizes for parasitoids of some host traits were small. Specifically: I had only 2 kleptoparasitic species, only 2 species that are parasitoids of only nocturnal spiders, and for host spider vertical stratification I have only 3 species parasitising ground-dwelling spiders only and 2 species parasitising vegetation-dwelling spiders only. Small sample sizes for some traits may explain why some between-group differences with medium or large effect sizes (as measured by Cohen's d_s) were not accompanied by a statistically significant ANOVA *F* test. Due to public health restrictions associated with the SARS-CoV-2 pandemic, the addition of more species of pompilid wasps to the dataset was not logistically feasible: prolonged and repeated Victorian Government lockdowns and public health restrictions prevented access to the University's electron microscopy facilities for most of 2020 and 2021; and there were multiple suspensions of the specimen loan services of various museum and other insect collections, which meant obtaining specimens was not practical. Regardless, the present dataset is robust with the current sample size and the persistence of strong patterns in this dataset indicates the potential pervasive nature of the selection pressures that host ecology impart on spider wasp sensory organ morphology.

More broadly, these results have implications for predictions that life history trade-offs may influence sensory organ morphology. Inverse resource allocation, potentially influenced by investment trade-offs driven by the energetically expensive nature of sensory systems (Keesey et al., 2019; Niven and Laughlin, 2008), are thought to influence the morphology of the sensory organs for each sensory modality, suggesting that limited energetic resources prevent increased investment in one sensory organ without decreased investment in another. Convincing patterns consistent with this theory have been documented in some ants (Jelley and Barden, 2021) and in *Drosophila* (Keesey et al., 2019) only, but patterns resembling inverse allocation can emerge from random genetic mutations (Keesey et al., 2019) and more recent studies have failed to observe long-theorised trade-offs between sensory organs for different modalities in the context of the photic environment (Freelance et al.,

2021b). The present study finds no evidence of such inverse resource allocation in the spider wasps: non-kleptoparasites have relatively larger eyes and relatively higher antennal sensilla densities, and spiders parasitising web-building spiders only or cursorial spiders only had increased antennal sensilla densities with no commensurate decrease in investment in the compound eye. In this light, my results add to growing evidence that it is the ecology of a species, and the availability of salient information in a given sensory modality, that is likely to exert the strongest selective influence on the morphology of their sensory organs.

My study is the first to explore in a single taxonomic family how the signal detection requirements of various parasitism strategies and host ecologies influence the morphology of the sensory organs that parasitoids require to detect host-related cues. In presenting the first simultaneous assessment of variation in eye and antenna morphology in relation to kleptoparasitism and to host species ecology, I provide direct evidence that both parasitism strategy and the ecology of the host species are associated with selection pressures on the evolution of sensory organs. These results add what was a missing dimension to our understanding of the behaviour of Pompilid wasps and expand our knowledge of the sensory ecology of parasites more broadly. This emphasises the need to consider not only the signals and cues utilised in interspecific parasitic interactions, but also the morphology of the sensory organs that parasites must use to detect that information.

2.6 Acknowledgements

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Chapter 3: The relationship between sensory organ morphology and mutualistic ant-association in Australian Lycaenid butterflies

3.1 Abstract

Communication is essential to mutualisms, allowing partners to correctly identify their cooperating partners. While many studies explore the evolution of the signals facilitating mutualisms, comparatively few explore the evolution of the sensory organs required for their detection. Here, I explore how variation in receptor organ morphology has been influenced by the evolution of myrmecophily in lycaenid butterflies. Myrmecophilous (ant-associated) lycaenids use ants as cues for oviposition by females and, in some species, mate location by males, and the use of these additional cues may select for changes in olfactory sensitivity, although the direction of change is unclear. This comparative analysis of a sample of Australian lycaenids reveals that males of obligately myrmecophilous species possess larger eyes but lower densities of olfactory antennal sensilla than non-myrmecophilous species. In contrast, I find no evidence that the evolution of myrmecophily had an impact on female receptor organ morphology, although there is a non-significant trend for species with greater host plant ranges to have higher densities of olfactory sensilla. These results are the first to provide direct evidence that the sensory organs required to detect the signals associated with interspecific mutualism are themselves subject to selection. More broadly, this emphasises the importance of examining both the signals used and the sensory organs that receive them to develop a more complete understanding of the communicative domain that facilitates mutualistic interactions.

3.2 Introduction

Communication is essential for cooperative associations between species: at the very least, each partner must produce and detect signals that identify themselves as appropriate partners. Nevertheless, studies of cooperation, or mutualism, typically place emphasis on the fitness benefits for each partner rather than on the enabling mechanisms (Frederickson, 2017; Kiers et al., 2010; Leigh Jr, 2010). Efficient communication requires reliable signals and receptor organs capable of detecting those signals (Gill et al., 2013), with selection acting on both the signals and the sensory organs that

detect them (Endler, 1992). Sensory receptor organs are energetically expensive to develop and maintain (Niven and Laughlin, 2008; Stöckl et al., 2016a) and investment in receptor organs is expected to be concomitant with the requirements to detect particular signals and cues (Elgar et al., 2018). For example, dim-light active insects have larger compound eye ommatidia, enhancing their photon capture capabilities (Freelance et al., 2021b), while a lower investment in antennal sensilla, used to detect chemical signals, is associated with transitions from social to solitary lifestyles in halictid bees (Wittwer et al., 2017).

The production and detection of signals play a significant role in establishing and maintaining symbioses involving myrmecophilous lycaenid butterfly larvae and their attendant ants. Amongst the estimated 6000 species of lycaenid butterflies (Lepidoptera: Lycaenidae), the majority are myrmecophilous: around 30% are obligately myrmecophilous (immatures (larvae and pupae) are always associated with ants during some developmental stage and dependent on them for survival), and 45% are facultatively myrmecophilous (immatures are only intermittently associated with ants and not dependent on ant attendants for survival) (Pierce et al., 2002). In these myrmecophilous relationships, the ants protect the butterfly larvae and pupae from natural enemies and the larvae and pupae, in return, provide their attendant ants with nutritious food rewards (Malicky, 1970; Pierece et al., 1987; Pierce et al., 2002). Epidermal glands on the larvae secrete ant attracting and appeasing compounds (Malicky, 1970), and distressed larvae emit volatile odours from their tentacular organs that recruit attendant ants (Axén et al., 1996; Pierce et al., 2002). The larvae can vary the production rate of secretions according to the perceived level of defence they require (Agrawal and Fordyce, 2000), the number of ants already in attendance (Agrawal and Fordyce, 2000) and the number of nearby larvae (Axén and Pierce, 1998). Later instar larvae and pupae can produce sounds, often when they have been disturbed, to recruit greater numbers of attending ants (Downey and Allyn, 1973; Hill, 1993; Pierce et al., 2002; Travassos and Pierce, 2000). Previous studies in this mutualistic system have typically emphasised identification of the costs and benefits of the mutualism, with limited focus on the role of communication in facilitating the mutualism. Moreover, the latter

studies typically examine and characterise the signals and cues themselves but do not examine sensory organs. For the signals or cues to effective, the receiver must possess sensory organs that are able to efficiently detect them, and so characterising the sensory organs is a crucial component of understanding the communicative domain that facilitates the mutualism.

Adult lycaenids avoid physically interacting with ants (Pierce et al., 2002), but nevertheless may use volatile intra-specific chemical signals produced by their mutualistic partners as cues for oviposition (Casacci et al., 2019; Fraser et al., 2002; Pierce et al., 2002; Pierce and Elgar, 1985; Wagner and Kurina, 1997) (but see also Fürst and Nash, 2010; Patricelli et al., 2011; Patricelli et al., 2015 and van Dyck et al., 2000), thereby increasing the likelihood that their eggs and young larvae are discovered and hence tended by their mutualist ants. Natural enemies also eavesdrop on these ant-derived odours (Elgar et al., 2016). Ant-dependent oviposition choices may explain the broader host-plant range of myrmecophilous lycaenids compared to those that are non-myrmecophilous (Pierce and Elgar, 1985). The additional complexity in the chemical signalling environment for mutualistic species, including recognising particular species of ants (Fraser et al., 2002), may require specialisation in sensory organs (Fiedler et al., 1996) in the form of a greater number and/or diversity of receptors and, thus, supporting sensilla on their antennae.

Accordingly, I ask whether the evolution of myrmecophily is associated with changes in the investment in sensory organs required to detect signals and cues associated with the mutualistic association. If adult females of myrmecophilous lycaenid species use ant odours in addition to plant odours as oviposition cues and thus have a greater diversity of odours to which they must be sensitive, I expect those species will invest more in antennal micro-morphology – reflected by a higher density of olfactory antennal sensilla – than non-myrmecophilous species. If, on the other hand, myrmecophilous lycaenids rely only on ant odours as oviposition cues, their antennal sensilla density may be similar to that of non-myrmecophilous species which must detect only plant but not ant odours as oviposition cues. While lycaenids likely rely on chemical cues to identify ants, the use of

visual cues to search for ants is possible (Fiedler et al., 1996; Mota and Oliveira, 2016) and butterflies in general are known to use visual signals. Hence, I explore investment in both olfactory (antennae) and visual (compound eyes) receptor organs.

3.3 Methods

3.3.1 Study species selection

I obtained female and male specimens for 20 Australian species of lycaenid butterflies for which sufficient female and male specimens (*N* = 3–5 per sex) were available from the Australian National Insect Collection (Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia). Species were selected based upon their known myrmecophilous tendencies (obligately myrmecophilous, facultatively myrmecophilous, non-myrmecophilous) following Eastwood and Fraser (1999): larvae of obligately myrmecophilous species are always attended by (usually large numbers of) ants and typically depend on them for survival (Fiedler et al., 1996) (only mutualistic, not parasitic, species are included in my study); facultatively myrmecophilous species associate with ants but not obligately and so vary in the frequency and stability of their associations with ants; and non-myrmecophilous species demonstrate no association with ants and do not typically interact with them if they're present on a food plant. With an association between host plant diversity and obligate myrmecophily amongst lycaenid butterflies (Fiedler, 1994; Pierce and Elgar, 1985) and the possibility for the diversity of host plants used by a species to influence the distribution of olfactory receptors, the number of taxonomic families of host plants used by each study species was ascertained from Braby (2016).

3.3.2 Sensory organ morphology metrics

I captured digital images of the specimens using a Canon 6D DSLR with Canon EF-L 100mm f2.8 macro lens (Canon, Tokyo, Japan), with a ruler included as a scale, in order to determine the average forewing length of each specimen as a measure of body size (van Hook et al., 2012). To image the sensory structures, each pinned specimen was mounted on an electron microscopy stub and underwent low-

vacuum scanning electron microscopy using a FEI Quanta 200F scanning electron microscope (10kV acceleration voltage, spot size 2.0, 0.50 mbar pressure) at the Bio21 Advanced Microscopy Facility or standard scanning electron microscopy using a Hitachi TM4000 benchtop scanning electron microscope (15kV acceleration voltage, charge reduction mode) at the BioSciences Microscopy Facility (The University of Melbourne). I then determined the following metrics for each specimen: the surface area of the eye (calculated as the area of half a spheroid with semi-axes equivalent to the greatest length and width of the compound eye; mm^2), the average diameter of the ommatidia of the eye (diameter of three ommatidium averaged; µm), the length of the antenna (only flagellomeres) (mm), and the density of each type of antennal sensilla on the dorsal surface of the apical antennomere (number of sensilla in a given area of antenna; sensilla per mm^2). Eye and ommatidia size provide information about sensitivity to visual information (reviewed by Warrant (2017)) and antennal sensilla density is a behaviourally relevant indicator of sensitivity to olfactory cues (Gill et al., 2013; Spaethe et al., 2007). To account for potential differences in ommatidia diameter between different regions of the compound eye (Perl & Niven, 2016), I measured the ommatidia from the anteromedial aspect (i.e. facing directly in front of the insect) of each compound eye. If an eye or antenna was damaged, that sensory structure was not imaged or assessed for that individual. All image analysis was performed using FIJI (Schindelin et al., 2012).

3.3.3 Statistical analysis

To control for the influence of phylogeny in my comparative analysis, I obtained a phylogenetic tree of my study species by pruning the maximum likelihood phylogenetic tree of Schär et al. (2018) and then mapping the myrmecophily trait of each species to the pruned tree (Figure 3.1). For each sensory morphology metric, I then fitted a phylogenetic least squares model including myrmecophily (obligate, facultative, non-myrmecophilous), the number of host plant families, and forewing length (as a measure of body size) as effects with variance partitioned using maximum likelihood. Each model was reduced by hierarchical removal of all terms with $p > 0.1$ except for myrmecophily. Females and males were analysed separately. These statistical analyses were performed using the PHYTOOLS (version 0.7-

80) (Revell, 2012), PICANTE (version 1.8.2) (Kembel et al., 2010) and GEIGER (version 2.0.7) (Pennell et al., 2014) packages in R version 4.1.0 for Windows (R Core Team, 2021). Myrmecophily, host plant families and female and male averages for each sensory morphology metric are listed for each species in Table 3.1; sensory organ morphology averages for each level of myrmecophily are listed in Table 3.2.

Table 3.1. Life history information and sensory organ morphology metrics for each study species of Lycaenid butterfly. Forewing length and antenna length are measured in mm, ommatidia diameter in µm, eye surface area in mm^2, and antennal sensilla densities as the number of sensilla per mm^2 of antenna. Forewing length and sensory morphology metrics are species averages. *N* = 5 specimens of each sex were examined for each species, except only *N* = 3 female *Ogyris subterrestris* were available and *N* = 10 female *Erysichton palmyra* were analysed. If a wing, eye, or antenna for a given specimen was damaged, it was not analysed.

Species	Myrmecophily	Host plant taxonomic families		Forewing length	Sensory organ morphology metrics						
					Ommatidia diameter	Compound eye surface area	Antenna length	Sensilla trichodea density	Sensilla basiconica density	Sensilla chaetica density	Sensilla auricillica density
Candalides helenita	Non-myrmecophilous	4	Female	12.9	18.0	1.2	8.1	6228	483	526	400
			Male	14.8	20.3	1.5	9.2	6460	446	510	337
Candalides margarita	Facultative	1	Female	15.6	17.8	1.0	8.9	8153	521	240	220
			Male	15.5	19.0	1.1	9.0	6731	447	286	292
Erysichton lineata	Facultative	5	Female	11.2	15.3	0.6	6.0	9021	567	668	303
			Male	11.5	16.4	0.8	6.8	10748	1092	782	455
Erysichton palmyra	Facultative	1	Female	12.8	17.5	1.0	6.2	7455	902	534	364
			Male	12.0	17.4	0.6	7.1	11979	1037	744	438
Hypochrysops byzos	Non-myrmecophilous	2	Female	13.1	17.9	1.0	7.7	8317	472	626	360
			Male	12.9	18.4	1.4	8.4	7604	375	596	306
Hypochrysops ignita	Obligate	17	Female	12.3	18.2	1.2	7.1	11754	631	786	482
			Male	12.3	20.4	1.6	8.3	9161	363	460	331
Hypolycaena danis	Non-myrmecophilous	1	Female	14.9	21.0	2.0	6.9	7437	739	372	242
			Male	13.2	20.4	1.8	6.7	7596	682	519	239
Hypolycaena phorbas	Obligate	12	Female	16.2	20.6	2.7	7.8	5584	557	278	151
			Male	15.8	21.7	2.5	8.1	5455	477	267	154

Jalmenus evagoras	Obligate	2	Female	20.4	17.2	0.9	8.9	5183	443	384	197
			Male	17.5	17.4	0.9	8.1	3844	471	390	246
Jalmenus ictinus	Obligate	2	Female	16.7	16.0	0.3	8.5	8273	697	627	314
			Male	14.2	17.4	0.4	7.5	5667	292	749	240
Neolucia agricola	Facultative	1	Female	11.0	14.7	0.8	6.4	8647	370	574	281
			Male	11.0	16.7	1.1	7.5	5581	206	304	154
Neolucia hobartensis	Non-myrmecophilous	1	Female	9.8	15.7	0.9	6.0	13158	1206	708	570
			Male	9.5	15.4	0.9	6.2	9470	889	648	477
Ogyris barnardi	Facultative	1	Female	18.1	21.1	2.3	9.6	7968	952	1037	473
			Male	16.5	20.3	2.2	9.1	7038	469	465	261
Ogyris olane	Facultative	1	Female	18.7	21.2	2.0	9.4	7592	854	1078	605
			Male	18.1	21.3	2.6	10.3	5272	565	672	353
Ogyris subterrestris	Obligate	1	Female	19.7	23.1	2.7	7.2	2049	285	334	132
			Male	19.9	24.3	3.1	7.5	1933	346	381	147
Paralucia pyrodiscus	Obligate	1	Female	12.5	18.0	1.7	7.5	7231	750	463	213
			Male	11.7	21.7	2.6	8.0	6285	547	401	236
Philiris innotatus	Facultative	1	Female	11.9	16.2	0.5	6.8	8975	549	723	377
			Male	11.2	15.8	0.8	7.4	8160	401	653	322
Philiris nitens	Non-myrmecophilous	2	Female	11.2	15.4	0.6	6.1	8712	413	598	382
			Male	10.3	16.6	0.6	6.4	10150	359	633	359
Prosotas dubiosa	Non-myrmecophilous	4	Female	8.7	13.4	0.3	5.0	16794	1343	754	794
			Male	8.7	15.3	0.5	5.4	18126	1370	1075	941
Prosotas felderi	Facultative	3	Female	9.3	14.0	0.4	5.3	18278	1082	748	596
			Male	9.8	16.1	0.8	6.1	16121	1639	814	937

Table 3.2. Sensory organ morphology metrics by sex for each level of myrmecophily. Forewing length and antenna length are measured in mm, ommatidia diameter in µm, eye surface area in mm^2, and antennal sensilla densities as the number of sensilla per mm^2 of antenna. Values are mean ± standard deviation.

Sensory organ morphology metric	Sex	Myrmecophily		
		Obligate	Facultative	None
Ommatidia diameter	Female	18.8 ± 2.56	17.2 ± 2.75	16.9 ± 2.62
	Male	20.5 ± 2.71	17.9 ± 2.06	17.7 ± 2.32
Compound eye surface are	Female	1.58 ± 0.96	1.08 ± 0.70	1.02 ± 0.58
	Male	1.86 ± 1.08	1.26 ± 0.72	1.12 ± 0.52
Antenna length	Female	7.84 ± 0.73	7.32 ± 1.69	6.63 ± 1.16
	Male	7.91 ± 0.35	7.91 ± 1.41	7.06 ± 1.45
Sensilla trichodea density	Female	6680 ± 3270	9510 ± 3590	10100 ± 4030
	Male	5390 ± 2430	8950 ± 3740	9900 ± 4250
Sensilla basiconica density	Female	560 ± 173	724 ± 254	776 ± 405
	Male	416 ± 97.1	732 ± 480	687 ± 393
Sensilla chaetica density	Female	479 ± 193	700 ± 271	597 ± 137
	Male	441 ± 163	590 ± 211	664 ± 209
Sensilla auricillica density	Female	248 ± 131	402 ± 143	458 ± 195
	Male	226 ± 68.1	402 ± 237	443 ± 256

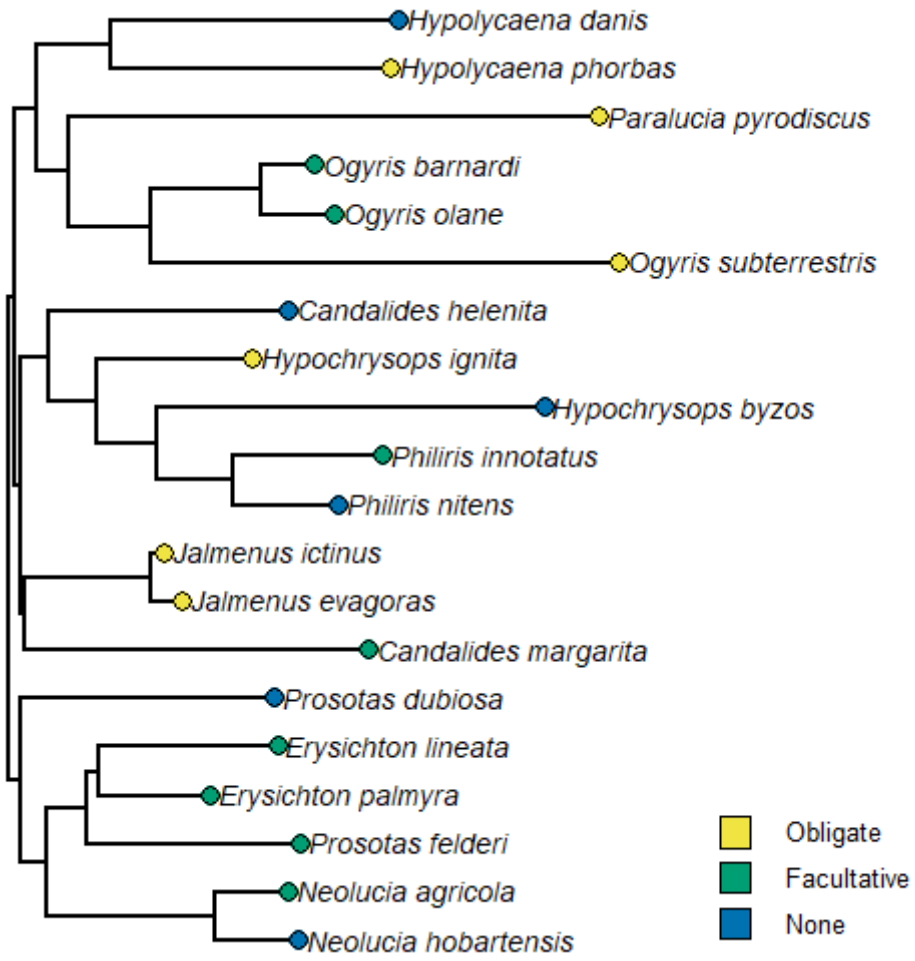

Figure 3.1. Phylogenetic tree of study species of Australian lycaenid butterflies, with the myrmecophilous tendency (obligate, facultative, none) mapped. The tree was pruned from the maximum likelihood phylogenetic tree of Schär et al. (2018).

3.4 Results

3.4.1 Antennal sensilla types

Four types of antennal sensilla were identified following Abu-shall and Tawfeek (2015), Xiangqun et al. (2014) and Seada (2015): trichodea (olfactory), basiconica (contact olfaction/gustation), chaetica (mechanosensory) and auricillica (olfactory) (Figure 3.2). Descriptions of the morphological characteristics of each sensillum type identified are in Appendix B.

3.4.2 Females

The diameter of the ommatidia (compound eye facets) is positively associated with forewing length as a measure of body size (β = 0.535, $F_{1,16}$ = 18.12, p = 0.0006) but is not associated with myrmecophily ($F_{2,16}$ = 1.718, p = 0.211). Similarly, the surface area of the compound eye is not related to myrmecophily ($F_{2,16}$ = 2.174, p = 0.146) but is larger for species with greater forewing length (β = 0.136, $F_{1,16}$ = 11.23, p = 0.004).

Antenna length increases with forewing length (β = 0.244, $F_{1,16}$ = 13.05, p = 0.002) but is not influenced by myrmecophily ($F_{2,16}$ = 1.475, p = 0.258). The density of sensilla trichodea on the antennae decreases significantly as forewing length increases (β = –870.2, $F_{1,15}$ = 12.42, p = 0.003), but the positive association with the number of taxonomic families of host plants is not statistically significant (β = 174.9, $F_{1,15}$ = 4.291, p = 0.056; Figure 3.3A) and myrmecophily does not explain any variation in this trait ($F_{2,15}$ = 1.644, p = 0.226). There is no effect of myrmecophily on the densities of sensilla basiconica ($F_{2,16}$ = 1.863, p = 0.187), sensilla chaetica ($F_{2,16}$ = 1.125, p = 0.349), or sensilla auricillica ($F_{2,16}$ = 2.769, p = 0.093). Similarly, there is no significant influence of forewing length on the densities of sensilla basiconica (β = –49.64, $F_{1,16}$ = 3.615, p = 0.075), sensilla chaetica (β = –31.23, $F_{1,16}$ = 3.094, p = 0.098) or sensilla auricillica (β = –26.72, $F_{1,16}$ = 4.097, p = 0.060).

3.4.3 Males

There is evidence that myrmecophily is associated with differential investment in ommatidia diameter ($F_{2,16}$ = 5.088, p = 0.020; Figure 3.4A), compound eye surface area ($F_{2,16}$ = 3.642, p = 0.05; Figure 3.4B)

and olfactory antennal sensilla density ($F_{2,15}$ = 4.451, p = 0.030; Figure 3.4C). Visual inspection of the data (Figure 3.4) reveals that compared to facultatively myrmecophilous and non-myrmecophilous species, obligately myrmecophilous species have greater ommatidia diameter and compound eye surface area, but a lower density of sensilla trichodea (olfactory sensilla) on the antennae. Both ommatidia diameter (β = 0.391, $F_{1,16}$ = 8.360, p = 0.011) and compound eye surface area (β = 0.138, $F_{1,16}$ = 9.657, p = 0.007) are also positively associated with forewing length as a measure of body size. Sensilla trichodea density is positively associated with the number of taxonomic families of host plants utilised (β = 221.1, $F_{1,15}$ = 4.880, p = 0.043; Figure 3.3B) and negatively associated with forewing length (β = –810.6, $F_{1,15}$ = 15.13, p = 0.002). Antenna length is positively associated with forewing length (β = 0.261, $F_{1,16}$ = 11.16, p = 0.004) but, despite a tendency for obligate myrmecophiles to have shorter antennae, the effect of myrmecophily of antenna length is not significant ($F_{2,16}$ = 2.968, p = 0.080).

There is no effect of myrmecophily on the densities of sensilla basiconica ($F_{2,17}$ = 1.305, p = 0.297), sensilla chaetica ($F_{2,16}$ = 2.245, p = 0.138), or sensilla auricillica ($F_{2,17}$ = 1.879, p = 0.183). The density of sensilla chaetica is lower for species with larger forewing lengths (β = –47.22, $F_{1,16}$ = 6.492, p = 0.022).

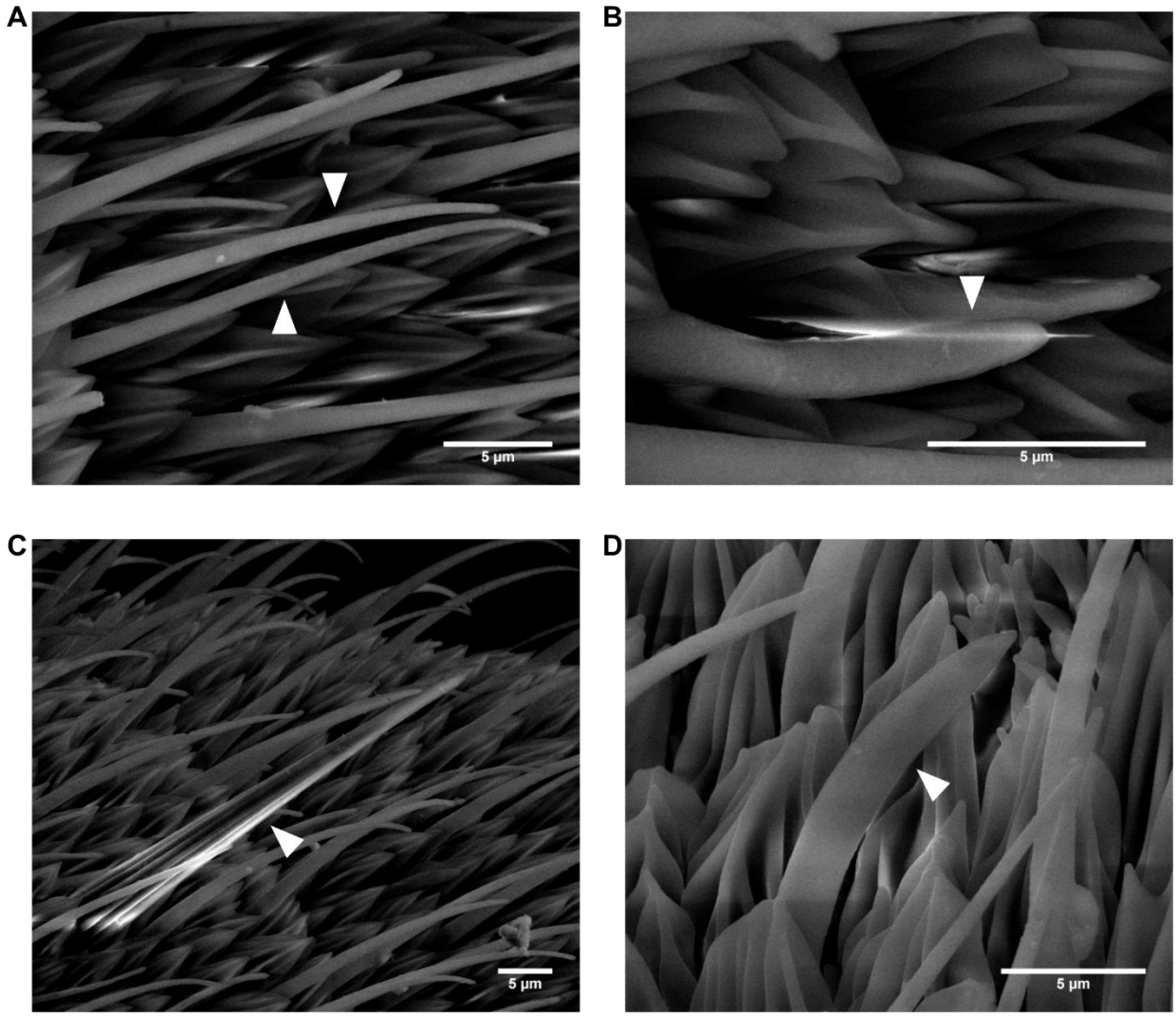

Figure 3.2. Scanning electron micrographs of the hair-like sensilla identified on the antennae of lycaenid butterflies. Scale bars = 5µm. **A:** Sensilla trichodea. **B:** Sensilla basiconica. **C:** Sensilla chaetica. **D:** Sensilla auricillica.

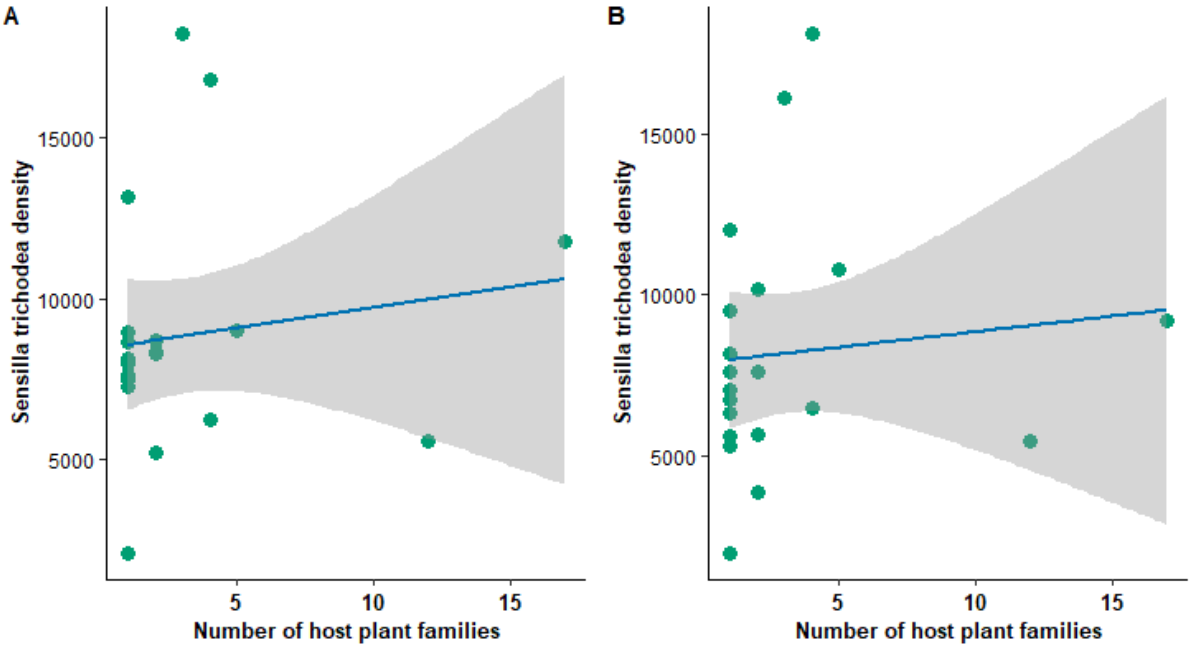

Figure 3.3. The influence of host plant diversity on antennal morphology amongst Australian lycaenid butterflies. Grey shaded area indicates standard error of the mean. Species using a greater number of taxonomic families of host plants possess a higher density of antennal sensilla trichodea in both **A.** females (β = 174.9, $F_{1,15}$ = 4.291, p = 0.056) and **B.** males (β = 221.1, $F_{1,15}$ = 4.880, p = 0.043).

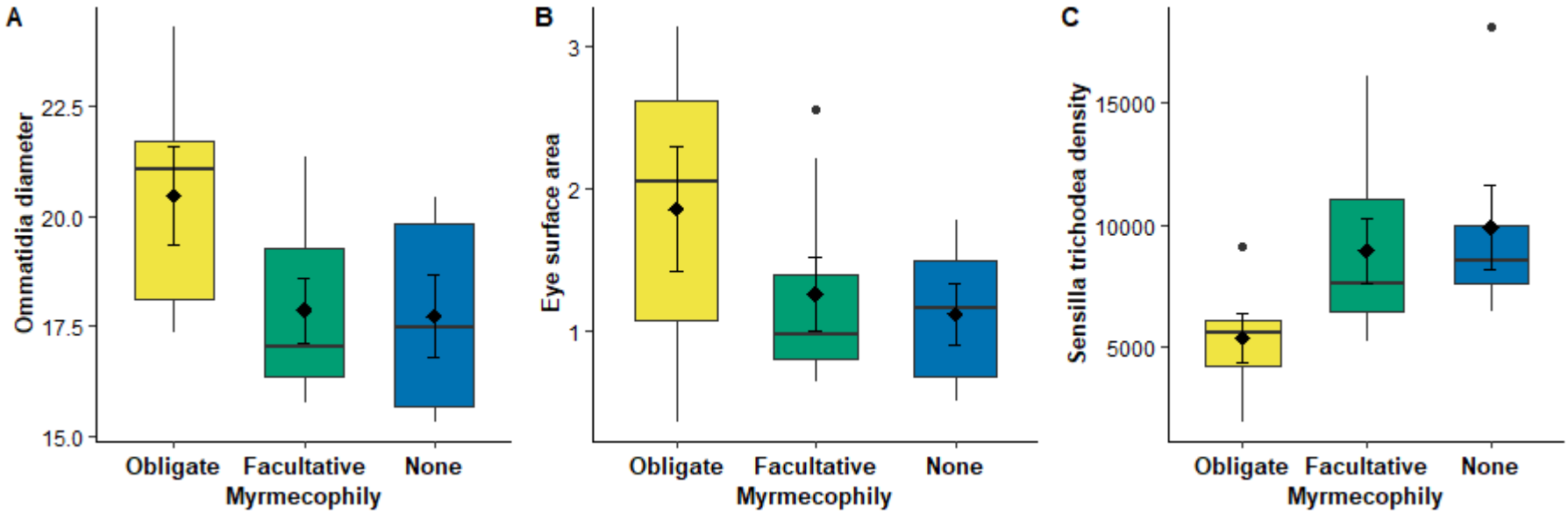

Figure 3.4. The influence of myrmecophily on eye and antennal morphology amongst male Australian lycaenid butterflies. Tails indicate the range; box indicates the interquartile range; horizontal line within the box indicates the median; black diamonds indicate the mean; black capped error bars indicated standard error of the mean. Compared to males of facultatively- or non- myrmecophilous species, males of obligately myrmecophilous species have **A.** larger ommatidia (µm) of the compound eye, **B.** larger compound eye surface area (mm^2), and **C.** a lower density of sensilla trichodea (no. of sensilla per mm^2 of antenna) on their antennae.

3.5 Discussion

I provide novel evidence that investment in sensory organ morphology is associated with systems of inter-specific mutualisms: males of obligately myrmecophilous species have larger eyes and fewer antennal olfactory sensilla. Lycaenid butterflies that use a broader number of taxonomic families of host plants have a higher density of a type of olfactory antennal sensilla.

The association between obligate myrmecophily and sensory organ morphology is confined to males, which was unexpected since myrmecophilous females are thought to use ant-derived odours as oviposition cues (Fraser et al., 2002; Pierce et al., 2002; Pierce and Elgar, 1985). The observed pattern – whereby males of obligately myrmecophilous species possess larger eyes but a lower density of antennal sensilla trichodea (an olfactory sensilla) – suggests that these males rely heavily on visual cues to locate mates. This is consistent with the general pattern that male butterflies often search for adult females visually (Rutowski, 1991), with the flashing of their iridescent wings acting as visual cues during courtship for many species (White et al., 2015), as well as with findings in the obligate myrmecophile *Jalmenus evagoras* that adult males use the presence of other adult males near pupae as a cue for mate searching (Elgar and Pierce, 1988). For obligately myrmecophilous lycaenids, there is evidence that males are more attracted to plants with ants present than those without ants regardless of the presence of pupae (Elgar and Pierce, 1988), suggesting these myrmecophilous males not only search directly for pupae or adult females but may also search for ants which could lead them to potential mates. While it is widely believed to be ant odours that lycaenids use to detect the presence of ants, there is no reason to presume that ants are emitting pheromone at any time butterflies are searching for them and it is possible that myrmecophilous lycaenids are instead detecting the ants visually. Thus, males of myrmecophilous species may require more sensitive (larger) compound eyes as they are searching not only for mates but also for ants that could lead them to potential mates. There is also evidence that males learn the location of plants on which larvae or pupae are present and tended by ants (Elgar and Pierce, 1988), which could be through visual

landmarking as in many insects that remember resource locations (Zeil, 2012) and thus further increase the need for greater visual sensitivity.

It is unsurprising that the density of sensilla trichodea was greater for individuals from species that have a greater taxonomic diversity of host plants: sensilla trichodea typically detect pheromones and plant volatiles (Gómez and Carrasco, 2008; Hallberg et al., 1994), and variation in the number of host plants used by an organism may influence differences in olfactory antennal sensilla (Chapman, 1982). These observations are consistent with predictions that the complexity of the signalling environment will necessarily drive the morphology of sensory organs to optimise the ability of those organs to detect salient signals and cues against the background noise in the environment (Endler, 1992): a greater complexity of cues to detect is expected to necessitate higher sensitivity in the relevant sensory modality. Intriguingly, while I observed lower sensilla densities in obligately myrmecophilous male lycaenids and no antennal adaptations to myrmecophily in females, myrmecophilous lycaenids typically have broader host-plant range than non-myrmecophilous lycaenids (Pierce and Elgar, 1985). However, this range expansion may be related to the presence of ants (and their associated odours) on plants (Fiedler, 1994; Forister et al., 2011; Pierce and Elgar, 1985) rather than to information provided by the floral chemical cues of the host plants: lycaenid larvae can survive on lower quality host plants if ant attendants are present (Forister et al., 2011), suggesting ant-derived cues may supersede plant derived cues in myrmecophilous species, and thus allow butterflies to make ovipositional 'mistakes' that might not be fatal for their offspring. Additionally, while having greater species-level host plant diversity, myrmecophilous lycaenids may confine their host plants to ones rich in nitrogen (Pierce, 1985; but see Fiedler, 1995), which may limit the diversity of plant odours to which such lycaenids require sensitivity. Nonetheless, the pattern I observe between host plant diversity and antennal sensilla density reinforces the need to examine variation in sensory organ morphology in relation to the main characteristic under investigation (myrmecophily) and in relation to include other life history traits (such as host plant diversity) that are associated with

elements of the main trait of interest and/or are likely to themselves exert a selective influence over sensory systems.

There is considerable evidence that females use ant-derived odours as oviposition cues (Fraser et al., 2002; Pierce et al., 2002; Pierce and Elgar, 1985). Assuming plant odours are also important to obligately myrmecophilous species, this increased complexity of the signalling environment is predicted to subject myrmecophilous females to selection for increased sensitivity to olfactory information (Endler, 1992; Fiedler et al., 1996). This prediction is consistent with what is observed in other systems of interaction: Wittwer et al. (2017) report that evolutionary transitions from social to solitary lifestyles (with a concomitant reduction in reliance on social odours) in halictid bees was associated with a decline in the density of olfactory sensilla. Additionally, differential sensitivity to host-related volatile odours (Chen and Fadamiro, 2007; Ngumbi et al., 2010; Ngumbi et al., 2009) in parasitoid braconid wasps (Hymenoptera: Braconidae) that differ in host specificity is associated with differential abundance of olfactory sensilla (Das et al., 2011). The reason I do not see a higher density of olfactory sensilla on the antennae of females of obligately myrmecophilous species in my study could be related to how female lycaenids use ant-derived odours as oviposition cues: the larvae of obligately myrmecophilous species are unlikely to survive without ant attendants, and so obligately myrmecophilous females may use almost exclusively ant odours as oviposition cues rather than also using plant odours. The olfactory complexity associated with the need to detect only ant odours, rather than both ant and host plant odours, may be functionally equivalent to that experienced by non-myrmecophilous species using only plant odours as oviposition cues, and so obligately myrmecophilous females would not experience strong selection pressures for specialisation of their antennal morphology. Additionally, myrmecophilous species may rely on the odours of several species of ants, the number and diversity of which may be a stronger predictor of sensilla density in obligate mutualists.

The lack of difference in sensory organ morphology between facultatively myrmecophilous and non-myrmecophilous species is noteworthy. The larvae of facultatively myrmecophilous species do not depend on ant attendants for survival (Eastwood and Fraser, 1999; Fiedler et al., 1996), meaning that the need for ant-mediated oviposition amongst adults is far lower than for obligately myrmecophilous species. Additionally, facultative myrmecophily represents a broad spectrum: the larvae of some facultatively myrmecophilous species may be more regularly tended by ants than not associated with them; and the larvae of some species may be more regularly not tended by ants with stable associations with ants being rare (Eastwood and Fraser, 1999). Consequently, selection pressures for adults of facultatively myrmecophilous species to be sensitive to ant odours for ant-mediated oviposition may be low or negligible, and so the morphology of their sensory organs equivalent to that of non-myrmecophilous species. The results of my study suggest that it is predominantly obligate interspecific mutualism that is associated with morphological specialisation of the sensory organs, and that the signal detection requirements of facultatively mutualistic interactions (with such interactions perhaps being context-dependent or even largely opportunistic) are not sufficient to act as a strong selection pressure on sensory organ morphology. Interestingly, facultatively myrmecophilous and non-myrmecophilous species in my sample generally exhibited greater variation in sensilla density than obligately myrmecophilous species (despite almost equal sample sizes; see Table 2), which is consistent with the view that they experience weaker selection on sensory organ morphology. Investigating sensory organ differences between obligately and facultative mutualistic species in other systems of interspecific mutualism may elucidate the generality of this phenomenon.

The negative relationship between body size (proxied by forewing length) and the densities of some antennal sensilla is not unusual and is seen in butterflies and other insects in other ecological contexts (Freelance et al., 2021b). While counterintuitive in that a larger antenna (antenna length increases with body size in my study) can theoretically support more sensilla, this pattern likely reflects that the absolute number of sensilla possessed by an antenna is conserved for a given taxa. The even

distribution of those sensilla across a larger antenna would necessarily reduce the number of sensilla per unit area of antenna. This pattern may represent a constraint posed by the energetically expensive nature of sensory systems (Niven and Laughlin, 2008): with an absolute number of sensilla for a given taxon, a larger antenna with a fixed number of sensilla distributed across it more sparsely would be energetically cheaper to develop and maintain than would be a larger antenna where sensilla number increased with antennal size.

In conclusion, this study provides the first direct evidence that the sensory organs required to detect the signals associated with mutualistic associations are themselves subject to selection pressures. Performing similar assessments to this study in other systems of mutualism may further elucidate how the associated selection pressures shape the sensory ecology of species that engage in such interactions.

3.6 Acknowledgements

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Chapter 4: The association between sensory organ morphology and the photic environment across multiple taxonomic orders of insects.

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4.1 Abstract

The perception of cues and signals in visual, olfactory and auditory modalities underpins all animal interactions and provides crucial fitness-related information. Sensory organ morphology is under strong selection to optimise detection of salient cues and signals in a given signalling environment; the most well studied example being selection on eye design in different photic environments. Many dim-light active species have larger compound eyes relative to body size, but little is known about differences in non-visual sensory organ morphology between diurnal and dim-light active insects. Here, we compare the micromorphology of the compound eyes (visual receptors) and antennae (olfactory and mechanical receptors) in representative pairs of day- and dim-light- active species spanning multiple taxonomic orders of insects. We find that dim-light activity is associated with larger compound eye ommatidia and larger overall eye surface area across taxonomic orders but find no evidence that in dim-light active insects, morphological adaptations that enhance the sensitivity of the eye are accompanied by morphological traits of the antennae that may increase sensitivity to olfactory, chemical or physical stimuli. This suggests that the ecology and natural history of species is a stronger driver of sensory organ morphology than is selection for complementary investment between sensory modalities.

4.2 Keywords

Antenna, compound eye, diurnal, nocturnal, photic environment, sensory ecology

4.3 Introduction

Animals perceive information about their extrinsic environment, including the location and nature of potential mates, food sources, shelter, or predators through a variety of modalities (e.g. light, odour, sound). As efficiency is essential for biological fitness, the considerable energetic resources required to develop and maintain elaborate sensory systems (Niven & Laughlin, 2008) mean that natural selection is expected to favour sensory organs with morphology optimised to detect salient cues and signals from the background information in the environment (Elgar et al., 2018; Endler, 1992).

With millions of years of a stable light/dark cycle, the photic environment is a consistent selection pressure shaping the evolution of sensory structures. Indeed, sensory adaptations to the photic environment in which an animal is active are well documented. For example, populations of Mexican cave fish (*Astyanax mexicanus*) living in lightless caves no longer have functional eyes, while eyes are retained in populations that remain on the surface (Dowling et al., 2002). Similarly, reduced investment in eyes is observed in arthropod troglofauna (cave-dwelling animals) including crustacea (Christiansen, 2012; Hobbs III, 2012), cave-crickets (Lavoie et al., 2007), leiodid beetles (Friedrich et al., 2011; Peck, 1973) and dytiscid beetles (Tierney et al., 2018).

Many crepuscular (active during twilight) or nocturnal (active beyond astronomical twilight) – collectively referred to as dim-light active – arthropods frequently have sensory adaptations specific to their photic environment (Tierney et al., 2017; Warrant & Dacke, 2011; Warrant, 2006; Wcislo & Tierney, 2009). Arthropod compound eyes are composed of ommatidia, each of which is an independent photoreceptive unit that distinguishes brightness and colour. For example, compared with their closest diurnal relatives, the average ommatidia diameter is greater in the compound eye of obligate dim-light foraging bees (order Hymenoptera, superfamily Apoidea) (Jander & Jander, 2002; Wcislo & Tierney, 2009) and wasps (Hymenoptera: Mutillidae, Polistinae, Vespinae) (Warrant, 2008), crepuscular or nocturnal *Myrmecia* ants (Hymenoptera: Formicidae) (Greiner et al., 2007; Narendra

et al., 2011), night-flying leafcutter ants of the genus *Atta* (Hymenoptera: Formicidae) (Moser et al., 2004) and night-flying onitine dung beetles (Coleoptera: Scarabaeidae) (McIntyre & Caveney, 1998). Larger ommatidia capture more photons and can thus detect changes in luminance in dimmer light (Greiner et al., 2007; Jander & Jander, 2002; Land, 1997; Tierney et al., 2017). Consequently, low levels of ambient light should favour larger ommatidia diameter to improve visual sensitivity. Larger ommatidia are often accompanied by distinct arrangement of the internal structure of the compound eye (Land, 1997; Warrant, 2017) as well as physiological adaptations of the photoreceptors and of the neural circuitry involved in the processing of spatial and temporal visual information (Stöckl et al., 2016b; Warrant, 2017).

While there appear to be consistent adaptations of insect compound eyes to dim-light activity, most studies are taxonomically limited to the Hymenoptera, Coleoptera and Lepidoptera. Furthermore, it is unclear how differences in compound eye morphology compare with differences in the sensory organs that insects use to detect odours and vibrations: the antennae. Selection pressures associated with dim-light activity may not only favour adaptations that increase the sensitivity to light but also adaptations that increase sensitivity to information in complementary modalities, such as odour and sound (provided that salient information is available in those modalities). For example, the nocturnal hawkmoth *Deilephila elpenor* (Lepidoptera: Sphingidae) preferentially uses olfactory rather than visual cues while the diurnal hawkmoth *Macroglossum stellatarum* shows the opposite preference (Balkenius et al., 2006). This behavioural difference is accompanied by differences in the abundance of types of antennal sensilla (Balkenius et al., 2006), which are the sensory hairs and pores on antennae that detect odours, vibrations, stretch, temperature, humidity and carbon dioxide (Chapman, 1982; Elgar et al., 2018). The density (number per unit area) of sensilla is an ecologically-relevant measure of resource investment in insect antennae, and is positively associated with the strength of both behavioural (Gill et al., 2013) and physiological (Spaethe et al., 2007) responses to olfactory stimuli. Differences in the abundance of antennal sensilla between the nocturnal bull ant *Myrmecia pyriformis* and similarly-sized diurnal ant species have also been documented (Ramirez-

Esquivel et al., 2014), although the observed differences may not be due solely to differences in the photic environment in which the ants are active (Ramirez-Esquivel et al., 2014). Interestingly, the antennae of nocturnal fireflies (Coleoptera: Lampyridae) are relatively shorter than those of their diurnal relatives (Stanger-Hall et al., 2018), although it is not known if this corresponds to differences in antennal sensilla density.

In this study, we simultaneously assess differences in the morphology of the compound eyes and antennae in representative pairs of diurnal and dim-light active species across multiple taxonomic orders of Australian insects. We ask whether there are consistent eye and antennal adaptations to behaviours in dim-light environments across taxonomic orders – specifically, is dim-light activity consistently associated with greater ommatidia diameter, greater overall size (area) of the compound eye and, as seen in moths (Balkenius et al., 2006) and bull ants (Ramirez-Esquivel et al., 2014), a greater density of antennal sensilla? Consistent patterns would suggest that changes to information availability in one sensory modality (e.g. vision) may not only favour morphological adaptations that increase the sensitivity in that modality but also adaptations that increase sensitivity to information in complementary modalities (e.g. olfaction). Alternatively, inconsistent investment into different sensory modalities would indicate that they are typically independent of each other and primarily driven by the ecology and natural history of the species/family rather than by complementary investment.

4.4 Materials and Methods

Compiling images of the eyes and antennae of a comprehensive sample of insects across taxonomic orders and fitting this into a phylogenetic comparative framework is not possible without a complete phylogeny of insects. Instead, we provide taxonomic generality by selecting 12 closely related pairs of species that vary in the photic environment in which they are active for foraging and reproduction. Thus, we compare a day-active (diurnal) and dim-light active (nocturnal and/or crepuscular) species in each pair, with each species pair belonging to a different family and spanning six taxonomic orders

of insects (Table 4.1). We ensured that species within a pair overlapped in habitat type (e.g. temperate forest) and geographic range. Two to six specimens of each species were obtained from Museum Victoria (Melbourne, Victoria, Australia) or the Australian National Insect Collection (Commonwealth Scientific and Industrial Research Organisation) for morphological analysis (Table 4.1); with the exception of the velvet ants (Hymenoptera: Mutillidae), our species pairs were confined to Australian taxa for sampling convenience and to provide continental consistency. As there are no nocturnal velvet ants found in Australia, the nocturnal species used in our analysis is North American with the specimens obtained from Utah State University (Utah, USA).

To image the sensory organs, each pinned uncoated specimen underwent low-vacuum scanning electron microscopy (SEM) using a FEI Quanta 200F scanning electron microscope (10kV acceleration voltage, spot size 2.0, 0.5 mBar pressure) at the Bio21 Advanced Microscopy Facility (Bio21 Institute, The University of Melbourne, Victoria, Australia) (Halictidae specimens) or a Hitachi TM3030 Plus tabletop scanning electron microscope (5kV acceleration voltage) at the Australian National Insect Collection. The katydid (Orthoptera: Tettigoniidae) specimens were too large to be imaged using SEM without removing the antennae from museum specimens, and instead underwent stereomicroscopy (160x magnification) using a Leica M205 A fitted with a Leica DFC 500 camera at the Australian National Insect Collection. Using the microscope images, we determined for each specimen: the average diameter of the ommatidia of the compound eye (diameter of three ommatidium averaged; μm); the average surface area of the compound eye (mm^2); and the average density of each type of antennal sensilla (number of sensilla in a given area of antenna; sensilla per mm^2). Eye ommatidia size provides information about sensitivity to visual information (Jander & Jander, 2002; Land, 1997; Warrant, 2017) and antennal sensilla density is a behaviourally relevant indicator of sensitivity to olfactory and tactile cues (Elgar et al., 2018; Gill et al., 2013; Spaethe et al., 2007).

We measured ommatidia from the anteromedial aspect of each compound eye (i.e. the ommatidia that face directly in front of the insect), thereby accounting for potential differences in ommatidia diameter between regions of the compound eye (Perl & Niven, 2016) and for potential differences between taxa related to whether a species spends most of its time looking above (terrestrial species) or below (aerial species). Antennal sensilla were identified and classified into three classes: olfactory (detects airborne odours), contact chemosensory (detects chemicals on a surface across which the antenna is palpated) and mechanosensory (responds to vibrations or mechanical deformation of the sensilla) (Figure 4.1, Table 4.2). Pore-like sensilla were not consistently observed on antennae and were not included in this analysis. This is unlikely to have affected our assessment of olfactory, chemosensory or mechanosensory sensilla because pore-like sensilla are often predominantly thermoreceptors or hygroreceptors. We accounted for differences in sensilla density between antennal segments/regions for each taxonomic family, by imaging the sensilla on the most populated part of the antenna that was consistently observable: ventro-lateral side of the proximal antennomer of the antennal flagellum for Odonata; dorso-lateral surface of the antennal club for Scarabaeidae (Coleoptera); ventro-lateral side of the 10th-most distal antennomer for Sphingidae (Lepidoptera); and the dorso-lateral surface of the distal antennomer for all other specimens. Focusing on the antennal region for each taxon that is the most densely populated minimises the potential for underestimating the diversity of sensilla types possessed by a given taxon and maximises the behavioural relevance of our data, as sensilla density on the most populated section of antennae can be a behaviourally relevant indicator of olfactory sensitivity (Elgar et al., 2018; Gill et al., 2013). While the abundance and distribution of types of sensilla along the length of antennae may vary, it is unlikely to consistently differ between day active and dim-light active species and to thus introduce a bias in our results. Only undamaged eyes or antennae were imaged and analysed.

As body size is generally larger for dim-light active species compared with closely related diurnal bee species (Wcislo & Tierney, 2009), we obtained relevant measures of body size to account

for the potential influence of body size allometry on ommatidia size (Jander & Jander, 2002; Schwarz et al., 2015) and antennal sensilla density (Spaethe et al., 2007). To obtain measures of body size, we either imaged the relevant body parts of the specimen under the scanning electron microscope or took digital images of the specimens using a Canon 6D DSLR with Canon EF-L 100mm f2.8 macro lens (Canon, Tokyo, Japan) with a ruler included as a scale. Body size was measured as average elytra length for the Coleoptera (Frank et al., 2005), as the ratio of average wing length to thorax length for the Diptera (Barker & Krebs, 1995), as head width just posterior to the compound eyes for the Hymenoptera (Boudinot & Fisher, 2013; Spaethe et al., 2007; Wild, 2007), as average forewing length for the Lepidoptera (van Hook et al., 2012) and Odonata (Johnson et al., 2013) and as average femur length for the Orthoptera (Whitman, 2008). All image analysis was performed using FIJI (Schindelin et al., 2012).

We used the natural log of each of ommatidia diameter, density of contact chemosensory antennal sensilla and density of mechanosensory antennal sensilla to normalise the distributions. For each metric, we fitted a linear model including active foraging time (day active, dim-light active) and body size as fixed effects and taxonomic family (equivalent to species pair ID) as a random effect, with variance partitioned using restricted maximum likelihood. All statistical analyses were performed using JMP 13.1.0 for Windows (SAS Institute, Cary, NC, USA).

Table 4.1. Sister pairs of day- and dim-light- active species used with species sample size for each sensory morphology metric. Members of a sister pair are from the same taxonomic family (or genus). Sample sizes represent the number of individuals of a given species for which an average ommatidia diameter, average eye surface area or average density of the specified class of antennal sensilla was calculated.

		Day active						Dim-light active					
			Sample size						Sample size				
Taxonomic order	**Taxonomic family**	**Species**	**Ommatidia**	**Eye surface area**	**Olfactory sensilla**	**Contact chemosensilla**	**Mechanosensilla**	**Species**	**Ommatidia**	**Eye surface area**	**Olfactory sensilla**	**Contact chemosensilla**	**Mechanosensilla**
Coleoptera	Carabidae	*Cicindela semicincta*	5	5	5	5	5	*Megacephala cylindrica*	4	5	4	4	4
	Scarabaeidae	*Phyllotocus macleayi*	6	6	6	6	6	*Sericesthis geminata*	5	5	5	5	5
Diptera	Culicidae	*Aedes albopictus*	3	3	3	3	3	*Culex quinquefasciatus*	3	3	2	2	2
Hymenoptera	Halictidae	*Mellitidia tomentifera*	6	6	6	6	6	*Reepenia bituberculata*	5	5	5	5	5
	Formicidae	*Myrmecia croslandi*	6	6	6	6	6	*Myrmecia pyriformis*	6	6	6	6	6
	Mutillidae	*Ephutomorpha ferruginata*	5	5	5	5	5	*Odontophotopsis melicausa*	3	3	3	3	3
Lepidoptera	Hesperiidae	*Netrocoryne repanda*	5	5	4	4	4	*Chaetocneme denitza*	4	4	3	3	3
	Sphingidae	*Macroglossum micacea*	4	4	4	4	4	*Macroglossum vacillans*	4	4	4	4	4
Odonata	Austrocorduliidae	*Austrocordulia refracta*	4	3	2	0	2	*Apocordulia macrops*	5	5	3	0	3
	Telephlebiidae	*Austroaeschna atrata*	5	5	4	0	4	*Telephlebia brevicauda*	5	5	2	0	2
Orthoptera	Gryllidae	*Bobilla victoria*	4	4	4	4	4	*Pteronemobius truncatus*	3	3	3	3	3
	Tettigoniidae	*Terpandrus jumbunna*	2	2	2	2	2	*Terpandrus calperum*	2	2	2	2	2
			55	54	51	45	51		49	50	42	37	42

Table 4.2. Types of antennal sensilla identified and included in the analysis for each taxonomic family pair. While pore-like types of sensilla may have been identified, they were not included because they were not consistently observed on the antennae of each individual specimen for a given species pair.

Taxonomic order	Taxonomic family	Antennal sensilla types identified and included for analysis			References for sensilla typing
		Olfactory	Contact chemosensory	Mechanosensory	
Coleoptera	Carabidae	Trichodea, coeloconica	Basiconica	Chaetica	Merivee et al. (2002)
	Scarabaeidae	Trichodea, coeloconica, auricillica	Basiconica	Chaetica	Handique et al. (2017); Romero-López et al. (2010); Shao et al. (2019)
Diptera	Culicidae	Trichodea, coeloconica	Basiconica	Chaetica	Ibrahim et al. (2018); Seenivasagan et al. (2009)
Hymenoptera	Halictidae	Trichodea, placodea	Basiconica	Chaetica	Carvalho, et al. (2017); Frasnelli et al. (2010); Freelance et al. (2019)
	Formicidae	Trichodea, trichodea curvata	Basiconica	Chaetica	Dumpert (1972); Freelance et al. (2019)
	Mutillidae	Trichodea, placodea	Basiconica	Chaetica	Undescribed; based on sensilla typing for Apiidae
Lepidoptera	Hesperiidae	Trichodea, auricillica	Basiconica	Chaetica	Abu-shall and Tawfeek (2015); Xiangqun et al. (2014)
	Sphingidae	Trichodea, auricillica, coeloconica	Basiconica	Chaetica	Balkenius et al. (2006)
Odonata	Austrocorduliidae	Coeloconica	N/A	Deeply-sunken	Rebora et al. (2008); Rebora et al. (2010)
	Telephlebiidae	Coeloconica	N/A	Deeply-sunken	Rebora et al. (2008); Rebora et al. (2010)
Orthoptera	Gryllidae	Trichodea	Basiconica	Chaetica	Kostromytska et al. (2015)
	Tettigoniidae	Trichodea	Basiconica	Chaetica	Schneider and Römer (2016)

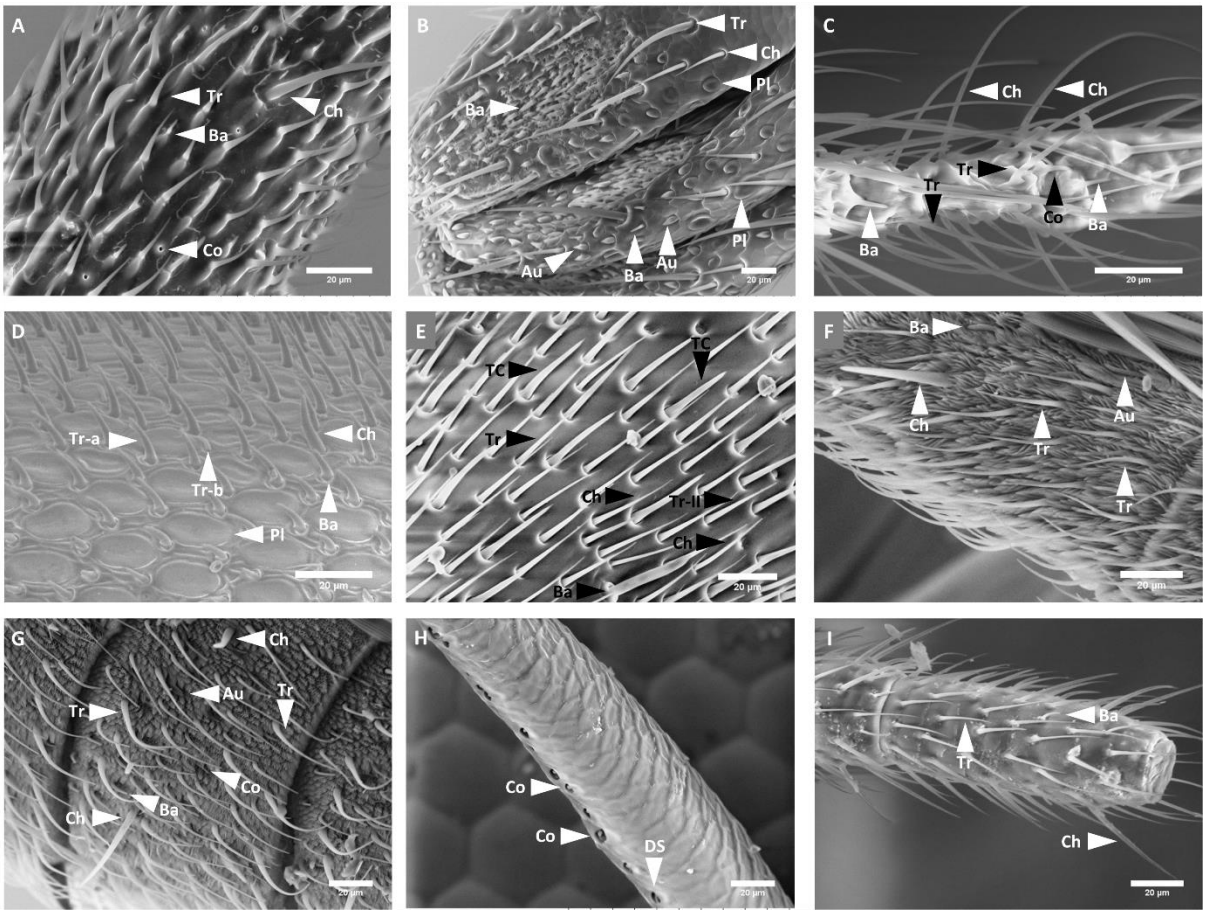

Figure 4.1. Electron micrographs displaying the types of antennal sensilla identified and included in the analysis for each taxon. Au = auricillica; Ba = basiconica; Ch = chaetica; Co = coeloconica; DS = deeply sunken; Pl = placodea; Tr = trichodea; Tr-a = trichodea type a; Tr-b = trichodea type b; TC = trichodea curvata; Tr-II = trichoid type II. The class (olfactory, contact chemosensory, mechanosensory) for each type of sensilla identified for each taxon is listed in Table 2; note that the antennae of Odonata do not possess contact chemosensilla. All scale bars are 20µm in length. **A.** *Cicindela semicincta* (tiger beetle; Coleoptera: Carabidae). **B.** *Phyllotocus macleayi* (flower scarab beetle; Coleoptera: Scarabaeidae). **C.** *Aedes albopictus* (Asian tiger mosquito; Diptera: Culicidae). **D.** *Mellitidia tomentifera* (an Australian native bee; Hymenoptera: Halictidae). **E.** *Myrmecia pyriformis* (bull ant; Hymenoptera: Formicidae). **F.** *Netrocoryne repanda* (butterfly; Lepidoptera: Hesperiidae). **G.** *Macroglossum micacea* (hawkmoth; Lepidoptera: Sphingidae). **H.** *Austrocordulia refracta* (eastern hawk dragonfly; Odonata: Austrocorduliidae). **I.** *Bobilla victoria* (cricket; Orthoptera: Gryllidae).

4.5 Results

As predicted, the ommatidia diameter (natural log transformed) is larger for dim-light active than for day active insects ($F_{1,94.73}$ = 8.794, p = 0.004; Table 4.3A; Fig 4.2A). The relationship between ommatidia size and body size was not statistically significant (β = 0.012, $F_{1,35.61}$ = 3.868, p = 0.057; Table 4.3A). The natural log of compound eye surface area is also larger for dim-light active than for day active insects ($F_{1,96.35}$ = 4.423, p = 0.038; Table 4.3B; Fig 4.2B) and was positively associated with body size (β = 0.088, $F_{1,63.98}$ = 46.50, p = <0.0001; Table 4.3B).

Day active and dim-light active insects do not differ in the density of olfactory, contact chemosensory or mechanosensory antennal sensilla (Table 4.3C–E; Fig 4.2C–E). There is a significant negative correlation between body size and the density of olfactory and contact chemosensory antennal sensilla: smaller individuals had higher densities of these antennal sensilla (Table 4.3C–E).

Taxonomic family explained 85.51% (p = 0.074), 89.70% (p = 0.026), 54.05% (p = 0.041), 64.43% (p = 0.052) and 75.97% (p = 0.031) of the variation in ommatidia diameter, compound eye surface area, olfactory sensilla density, contact chemosensory sensilla density and mechanosensory sensilla density respectively (Table 4.3A–E).

For each sensory organ metric, means and standard deviations of day and dim-light active groups for each taxonomic order and family are described in Table C1 (eyes) and Table C2 (antennae) of Appendix C.

Table 4.3. Mixed effects models explaining variation in the compound eye ommatidia diameter, compound eye surface area and the densities of antennal sensilla between day active and dim-light active insects.

Model/parameter			**Statistics**	
A. Ln (ommatidia diameter)				
Model fit			R^2 adjusted: 0.917	n = 104
Parameter estimates	***β***	***SE***	***t ratio***	***p > |t|***
Intercept	3.194	0.131	24.45	<0.0001
Foraging time [day]	-0.045	0.015	-2.970	0.004
Body size (mm)	0.011	0.005	1.970	0.057
Random effects			***% variation explained***	***Wald's p-value***
Taxonomic family			85.51	0.074
Fixed effects		***df***	***F ratio***	***p > F***
Foraging time (day, dim-light)		1,94.73	8.794	0.004
Body size		1,35.61	3.868	0.057
B. Ln (compound eye surface area)				
Model fit			R^2 adjusted: 0.966	n = 104
Parameter estimates	***β***	***SE***	***t ratio***	***p > |t|***
Intercept	-0.392	0.341	-1.150	0.267
Foraging time [day]	-0.073	0.035	-2.100	0.038
Body size (mm)	0.088	0.013	6.820	<0.0001
Random effects			***% variation explained***	***Wald's p-value***
Taxonomic family			89.70	0.026
Fixed effects		***df***	***F ratio***	***p > F***
Foraging time (day, dim-light)		1,96.35	4.423	0.038
Body size		1,63.98	46.50	<0.0001
C. Olfactory antennal sensilla density				
Model fit			R^2 adjusted: 0.700	n = 93
Parameter estimates	***β***	***SE***	***t ratio***	***p > |t|***
Intercept	11422.2	1823.06	6.270	<0.0001
Foraging time [day]	-597.3	409.2	-1.460	0.148
Body size (mm)	-311.9	88.47	-3.530	0.003
Random effects			***% variation explained***	***Wald's p-value***
Taxonomic family			54.05	0.041
Fixed effects		***df***	***F ratio***	***p > F***
Foraging time (day, dim-light)		1,85.20	2.131	0.148
Body size		1,16.39	12.43	0.003

D. Ln (contact chemosensory antennal sensilla density)

Model fit			R^2 adjusted: 0.736	n = 82
Parameter estimates	***β***	***SE***	***t ratio***	***p > |t|***
Intercept	6.701	0.234	28.64	<0.0001
Foraging time [day]	0.073	0.047	1.550	0.126
Body size (mm)	-0.043	0.015	-2.950	0.007
Random effects			***% variation explained***	***Wald's p-value***
Taxonomic family			64.43	0.052
Fixed effects		***df***	***F ratio***	***p > F***
Foraging time (day, dim-light)		1,77.23	2.399	0.126
Body size		1,25.51	8.680	0.007

E. Ln (mechanosensory antennal sensilla density)

Model fit			R^2 adjusted: 0.811	n = 92
Parameter estimates	***β***	***SE***	***t ratio***	***p > |t|***
Intercept	6.545	0.275	23.78	<0.0001
Foraging time [day]	0.064	0.042	1.530	0.130
Body size (mm)	-0.024	0.013	-1.880	0.072
Random effects			***% variation explained***	***Wald's p-value***
Taxonomic family			75.97	0.031
Fixed effects		***df***	***F ratio***	***p > F***
Foraging time (day, dim-light)		1,85.28	2.341	0.130
Body size		1,25.29	3.516	0.072

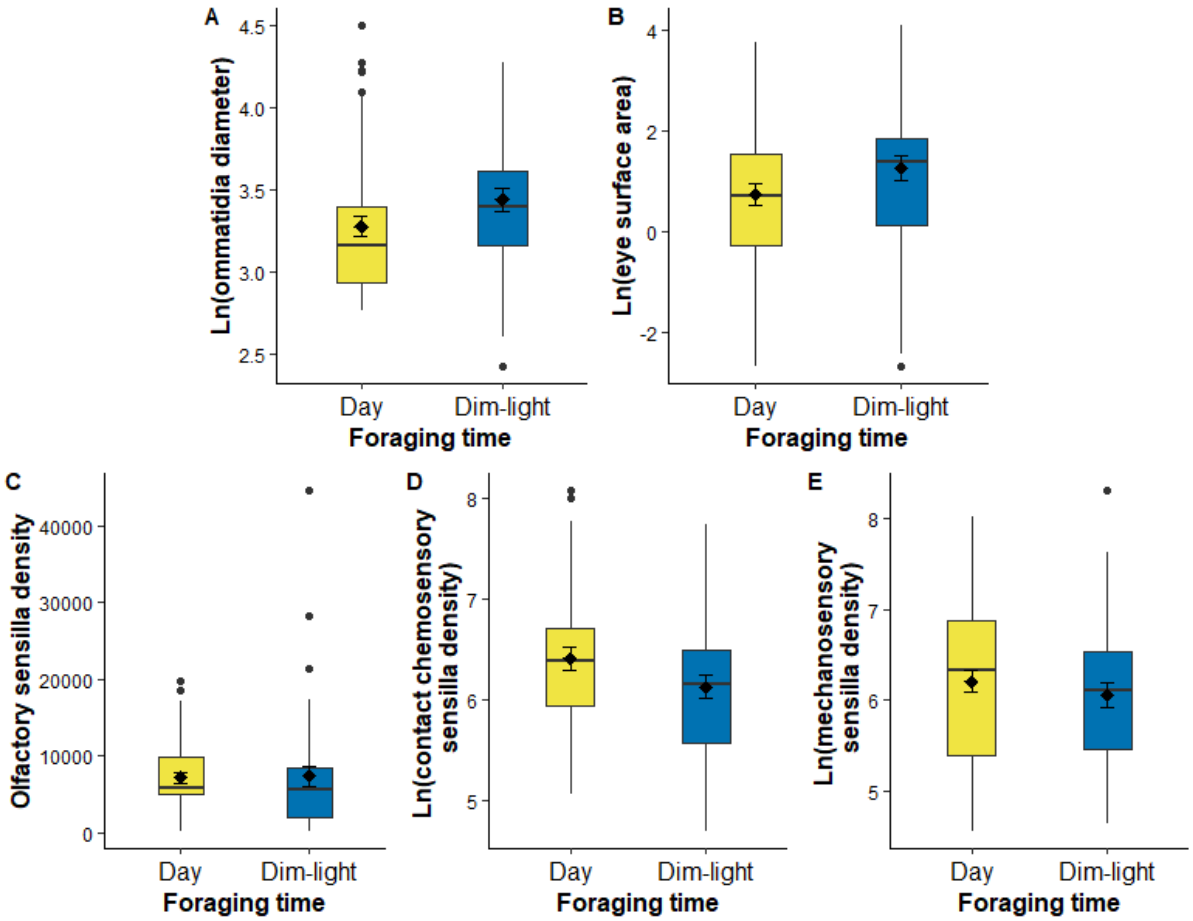

Figure 4.2. The influence of the photic environment on compound eye and antennal morphology. Tails indicate the range; box indicates the interquartile range; horizontal line within the box indicates the median; black diamonds indicate the mean; black capped error bars indicate standard error of the mean. **A.** The natural log of average compound eye ommatidia diameter (µm) is larger for dim-light active than for day active species ($F_{1,94.73}$ = 8.794, p = 0.004). **B.** The natural log of compound eye surface area (mm^2) is larger for dim-light active than for day active species ($F_{1,96.35}$ = 4.423, p = 0.038). **C.** The density of olfactory antennal sensilla does not vary between day active and dim-light active insects ($F_{1,85.20}$ = 2.131, p = 0.148). **D.** The density of the natural log of contact chemosensory sensilla does not vary between day active and dim-light active insects ($F_{1,77.23}$ = 2.399, p = 0.126). **E.** The density of the natural log of mechanosensory sensilla does not vary between day active and dim-light active insects ($F_{1,85.28}$ = 2.341, p = 0.130).

4.6 Discussion

Our results show that dim-light activity is associated with larger compound eye ommatidia and larger overall compound eye size across taxonomic orders of insects, but there is no corresponding difference in antennal sensilla densities. There was evidence of body size allometry related to the distribution of some classes of antennal receptors.

The predicted and observed association between dim-light activity and larger compound eye ommatidia is consistent with results in bees (Jander & Jander, 2002; Wcislo & Tierney, 2009) and ants (Greiner et al., 2007). Larger ommatidia enable greater photon capture, and therefore sensitivity, though at the expense of spatial resolution (Jander & Jander, 2002; Warrant, 2017); however, rhabdomere size and receptor photon-responses are also important considerations when assessing visual sensitivity (Horridge, 2005). Spatial (across ommatidia) and temporal (across time) summation of photons during visual information processing is also beneficial for vision in dim light for insects (Stöckl et al., 2016a; Warrant, 2017): future studies might explore whether the increased ommatidia diameter in dim-light active insects is consistently accompanied by this visual processing adaptation. The observation that dim-light activity is associated with larger compound eye size in unsurprising, as an increase in ommatidia diameter would result in an increase in overall eye size unless the number of ommatidia were reduced, which is unlikely to be favoured by selection as it would reduce the visual acuity of the eye (Jander & Jander, 2002).

Enhanced sensitivity in other sensory modalities – manifested as elaborated antennae and/or more numerous antennal sensilla – in response to dim-light living may be expected, with such adaptations thought to compensate for reduced availability of visual information. For example, nocturnal hawkmoths (Lepidoptera: Sphingidae) tend to preferentially use olfactory cues over visual cues while their diurnal counterparts show the opposite preference (Balkenius et al., 2006), suggesting increased reliance on non-visual sensory systems. However, our results do not provide evidence that dim-light activity is associated with increased antennal sensilla density and thus contradict the view

that dim-light activity is associated with increased morphological investment in antennae. This view is also contradicted by recent findings in fireflies (Coleoptera: Lampyridae) that were in the opposite direction of the predicted pattern, with nocturnal firefly species having relatively shorter antennae than diurnal species (Stanger-Hall et al., 2018). Investment in non-visual sensory organs may also depend on whether species are obligately or facultatively dim-light active, as selection for morphological specialisation is expected to be stronger for obligately dim-light active species (Wcislo & Tierney, 2009). Indeed, facultatively nocturnal bees do not have the visual morphology adaptations that are typical of obligately nocturnal species, suggesting that behavioural change precedes structural adaptations (Wcislo & Tierney, 2009). Sufficiently detailed natural history information is not available for all species in our analysis to determine whether each dim-light active species was obligately or facultatively dim-light active, however future studies of this nature would ideally make this distinction. The availability of information in non-visual sensory modalities is also likely to influence investment in antennal morphology, as the benefit of increasing sensitivity for a given sensory channel (e.g. olfaction) would depend on the availability of salient cues and signals in that sensory channel. Indeed, information in non-visual sensory channels may not be equally available for dim-light active species across taxonomic orders of insects, and such natural history differences potentially explain why our results do not support the view that dim-light activity is consistently associated with a higher density of antennal sensilla.

While our results do not provide evidence of increased investment in non-visual sensory organs in dim-light active insects, they also do not support inverse resource allocation between ommatidia and antennal sensilla that has been documented in fireflies (diurnal species have smaller eyes and longer antennae compared with nocturnal species) (Stanger-Hall et al., 2018) and multiple species of *Drosophila* (Diptera: Drosophilidae) (Keesey et al., 2019), and is frequently characteristic of the troglomorphy exhibited of cave-dwelling arthropods (Christiansen, 2012; Hobbs III, 2012) including leiodid beetles (Peck, 1973; Peck, 1977; Peck, 1998). Finite energetic resources mean that elaboration of one morphological structure may be at the expense of another structure (Emlen, 2001;

Nijhout & Emlen, 1998), and this might be especially evident across different sensory modalities, given the energetically expensive nature of complex sensory systems (Keesey et al., 2019; Niven & Laughlin, 2008). Nonetheless, our findings may be unsurprising in three ways. Firstly, animals typically use information in multiple sensory modalities (e.g. light and odour) simultaneously (Partan & Marler, 1999) and so it may be disadvantageous to invest heavily in receptors for one sensory modality at the expense of receptors for another modality; we note that taxa often differ in their reliance on information in a given sensory modality. Secondly, predicted negative correlations between investment in morphological structures are not commonly observed (Nijhout & Emlen, 1998; van Noordwijk & de Jong, 1986): inverse resource allocation between traits can be context dependent with variation in the direction of relationships between structures influenced by environmental conditions (Sgrò & Hoffmann, 2004) and, at least for animals which feed continuously, by changes in resource acquisition (Nijhout & Emlen, 1998). Thirdly, many instances of negative relationships in investment between sensory systems involve an absence of information in one sensory modality. For example, many species of cave-dwelling arthropods exhibit regressed visual systems (e.g. smaller compound eyes with smaller and/or fewer ommatidia) and enhanced olfactory systems (e.g. longer antennae or higher densities of antennal sensilla) (Christiansen, 2012; Hobbs III, 2012; Peck, 1973; Peck, 1977; Peck, 1998). In the cavernous environment, characterised by the absence of natural light, natural selection would likely favour diversion of energetic resources from visual to non-visual sensory systems, as the energetically expensive visual system can no longer detect information that contributes to an individual's fitness (Niven & Laughlin, 2008; Stearns, 1989).

In conclusion, we show that dim-light active insects, across multiple taxonomic families, have larger compound eyes and ommatidia but no commensurate increase in the density of antennal receptors. This association of dim-light activity with comparatively greater ommatidia diameter and larger overall compound eye size is consistent with the predicted close relationship between sensory organ morphology, signal perception and the signalling environment (Endler, 1992), excluding radical departures in natural history among the taxa being compared. Given the potential for the use of

insects to enhance restoration of anthropogenically-degraded habitats (Prather & Laws, 2018; Elizalde et al., 2020) and the need to inform conservation efforts with sensory ecology in response to the prevalence of anthropogenically-induced environmental change (Lim et al., 2008), understanding the interaction between sensory system adaptation and life history specialisation is of increasing relevance. Knowledge of sensory organ morphology has implications for understanding how long-term anthropogenic changes to the photic environment – such as the penetration of artificial light at night into the once-dark night-time environment (reviewed by Hopkins et al., 2018; Tierney et al., 2017) or the presence of daytime light-reducing smog (White, 1976) – may influence species responses, especially since many insect species have relatively short generation times and temporally specific mating patterns. Such influences may have cascading effects upon insect community dynamics through altered signalling and communication behaviours. Insects provide important ecosystem services, including pollination, nutrient cycling, seed dispersal and bioturbation that are not only of obvious environmental importance but also considerable economic value (Elizalde et al., 2020; Losey & Vaughan, 2006): effective signalling and communication within insect communities, at least for social insects, is essential to the efficiency of the provision of these ecosystem services (Dyer, 2002; Elizalde et al., 2020).

4.7 Acknowledgements

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Chapter 5: The association between sensory organ morphology and long-term conservation captive breeding in the critically endangered Lord Howe Island stick insect *Dryococelus australis*

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5.1 Abstract

1. Captive breeding programs are key to many threatened species reintroduction strategies but could potentially be associated with adaptations to captivity that are maladaptive in their natural habitat. Despite the importance of sensory ecology to biological fitness, few studies explore sensory system adaptations to captivity. Captive environments are devoid of predators and provide ready access to food sources and potential mates, thus reducing the need for individuals to use signals and cues to identify and locate resources or detect potential threats. With reduced complexity of the signalling environment, relaxation of selective pressures may favour reduced investment in sensory organs in captivity.
2. We test this prediction in an iconic critically endangered invertebrate, the Lord Howe Island stick insect *Dryococelus australis*, which was extirpated from the island in the 1920s/30s and rediscovered on a nearby volcanic stack, Ball's Pyramid, in 2001.
3. Using historical specimens from these populations and specimens from the 8–10th and 14th generations of a long-term conservation captive breeding program, we examine differences in behaviourally relevant morphological traits of the compound eyes (visual organs) and antennae (olfactory organs).

4. We find that captivity is associated with smaller compound eye size, smaller eye ommatidia and reduced density of antennal odour receptors. These morphological changes are indicative of reduced sensitivity to visual and olfactory signals and cues, and therefore are likely to have fitness implications when reintroducing a captive population into the wild.
5. Synthesis and applications: We observe differences in sensory organ morphology between wild and captive bred populations of the critically endangered Lord Howe Island stick insect. Our results emphasise the importance of incorporating evolutionary biology and sensory ecology into conservation program design: to minimise the potential for captive breeding environments to compromise sensory systems that support appropriate behaviours upon reintroduction of populations into a natural habitat.

5.2 Keywords

Antenna, captive breeding, compound eye, conservation, *Dryococelus australis*, Lord Howe Island stick insect, sensory ecology

5.3 Introduction

We are amidst the Earth's sixth mass extinction event (Barnosky et al., 2011), with an unprecedented number of species being driven to extinction via rapid environmental change resulting from anthropogenic activities (Dirzo et al., 2014). The increasing number of threatened species (IUCN, 2021) has encouraged a multitude of conservation strategies, one of the more important of which is captive breeding programs for species reintroductions (Seddon et al., 2007). Such breeding programs, established with individuals from wild populations of threatened species, provide insurance against extinction (Jakob-Hoff et al., 2015) and are commonly used to reinforce existing wild populations or to provide a founding population to re-introduce the species once threats are removed (IUCN/SSC, 2013; Jakob-Hoff et al., 2015). Captive breeding programs provide an environment typically free from extinction drivers and provide the opportunity for program managers to regulate reproduction to retain genetic diversity, and thus increase the likelihood of the successful establishment of new wild

populations (Frankham, 1995; Weeks et al., 2015). While most often used for vertebrate species, captive breeding programs are increasingly used as conservation strategies for invertebrates (Dojnov et al., 2012; Holwell & Andrew, 2015; Honan, 2007; Leather et al., 2008; Pearce-Kelly et al., 1998; Stringer & Chappell, 2008).

Captive breeding programs typically create benign living environments that can result in selection for survival in environments that have little resemblance to natural habitats (Frankham et al., 2010; Lacy, 1987; Williams & Hoffman, 2009). This can have significant fitness consequences for individuals subsequently released into the wild, with adaptations to captivity in these contexts typically being non-adaptive in the natural environment (Lewis & Thomas, 2001; Sutherland, 1998) and often resulting from the relaxation of natural selection pressures in captivity. Invertebrates are vulnerable to such effects (Dojnov et al., 2012; Frankham & Loebel, 1992; Lewis & Thomas, 2001; Woodworth et al., 2002), especially due to their comparatively short generation times (Lewis & Thomas, 2001). Studies of adaptation to captivity focus mostly on anti-predator responses (Kraaijeveld-Smit et al., 2006) and reproductive traits (Frankham & Loebel, 1992; Heath et al., 2003; Joron & Brakefield, 2003; Lewis & Thomas, 2001; Woodworth et al., 2002), with other morphological traits usually analysed only in the context of life history trade-offs with reproductive investment (Lewis & Thomas, 2001). Despite the importance of considering sensory ecology when designing and implementing conservation strategies (Lim et al., 2008), sensory system adaptations to captivity have not been investigated in a conservation context.

Animals depend on their ability to detect information from their environment, including the location of appropriate food sources, potential mates or approaching predators. Insects have diverse and complex sensory organs to achieve this (Elgar et al., 2018): the ommatidia (facets) of the compound eye are the primary sensory receptors for detecting visual cues, and the sensilla on the antennae are used to detect odours, movement and tactile information. Elaborate sensory systems require considerable energetic resources to develop and maintain, due largely to the associated neural circuitry (Niven & Laughlin, 2008), and sensory organ morphology is optimised to detect salient signals

and cues from the background noise in the signalling environment (Elgar et al., 2018; Endler, 1992). For example, insects living in environments characterised by low ambient light levels have larger compound eye ommatidia to enhance sensitivity to light (Freelance et al., 2021b) while halictid bees that evolved from a social to solitary lifestyle, and thus no longer need to frequently detect diverse social odours, have a lower density of antennal sensilla (Wittwer et al., 2017). Captive breeding environments, typically characterised by ready access to suitable food sources, proximity of potential mates and an absence of predators, effectively simplify the sensory environment and thus may relax natural selection pressures on sensory morphology that would be present in the wild. Accordingly, selection should favour changes to the morphology of sensory organs such that they are optimised (sufficiently but not unnecessarily sensitive) and/or adapted to the signal detection requirements of this new, sensory depauperate, environment.

To test this prediction, we explored differences in sensory organ morphology between wild and captive bred populations of a critically endangered insect for which a captive conservation breeding program has been ongoing since 2003. The iconic Lord Howe Island stick insect (LHISI), *Dryococelus australis* (Phasmatodea: Phasmatidae), is a large, black, flightless phasmid that was historically endemic to Lord Howe Island off the coast of New South Wales, Australia (31°33'15" S, 159°05'06" E) (Lea, 1916). Rats were accidentally introduced to the island in a 1918 shipwreck, leading to the supposed extinction of the insect in the 1920s (Priddel et al., 2003). However, a small population of the LHISI was re-discovered some 80 years later on a nearby volcanic stack, Ball's Pyramid (31°45'15" S, 159°15'06" E) (Priddel et al., 2003). Recent genetic studies confirmed that the stick insects on Ball's Pyramid are the LHISI (Mikheyev et al., 2017). In 2003, two adult breeding pairs were removed from Ball's Pyramid to start a captive conservation breeding program at Zoos Victoria's Melbourne Zoo (Parkville, Victoria, Australia) and at Insektus (Sydney, New South Wales, Australia) (Carlile et al., 2009; Honan, 2007). The Melbourne Zoo population, currently maintained free-ranging in glasshouses, reached its 14th captive bred generation in 2018. This captive population is intended

to be the source of LHISI for reintroduction to Lord Howe Island (Bower et al., 2018) following a rodent eradication program in 2019 (Lord Howe Island Board, 2020).

The Lord Howe Island, Ball's Pyramid and captive environments differ in the complexity of the sensory environment. Firstly, Lord Howe Island has diverse vegetation with which the LHISI historically interacted, including both food and non-food plants (Honan, 2008; McGrath et al., 2017), while the LHISI on Ball's Pyramid is known to associate only with the Lord Howe Island Melaleuca *Melaleuca howeana*. This plant is also one of only a few host (food and shelter) plant species provided to the Melbourne Zoo captive population (Honan, 2008; McGrath et al., 2017), meaning that both the Ball's Pyramid and the captive populations rarely use odours to differentiate among food and non-food plants. Secondly, the captive breeding environment is devoid of potential predators in contrast with Lord Howe Island (spiders, birds, and small mammals) and Ball's Pyramid (seabirds), and so captive bred individuals are not disadvantaged if they lose sensitivity to predator-related cues. Thirdly, while there is evidence of gregarious living from both the wild (Lea, 1916) and captive (Honan, 2008) populations, the maximum possible distance between two individuals in the captive breeding environment is significantly reduced, thereby reducing reliance on location-revealing sex pheromones to locate a mate.

The complexity of the sensory environment is evidently greater on Lord Howe Island than on Ball's Pyramid and is least for populations bred in captivity. Accordingly, we predicted that (i) individuals from the Lord Howe Island wild population (pre-extirpation) will have morphology indicative of greater sensitivity of the compound eyes and antennae compared to individuals from the Ball's Pyramid wild population and (ii) captive breeding will be associated with morphology indicating reduced sensitivity of the compound eyes and antennae compared to both wild populations.

5.4 Materials and Methods

5.4.1 Study populations

We accessed, from the Australian Museum entomology collection (Sydney, Australia), ethanol-preserved historical specimens from both the Lord Howe Island (LHI) and Ball's Pyramid (BP) wild populations. Seven specimens (*n* = 4 females; 3 males) had been collected from Lord Howe Island in the late 1800s pre-extirpation, and we examined the only two available specimens (*n* = 1 female; 1 male) of the four individuals collected from Ball's Pyramid in 2003 to establish the captive populations (Honan, 2007). The latter pair is believed to be the individuals provided to the Insektus organisation: we were unable to locate the breeding pair which founded the Melbourne Zoo captive population in any museum or zoo collections.

We examined the effects of long-term captive breeding on sensory morphology over generations by accessing representative specimens from two generations of the Melbourne Zoo captive population. These specimens had been preserved by freezing from 2011–2013, providing us with individuals (*n* = 10 females; 5 males) from generations 8–10 of captive breeding (MZ generations 8–10) since the establishment of the population with wild stock from Ball's Pyramid. In late 2018 when this study was initiated, the invertebrate keepers collected and froze all naturally deceased individuals until the end of that year, providing us with 6 females and 9 males from the 14th captive bred generation (MZ generation 14). Only adult stick insects were included in the study. Our study did not require animal research ethics approval.

5.4.2 Data collection

Eye ommatidia is positively associated with sensitivity to light (Jander & Jander, 2002; Land, 1997; Warrant, 2017) and eye size can indicate investment in photic sensitivity versus visual acuity, as a larger compound eye with smaller but more numerous ommatidia theoretically has greater visual acuity (Jander & Jander, 2002). Antennal sensilla density is a behaviourally relevant indicator of sensitivity to olfactory and tactile cues (Elgar et al., 2018; Gill et al., 2013; Spaethe et al., 2007).

Therefore, we used these three metrics of sensory capacity to compare across the study populations. Only undamaged eyes or antennae were analysed.

The compound eyes were imaged using a Leica MZ16 A stereomicroscope with Leica DFC500 camera (Leica Microsystems) at the Australian Museum (Sydney, Australia) or a Leica M205 stereomicroscope with Leica DFC500 camera at the BioSciences Microscopy Unit (The University of Melbourne, Australia). Using the images, we determined for each specimen the surface area of the compound eye (calculated as half of the surface area of a spheroid with semi-axes equivalent to the length and depth of the compound eye; mm^2) and the average diameter of the ommatidia of the compound eye (diameter of three ommatidium averaged; μm). As differences in ommatidia diameter between regions of the compound eye are not uncommon (Perl & Niven, 2016), for consistency we measured ommatidia from the dorso-medial (skyward-facing) region of the compound eyes.

To image the antennae, the left antenna was removed from each specimen and affixed on black matte cardboard on a scanning electron microscope stub using double-sided carbon sticky dots. If the left antenna was not intact for a specimen, the intact right antenna was used to maximise sample size. Mounted antennae were made conductive by gold coating using a Dynavac Xenosput gold coater and subsequently imaged using a FEI/Philips XL30 FEG scanning electron microscope (10kV acceleration voltage, spot size 3.0) at the BioSciences Microscopy Unit. From the electron micrographs, we determined for each specimen the average density of each type of antennal sensilla (number of sensilla per mm^2 of antenna) on the apical (1st) and 8th-most apical antennal segments (antennomers). As the antennal sensilla of the LHISI have not been previously examined, we first had to identify and describe the sensilla present before we could calculate sensilla density to compare across populations. Antennal sensilla were classified into four categories: olfactory/chemoreceptive sensilla detect airborne odours and chemicals in solution (Slifer, 1966), hair-like mechanoreceptive sensilla (tactile hairs) are involved in the detection and localisation of objects in the near-range environment during antennation (Dürr & Krause, 2013), campaniform sensilla detect stretch forces

due to mechanical deformation of the cuticle due to external forces or movement of the antenna initiated by the insect (Chapman, 1998; Zill et al., 2011), and hygro- and thermo-receptive sensilla (sensory pores) detect changes in humidity and temperature. Antenna length was not measured as meaningful comparison of this metric was precluded by the antennae having an inconsistent number of antennomers and by the inconsistent length of antennomers.

Using a Canon 6D DSLR with Canon EF-L 100mm f2.8 macro lens (Canon, Tokyo, Japan), we took digital images of the femurs of each individual, with a ruler included as a scale, as a measure of body size. All image analyses were performed using the software package FIJI (Schindelin et al., 2012).

5.4.3 Statistical analysis

One sensory trait, the density of campaniform sensilla, required natural log transformation to normalise the distribution for an ANOVA. For each sensory trait, we fitted a linear model including population (LHI, BP, MZ generations 8–10, MZ generation 14), sex (female, male) and body size as fixed effects with variance partitioned using ordinary least squares. In the event of a significant ANOVA (type III) *F* test for population, four planned pairwise comparisons were performed with any significant differences reported: LHI against BP to explore difference between the two wild populations; BP against MZ generations 8–10 to explore differences between the source population and the closest available generations of the derived captive population; MZ generations 8–10 against MZ generation 14 to explore changes across generations in captivity; MZ generation 14 against LHI as MZ generation 14 represents the most recent studied generation of the captive population which may be introduced onto Lord Howe Island. Statistical analysis was performed using the CAR (version 3.0-11) (Fox & Weisberg, 2019), EFFECTSIZE (version 0.4.5) (Ben-Shachar et al., 2020) and MULTCOMP (version 1.4-17) (Hothorn et al., 2008) packages in R version 4.1.0 for Windows (R Core Team, 2021).

5.5 Results

5.5.1 Description of antennal sensilla morphology

We identified seven types of antennal sensilla (Fig. 5.1): three types of chemoreceptive sensilla (sensilla basiconica, thick-walled chemoreceptors (TWC), sensilla trichotomous), two types of tactile hairs (sensilla trichodea, sensilla chaetica), one type of plate-like mechanoreceptive sensilla (sensilla campaniforma), and one type of pore-like thermo- and hygro-receptors (sensilla coeloconica). Because trichotomous sensilla were only identified on some specimens from the MZ captive population and their function is uncertain, they were excluded from the sensilla density analysis. Descriptions of the morphological characteristics of each sensillum type identified are in Table D1 in Appendix D.

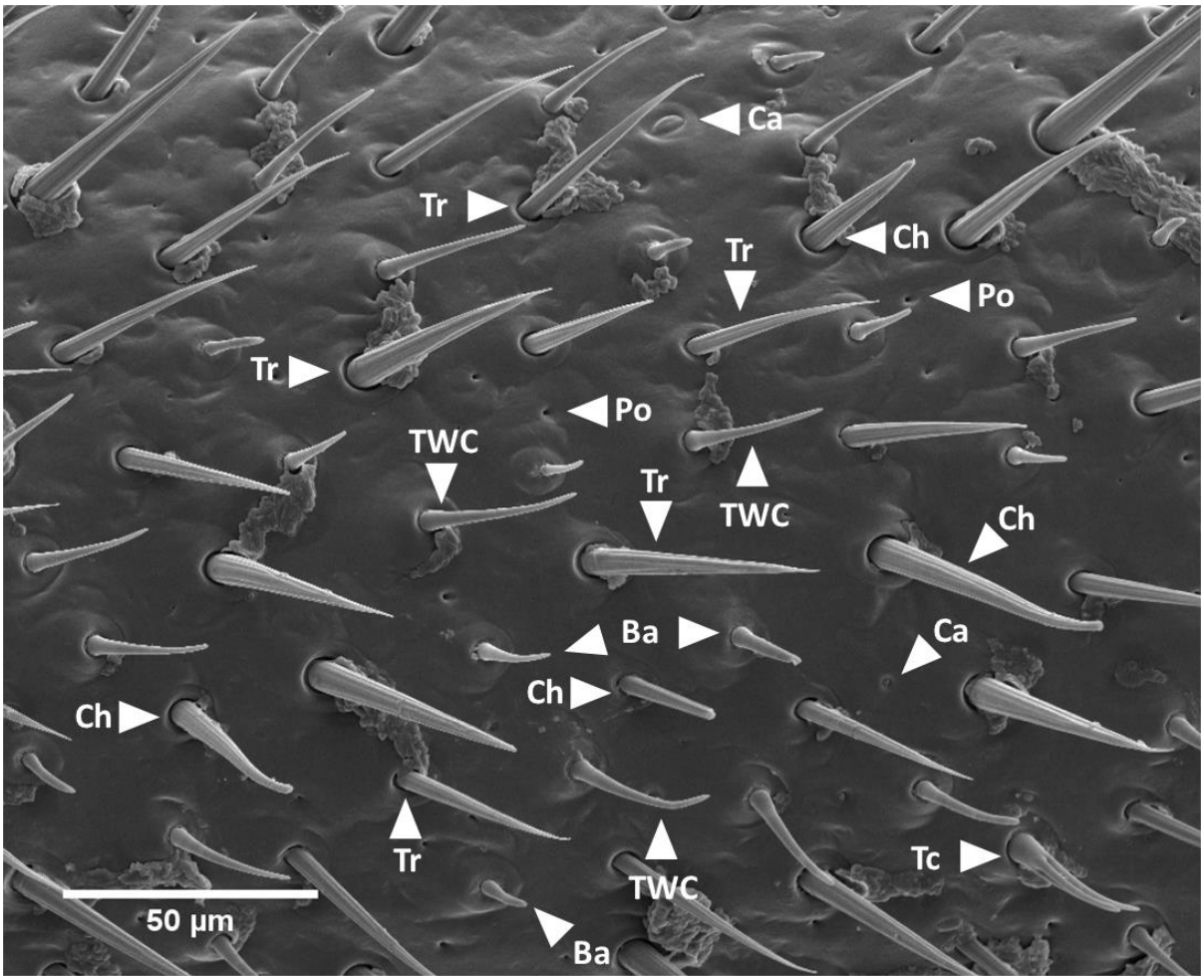

Figure 5.1. Electron micrograph displaying the types of antennal sensilla identified for the Lord Howe Island stick insect. Ba = sensilla basiconica (olfactory sensilla/chemoreceptors); Ca = sensilla campaniforma (stretch receptors); Ch = sensilla chaetica (mechanoreceptor/tactile hair); Po = sensory pore (thermo-/hygro-receptors); Tr = sensilla trichodea (mechanoreceptor/tactile hair); TWC = thick-walled chemoreceptor (olfactory sensilla/chemoreceptors); Tc = sensilla trichotomous (likely olfactory sensilla/chemoreceptors).

5.5.2 Population differences in sensory organ morphology

As predicted, the surface area of the compound eye was explained by population ($F_{3,31}$ = 3.354, p = 0.031, η^2_p = 0.25 (0.00, 0.45) (partial eta squared (95% confidence intervals))) (Fig. 5.2A): planned pairwise comparisons reveal the LHI population had significantly larger eyes than MZ generation 14 population (t = 2.235, p = 0.033). Eye size did not differ significantly by sex ($F_{1,31}$ = 4.132, p = 0.051, η^2_p = 0.12 (0.00, 0.35)) or with femur length as a measure of body size ($F_{1,31}$ = 1.05, p = 0.314, η^2_p = 0.03 (0.00, 0.22)).

Consistent with our prediction, the diameter of the ommatidia of the compound eye also varied significantly by population ($F_{3,32}$ = 4.491, p = 0.0097, η^2_p = 0.30 (0.03, 0.49); Fig. 5.2B): planned pairwise comparisons reveal the LHI population had significantly larger ommatidia than the wild BP population (t = 2.931, p = 0.006) and the captive MZ generation 14 (t = 2.056, p = 0.048). Females had significantly larger ommatidia than males (females: 68.31 ± 1.316 µm (mean ± SE), males: 58.38 ± 1.505 µm, $F_{1,32}$ = 9.470, p = 0.004, η^2_p = 0.23 (0.03, 0.45)), but ommatidia diameter was not explained by femur length ($F_{1,32}$ = 0.381, p = 0.542).

The density of chemoreceptive sensilla on the apical antennomer differed significantly by population ($F_{3,30}$ = 2.984, p = 0.047, η^2_p = 0.23 (0.00, 0.44); Fig. 5.2C), with planned pairwise comparisons revealing a significantly higher density in the LHI population compared to the captive MZ generation 14 (t = 2.925, p = 0.007). It should be noted that the LHI sample for this trait includes an outlier leveraging the result; this value may indicate the existence of even greater variation in the extirpated LHI population that would be apparent in a larger sample. Separate analyses of each type of chemoreceptor – TWCs and sensilla basiconica – were conducted to determine which type drive the pattern. The density of sensilla basiconica on the apical antennomer did not differ between populations ($F_{3,30}$ = 0.574, p = 0.637) but the density of TWCs did ($F_{3,30}$ = 2.972, p = 0.048, η^2_p = 0.23 (0.00, 0.44); Fig. 5.2D): the LHI population had a higher TWC density than MZ generation 14 (t = 2.844, p = 0.008). The density of chemoreceptive sensilla on the apical antennomer was also related to sex

(females: 523.9 ± 32.93 sensilla/mm^2, males: 585.5 ± 38.02, $F_{1,30}$ = 6.344, p = 0.017, η^2_p = 0.17 (0.00, 0.41)), but sex did not explain the density of sensilla basiconica ($F_{1,30}$ = 3.435, p = 0.074, η^2_p = 0.10 (0.00, 0.33)) or TWCs ($F_{1,30}$ = 4.022, p = 0.054, η^2_p = 0.12 (0.00, 0.35)) individually. The density of chemoreceptive sensilla, and of TWCs and sensilla basiconica when analysed separately, was negatively associated with body size: smaller individuals had higher densities (chemoreceptors: β = -28.59, $F_{1,30}$ = 13.26, p = 0.001, η^2_p = 0.31 (0.07, 0.53); sensilla basiconica: β = -10.30, $F_{1,30}$ = 9.535, p = 0.004, η^2_p = 0.24 (0.03, 0.47); TWCs: β = -18.28, $F_{1,30}$ = 7.278, p = 0.011, η^2_p = 0.20 (0.01, 0.43)).

The variation in the densities of no other antennal sensilla, on either the apical or 8th antennomer, was explained by population, sex or body size (Table 5.1). The 8th antennomer density of sensory pores was positively associated with femur length (β = 24.40, $F_{1,28}$ = 4.273, p = 0.048, η^2_p = 0.13 (0.00, 0.37)).

Table 5.1. Ordinary least squares models for the densities of types of antennal sensilla for which population or sex did not explain a significant amount of variation.

Model/parameter	Statistics		
8th antennomer chemoreceptors			
Fixed effects	***df***	***F ratio***	***p > F***
Population (LHI, BP, MZ gen 8–10, MZ gen 14)	3,28	0.226	0.878
Sex (female, male)	1,28	2.370	0.135
Femur length	1,28	0.149	0.702
Apical antennomer tactile hairs			
Fixed effects	***df***	***F ratio***	***p > F***
Population	3,30	0.038	0.990
Sex	1,30	0.003	0.954
Femur length	1,30	0.168	0.685
8th antennomer tactile hairs			
Fixed effects	***df***	***F ratio***	***p > F***
Population	3,28	0.692	0.565
Sex	1,28	0.512	0.480
Femur length	1,28	0.893	0.353
Ln(Apical antennomer sensilla campaniforma)			
Fixed effects	***df***	***F ratio***	***p > F***
Population	3,30	1.04	0.389
Sex	1,30	0.567	0.457
Femur length	1,30	2.93	0.097
Ln(8th antennomer sensilla campaniforma)			
Fixed effects	***df***	***F ratio***	***p > F***
Population	3,28	1.890	0.154
Sex	1,28	0.067	0.798
Femur length	1,28	0.211	0.650
Apical antennomer sensory pores			
Fixed effects	***df***	***F ratio***	***p > F***
Population	3,30	0.075	0.973
Sex	1,30	0.140	0.711
Femur length	1,30	0.667	0.421
8th antennomer sensory pores			
Fixed effects	***df***	***F ratio***	***p > F***
Population	3,28	2.625	0.070
Sex	1,28	0.151	0.700
Femur length	1,28	4.273	0.048

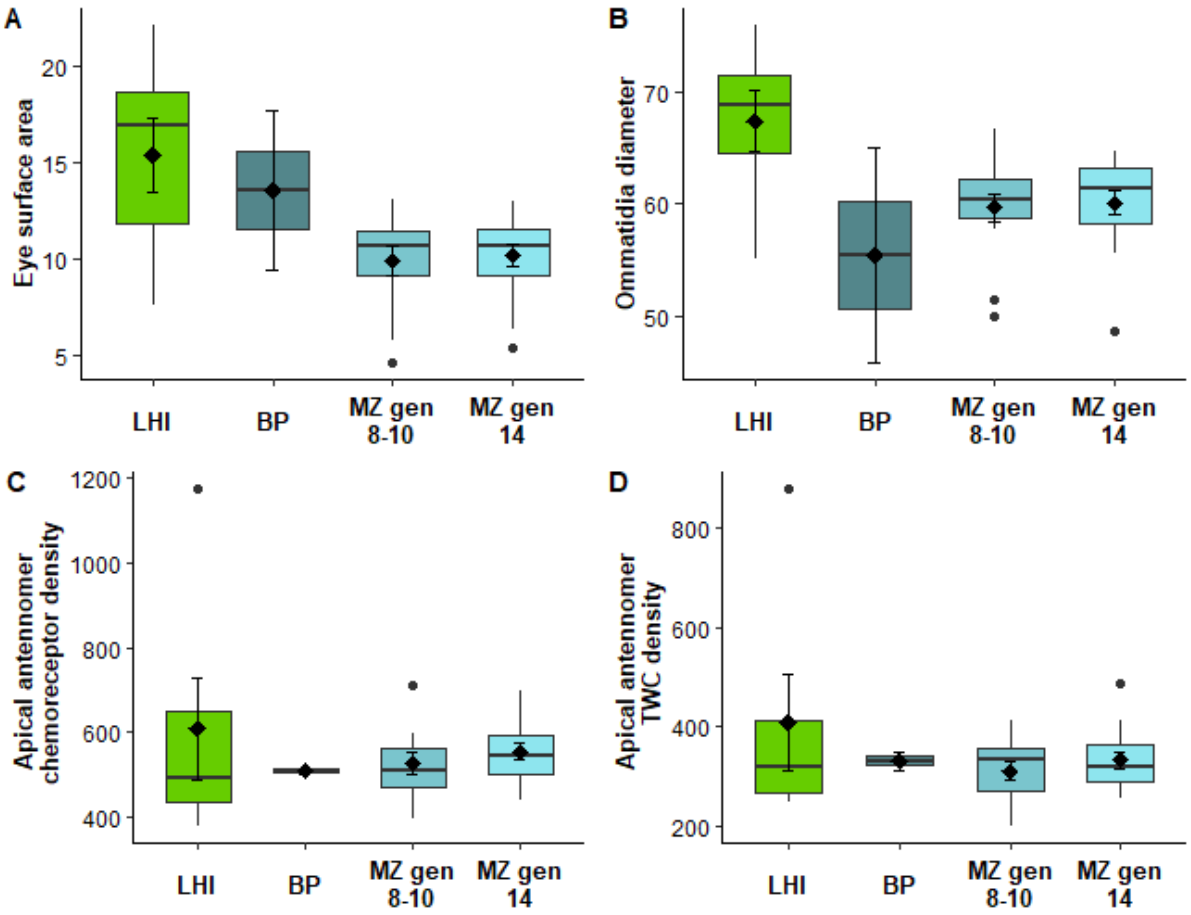

Figure 5.2. Comparison of compound eye and antennal micromorphology between populations of the Lord Howe Island stick insect. Tails indicate the range; box indicates the interquartile range; horizontal line within the box indicates the median; black diamonds indicate the mean; black capped error bars indicate standard error of the mean; black filled circles represent outliers. Sensilla densities were calculated as the number of sensilla per mm^2 of antenna. **A.** Individuals from the LHI had larger compound eyes by surface area (mm^2) than the captive MZ generation 14. **B.** Individuals from LHI had significantly larger ommatidia (µm) than the wild BP and captive MZ generation 14 populations. **C.** Individuals from LHI had a higher density of antennal chemoreceptors on the apical antennomer compared to the captive bred MZ generation 14; this pattern was driven specifically by **D.** the TWC type of chemoreceptor.

5.6 Discussion

Our results support the prediction that the simplification of the sensory environment, reflective in the captive and, to a lesser extent, BP populations, selects for smaller eyes and/or ommatidia and for antennae with a lower density of chemoreceptors. Investment in sensory organs is expected to reflect a balance between the energetic costs of sensory organs (Niven & Laughlin, 2008) and their capacity to detect salient cues and signals against the background noise of the sensory environment (Endler, 1992). Investing in less elaborate and costly sensory organs in comparatively less complex sensory environments allows individuals to redirect energetic resources to other fitness related traits. These changes in sensory organ morphology associated with the simplified wild (BP) or captive (MZ) sensory environment could be the result of evolutionary change or phenotypic plasticity. Indeed, plasticity is often proposed to precede, and possibly facilitate, evolutionary adaptation (Levis & Pfennig, 2016) and is known to drive changes in insect sensory organ morphology (Bernays & Chapman, 1998; Chapman, 2002). Elucidating whether the patterns we observe are fixed or plastic would require experimental manipulations such as changing the complexity of the captive breeding environment and assessing sensory organ morphology over subsequent generations; the limited captive population size and husbandry requirements of this highly threatened species make such an experiment challenging. Regardless of whether the differences we observe reflect evolutionary change or plasticity (Hendry et al., 2008; Hendry et al., 2017), our data are consistent with the notion that the complexity of the sensory environment influences sensory organ morphology and our findings regarding eye size in particular support growing evidence of adaptations to breeding in captivity (Dojnov et al., 2012; Frankham & Loebel, 1992; Heath et al., 2003; Joron & Brakefield, 2003; Kraaijeveld-Smit et al., 2006; Lewis & Thomas, 2001; Woodworth et al., 2002).

The reduced compound eye surface area in the captive population of the LHISI may be due to an absence of predators in captivity: a relatively large compound eye with many ommatidia theoretically confers greater visual acuity, and larger ommatidia provide greater photon capture ability (Jander & Jander, 2002). Visual acuity and visual sensitivity are likely of greater benefit in

populations that need to detect approaching predators, such as birds. In contrast, individuals reared in captivity experience no selection pressure to detect predators. As each ommatidium is associated with energetically expensive neural architecture required to detect photons and convey this information to the brain to form a visual map (Agi et al., 2014; Niven & Laughlin, 2008), a simultaneous decrease in overall eye size reduces the energetic cost of the eye.

While leveraged by a statistical outlier in the extirpated LHI population, the between-population differences in antennal sensilla density are consistent with our prediction. This was driven by the TWC type of chemoreceptor which is associated with detecting airborne odours and compounds in solution (Slifer, 1966) and is likely the predominant sensilla for detection of plant volatiles and pheromones. *D. australis* feeds on a range of food plants found on LHI but clearly prefers a few species (McGrath et al., 2017), whereas *M. howeana* may be the only food plant on BP and is the most frequently provided in captivity (Honan, 2008; McGrath et al., 2017). Perhaps individuals on LHI relied on plant odours to identify and locate their preferred food plants, while those on BP and in captivity have little or no requirement to exercise a choice. Dietary composition can also influence the distribution of chemoreceptors on the antennae of invertebrates (Bernays & Chapman, 1998; Chapman, 2002). Finally, the maximum possible distance between females and males in the BP and captive environments is substantially less than that on LHI, perhaps eliminating the need for males (with a higher density of chemoreceptors than females) to rely heavily on location-revealing female sex pheromones.

While the patterns of sensory organ investment across the populations are consistent with our prediction and with differences in the sensory environment, other variables may also be responsible. Firstly, museum specimens often derive from opportunistic or selective collecting which may be biased towards larger specimens (Pyke & Ehrlich, 2010). This could explain why specimens from LHI possess larger eyes and higher densities of antennal sensilla compared to the captive population specimens that were more representative of the population. However, the absence of

positive body size allometry with eye or antennal metrics suggests that such bias is unlikely to explain our results. Secondly, our LHI and especially BP sample sizes are very small and may not be an accurate representation of the entire population. Small sample sizes for these populations, while generally unavoidable with critically endangered species, may also have impacted our ability to detect statistically all population differences. Thirdly, the captive population, known to be derived from a maximum of only four individuals, is likely to have been influenced by a founder effect and will have very limited genetic variation; this may also be true of the BP population, which has an unknown number of founders (indeed, the separation time between the BP and extirpated LHI populations is unknown (Mikheyev et al., 2017) and there is uncertainty whether differences between the BP and LHI populations result from historical founder effects). Consequently, through founder effects there may have been insufficient genetic diversity for adaptation (as opposed to plasticity) to explain the differences we observe between the wild and captive populations. This could account for the absence of differences between the 8–10th and 14th generations of the MZ captive population in any of the traits we examined, although it is also possible that the sensory morphology of the 8–10th generations had already become optimised to the captive environment and thus remained unchanged in subsequent generations. Inbreeding depression, resulting from low genetic variation and multiple generations in captivity, could also contribute to sub-optimal expression of morphological traits. Despite these caveats, our data provide novel practical insights from a real-world conservation captive breeding program of a critically endangered species, and thus relate to an actual situation that must be managed rather than being derived from a laboratory experiment with a model organism.

Differences in sensory organ morphology in captive compared with natural populations have implications for species reintroduction programs, since reintroduced individuals adapted to captivity may well be less equipped to respond to their new, natural, environment (Lewis & Thomas, 2001; Sutherland, 1998). However, captive populations, especially if added to with further founders over the course of the breeding program, may retain sufficient genetic diversity to respond to selection pressures when reintroduced into the natural environment. Moreover, if the differences reflect

plasticity rather than solely evolutionary change (Hendry et al., 2008), the recovery of visual and olfactory sensitivity following reintroduction could be rapid. Changes to captive population husbandry, such as increasing the complexity of the sensory environment by presenting a variety of both food and non-food plants in captivity, may facilitate changes in the direction of greater sensitivity before wild reintroduction begins. Additionally, a dietary composition similar to that of the natural habitat may further favour more appropriate antennal morphology due to the direct influence of diet on antennal chemoreceptor density (Bernays & Chapman, 1998; Chapman, 2002). Presenting a variety of plants similar to those in the natural habitat may also facilitate greater expression of natural exploratory behaviours in captivity, which promotes general welfare (Freelance, 2019). In the case of the LHISI, introducing a variety of food and non-food plants is unlikely to compromise the fitness of the captive population, as captive individuals can thrive on a variety of food plants native to LHI (McGrath et al., 2017).

In conclusion, our findings from an ongoing conservation captive breeding program are consistent with the prediction that long-term captive breeding of invertebrates may be associated with adaptation of sensory organs to the captive environment. With the increasing use of captive breeding programs as part of threatened species insurance and recovery strategies, these findings provide a cautionary tale: that simplified environments may compromise sensory systems that support efficient expression of appropriate behaviours in a natural habitat. These results highlight the need to consider invertebrate models in evaluating captive breeding and reintroduction programs, emphasise the importance of employing evolutionary biology when undertaking such programs for species reintroductions, and echo long-neglected calls to consider sensory ecology when designing conservation programs (Lim et al., 2008).

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Appendix A: Chapter 2 supporting information

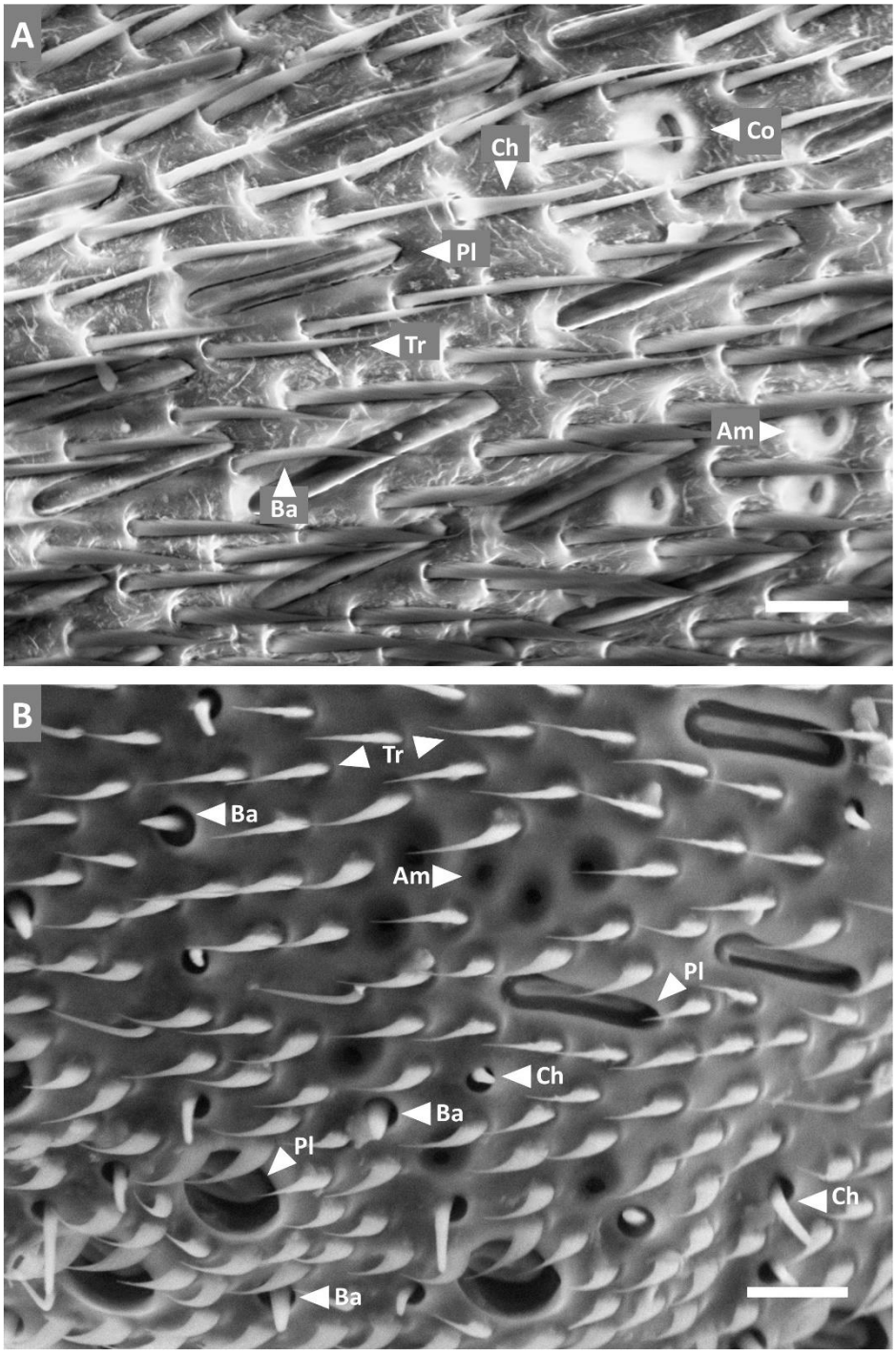


Figure A1. Scanning electron micrographs of antennae of **A.** *Priochilus* regius and **B.** *Pompilinus marginatus* displaying the types of antennal sensilla identified for spider wasps. Scale bars = 10 µm. Sensilla ampullacea (Am) appear as clusters of pore-like sensilla, with the opening into the pore clearly visible. Sensilla coeloconica (Co) appear similar to sensilla ampullacea, except they do not occur in clusters and the size of the opening is typically larger. Sensilla basiconica (Ba) are hair-like sensilla

with a rounded (not sharp/pointed) tip that lean in the direction of the antennal apex (A1.A) or stand upright (A1.B). Sensilla chaetica (Ch) are hair-like sensilla and can be distinguished from the other types as the tip of the sensillum, if not the entire sensillum, points towards the base of the antenna rather than towards the antennal apex. The remaining hair-like sensilla are sensilla trichodea (Tr). Sensilla placodea (Pl) are plate-like sensilla that can appear as an elongated, oval or round plate-like structure either on the antennal surface (A1.A) or sunken below the surface of the antenna (A1.B).

Appendix B: Chapter 3 supporting information

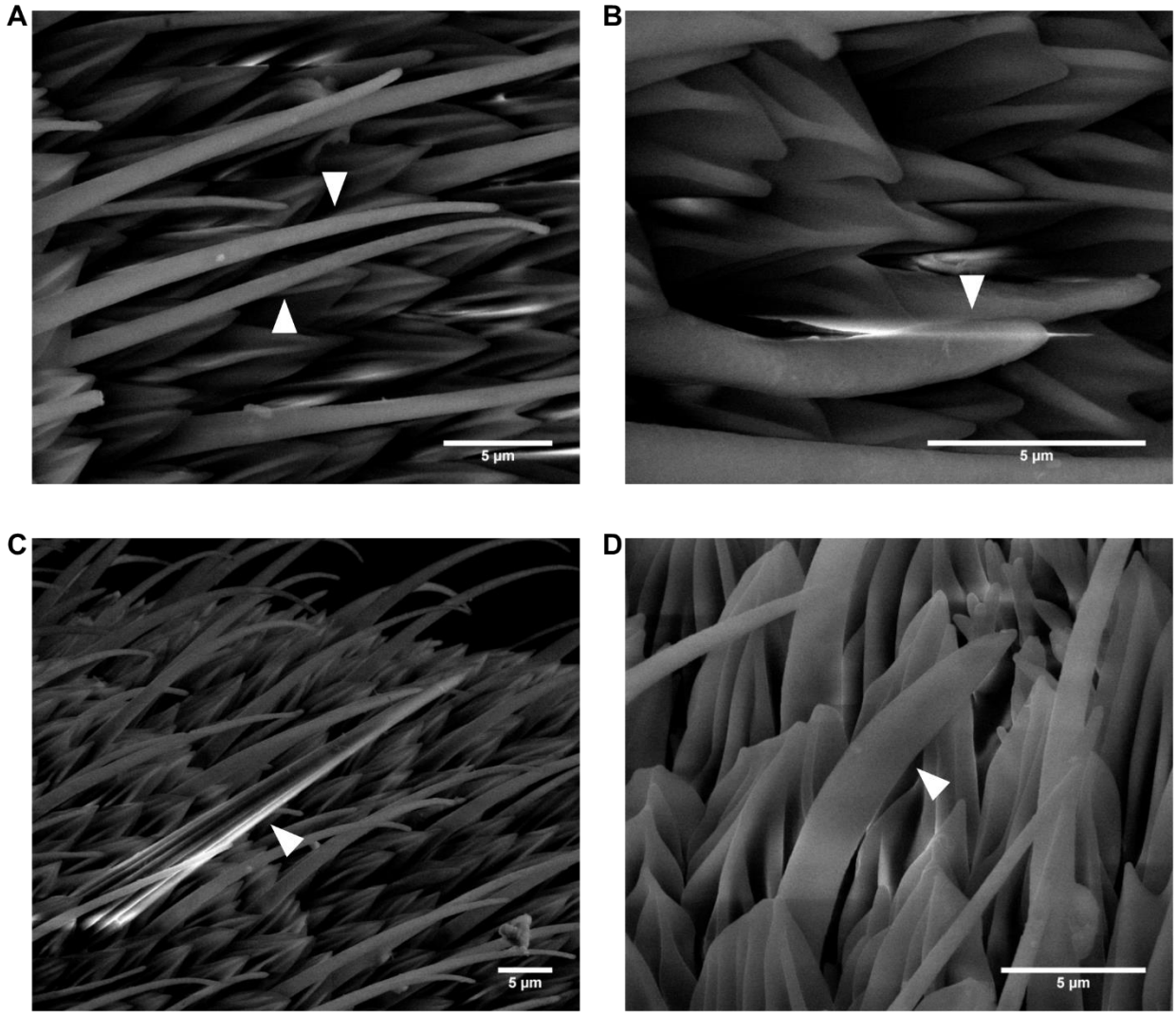


Figure B1. Scanning electron micrographs of the hair-like sensilla identified on the antennae of lycaenid butterflies. Scale bars = 5µm. **A:** Sensilla trichodea are slender and smooth hair-like sensilla that taper to a rounded tip. They are typically longer than the other hair-like sensilla. **B:** Sensilla basiconica are relatively short with a consistent thickness throughout their length. They are smooth and possess a rounded tip. **C:** Sensilla chaetica are rigid sensilla with a relatively thick base. The length of the sensillum is prominently ridged, and each sensillum tapers to a pointed tip. **D:** Sensilla auricillica, sometimes referred to as "rabbit eared sensilla" for their flat appearance and shape resembling a rabbit's ear, are wide and relatively flat rather than round. Their width is consistent around most of the length except for the tip, where the sensillum tapers but in a less rounded fashion than sensilla basiconica.

Appendix C: Chapter 4 supporting information

Table C1. Mean and standard deviation for each compound eye morphology metric by active time, taxonomic order and taxonomic family. Note that where a metric has been Ln transformed (to normalise the distribution), this transformed metric rather than the untransformed metric was used in the statistical analysis.

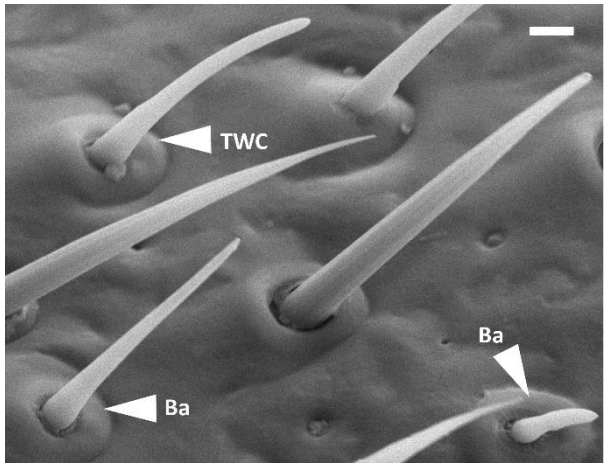
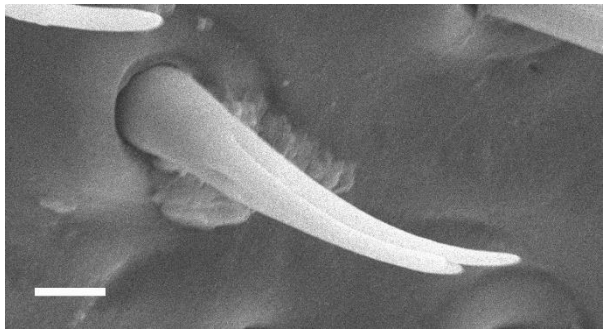
Taxonomic order	Taxonomic family	Ommatidia diameter		Ln(ommatidia diameter)		Compound eye surface area		Ln(compound eye surface area)	
		Day	Dim-light	Day	Dim-light	Day	Dim-light	Day	Dim-light
Coleoptera		*20.38 ± 2.598*	*20.95 ± 3.662*	*3.007 ± 0.125*	*3.029 ± 0.169*	*1.536 ± 1.611*	*2.580 ± 1.655*	*-0.125 ± 1.132*	*0.728 ± 0.721*
	Carabidae	22.22 ± 2.586	23.08 ± 4.528	3.095 ± 0.117	3.123 ± 0.208	2.959 ± 1.354	4.092 ± 0.654	1.012 ± 0.412	1.398 ± 0.168
	Scarabaeidae	18.84 ± 1.392	19.26 ± 1.830	2.934 ± 0.076	2.954 ± 0.095	0.350 ± 0.078	1.068 ± 0.141	-1.073 ± 0.237	0.058 ± 0.137
Diptera	Culicidae	18.23 ± 0.531	19.67 ± 3.34	2.903 ± 0.029	2.970 ± 0.163	0.083 ± 0.015	0.090 ± 0.023	-2.501 ± 0.182	-2.427 ± 0.253
Hymenoptera		*20.81 ± 5.176*	*27.14 ± 8.630*	*3.009 ± 0.228*	*3.239 ± 0.393*	*1.392 ± 0.761*	*2.481 ± 1.469*	*0.192 ± 0.548*	*0.603 ± 0.968*
	Halictidae	26.39 ± 4.610	33.56 ± 5.341	3.261 ± 0.171	3.502 ± 0.171	2.296 ± 0.458	3.711 ± 0.610	0.815 ± 0.200	1.300 ± 0.175
	Formicidae	18.98 ± 1.625	28.90 ± 1.955	2.941 ± 0.083	3.362 ± 0.068	0.942 ± 0.247	2.522 ± 1.030	-0.089 ± 0.270	0.859 ± 0.394
	Mutillidae	16.29 ± 0.319	12.93 ± 1.445	2.790 ± 0.020	2.555 ± 0.116	0.848 ± 0.283	0.348 ± 0.071	-0.219 ± 0.386	-1.069 ± 0.196
Lepidoptera		*27.71 ± 2.618*	*29.62 ± 3.26*	*3.318 ± 0.097*	*3.383 ± 0.108*	*4.446 ± 0.689*	*5.249 ± 1.316*	*1.481 ± 0.162*	*1.626 ± 0.281*
	Hesperiidae	25.79 ± 1.782	31.49 ± 3.717	3.248 ± 0.070	3.444 ± 0.123	3.970 ± 0.513	5.932 ± 1.164	1.372 ± 0.131	1.767 ± 0.187
	Sphingidae	30.11 ± 0.463	27.75 ± 1.279	3.405 ± 0.015	3.322 ± 0.046	5.042 ± 0.257	4.566 ± 1.201	1.617 ± 0.051	1.486 ± 0.311
Odonata		*65.69 ± 10.91*	*66.74 ± 3.316*	*4.174 ± 0.154*	*4.200 ± 0.050*	*34.72 ± 5.169*	*46.89 ± 6.844*	*3.537 ± 0.153*	*3.838 ± 0.145*
	Austrocorduliidae	67.08 ± 5.048	68.04 ± 3.503	4.204 ± 0.077	4.219 ± 0.052	31.16 ± 4.752	44.89 ± 5.523	3.431 ± 0.154	3.798 ± 0.120
	Telephlebiidae	64.58 ± 14.67	65.44 ± 2.875	4.150 ± 0.204	4.180 ± 0.044	36.85 ± 4.504	48.90 ± 8.052	3.601 ± 0.124	3.878 ± 0.170
Orthoptera		*27.70 ± 5.364*	*36.38 ± 2.924*	*3.307 ± 0.186*	*3.591 ± 0.080*	*2.382 ± 2.167*	*3.482 ± 4.062*	*0.530 ± 0.881*	*0.470 ± 1.468*
	Gryllidae	24.34 ± 1.421	34.80 ± 2.043	3.191 ± 0.058	3.549 ± 0.058	1.012 ± 0.275	0.593 ± 0.250	-0.018 ± 0.288	-0.578 ± 0.401
	Tettigoniidae	34.43 ± 1.53	38.75 ± 2.682	3.538 ± 0.044	3.656 ± 0.069	5.121 ± 0.860	7.814 ± 1.819	1.626 ± 0.169	2.042 ± 0.235

Table C2. Mean and standard deviation for each antennal sensilla density metric by active time, taxonomic order and taxonomic family. Note that where a metric has been Ln transformed (to normalise the distribution), this transformed metric rather than the untransformed metric was used in the statistical analysis.

Taxonomic order	Taxonomic family	Olfactory sensilla density		Contact chemosensilla density		Ln(contact chemosensilla density)		Mechanosensilla density		Ln(mechanosensilla density)	
		Day	Dim-light	Day	Dim-light	Day	Dim-light	Day	Dim-light	Day	Dim-light
Coleoptera		*4726 ± 965.8*	*4008 ± 2094*	*1575 ± 1044*	*737.7 ± 406.3*	*7.142 ± 0.707*	*6.465 ± 0.565*	*660.1 ± 534*	*413.5 ± 358.5*	*6.146 ± 0.892*	*5.657 ± 0.924*
	Carabidae	3949 ± 865.1	2510 ± 1918	2620 ± 448.0	1131 ± 238.2	7.860 ± 0.166	7.016 ± 0.197	1187 ± 274.0	758.3 ± 236.9	7.056 ± 0.244	6.599 ± 0.282
	Scarabaeidae	5373 ± 402.5	5207 ± 1402	703.8 ± 124.1	422.9 ± 94.07	6.543 ± 0.178	6.024 ± 0.251	221.0 ± 36.61	137.7 ± 32.28	5.388 ± 0.156	4.904 ± 0.224
Diptera	Culicidae	12367 ± 1015	11563 ± 1829	1319 ± 377.2	1479 ± 637.1	7.159 ± 0.269	7.25 ± 0.445	2557 ± 622.0	2875 ± 1641	7.825 ± 0.265	7.875 ± 0.606
Hymenoptera		*11634 ± 4452*	*13811 ± 11090*	*586.7 ± 199.3*	*653.6 ± 496*	*6.309 ± 0.392*	*6.314 ± 0.567*	*657.6 ± 275.5*	*472.5 ± 171.6*	*6.381 ± 0.521*	*6.086 ± 0.413*
	Halictidae	10091 ± 2862	12006 ± 9309	561.2 ± 219.3	946.7 ± 752.3	6.260 ± 0.418	6.672 ± 0.611	420.8 ± 241.8	368.4 ± 193.6	5.907 ± 0.576	5.809 ± 0.489
	Formicidae	8490 ± 2405	8308 ± 2608	512.6 ± 216.2	480.6 ± 219.8	6.157 ± 0.458	6.061 ± 0.557	796.4 ± 196.0	513.8 ± 161.6	6.654 ± 0.252	6.196 ± 0.341
	Mutillidae	17257 ± 1972	27826 ± 14680	706.1 ± 115.8	511.1 ± 106.5	6.550 ± 0.155	6.223 ± 0.201	775.3 ± 228.3	563.2 ± 81.06	6.624 ± 0.257	6.326 ± 0.149
Lepidoptera		*6615 ± 1420*	*4653 ± 2216*	*320.3 ± 134.5*	*215.6 ± 60.00*	*5.686 ± 0.446*	*5.332 ± 0.328*	*279.7 ± 157.9*	*359.6 ± 279.2*	*5.492 ± 0.57*	*5.637 ± 0.752*
	Hesperiidae	5987 ± 1196	2856 ± 2333	374.8 ± 161.5	235.3 ± 40.99	5.836 ± 0.522	5.45 ± 0.185	333.5 ± 194.5	590.7 ± 292.8	5.663 ± 0.640	6.267 ± 0.632
	Sphingidae	7243 ± 1490	6001 ± 732.3	265.9 ± 90.51	200.8 ± 73.47	5.536 ± 0.362	5.244 ± 0.41	225.8 ± 112.5	186.3 ± 72.86	5.322 ± 0.521	5.165 ± 0.414
Odonata		*296.2 ± 144.3*	*436.9 ± 311.2*	*0 ± 0*	*0 ± 0*	*0 ± 0*	*0 ± 0*	*321.8 ± 131.8*	*236.7 ± 145.6*	*5.711 ± 0.376*	*5.665 ± 0.272*
	Austrocorduliidae	382.8 ± 226.1	593.6 ± 318.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	487.6 ± 59.16	213.3 ± 184.8	6.186 ± 0.122	5.768 ± 0.014
	Telephlebiidae	253.0 ± 100.9	201.7 ± 12.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	238.9 ± 17.29	271.9 ± 111.5	5.474 ± 0.073	5.561 ± 0.422
Orthoptera		*4238 ± 2939*	*4480 ± 4015*	*418.1 ± 207.9*	*309.7 ± 135.3*	*5.928 ± 0.523*	*5.668 ± 0.398*	*996.9 ± 466.6*	*1113 ± 769.9*	*6.774 ± 0.613*	*6.734 ± 0.917*
	Gryllidae	6120 ± 473.6	7246 ± 1882	492.2 ± 205.7	362.1 ± 158.7	6.137 ± 0.402	5.826 ± 0.449	1275.8 ± 202.7	1641 ± 369.0	7.141 ± 0.169	7.386 ± 0.224
	Tettigoniidae	472.9 ± 43.60	331.3 ± 88.40	269.9 ± 152.6	231.3 ± 48.57	5.511 ± 0.599	5.432 ± 0.212	439.2 ± 180.2	320.9 ± 82.46	6.041 ± 0.422	5.754 ± 0.260

Appendix D: Chapter 5 supporting information

Table D1. Description of the morphological characteristics of each of the 7 types of antennal sensillum identified on the antennae of the Lord Howe Island stick insect *Dryococelus australis* (Phasmatodea: Phasmatidae). Scale bars = 5µm.

Sensilla category	Types and morphological characteristics	Representative electron micrographs
Chemoreceptor	**Thick-walled chemoreceptor pegs** (TWC) have a rounded tip and smooth surface (Slifer, 1966). **Sensilla basiconica** (Ba), sometimes called thin-walled chemoreceptor pegs (Slifer, 1966), are similar in appearance to TWCs but have more prominent curvature, more variation in length and have a small bump near the base where each sensillum inserts into the antenna.	
	On the antennae of a few specimens from the captive population, we identified a type of sensillum resembling sensilla basiconica but splitting into three hairs partway up the length of the sensillum. We term this type the "**trichotomous sensillum**" and, due to the lack of surface grooves and similar length and curvature to TWCs, trichotomous sensilla are likely to be chemoreceptors.	

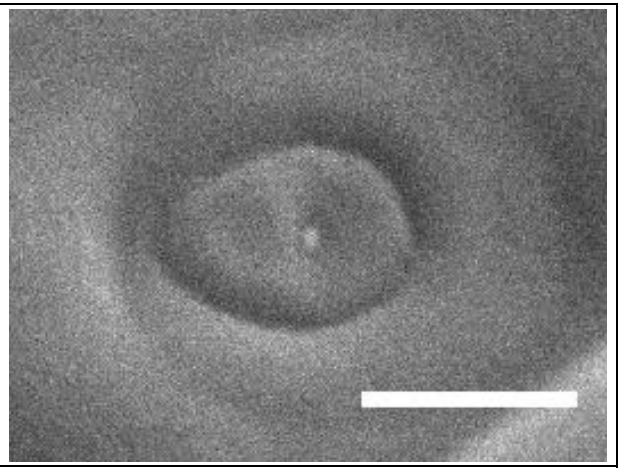
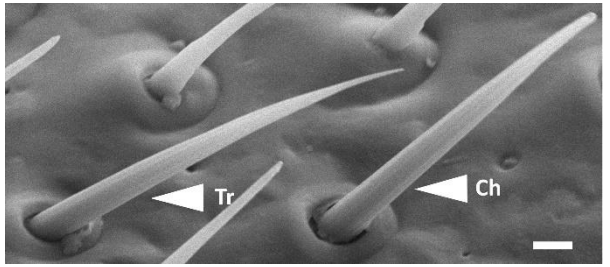
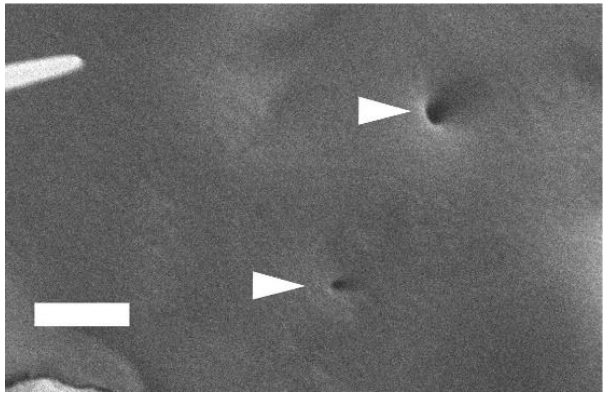
Stretch receptor (campaniform sensilla)	**Campaniform sensilla** are a type of plate-like mechanoreceptive sensilla which are an oval-shaped pore in which the moulting cap can be clearly seen remaining (Zill et al., 2011).	
Hair-like mechanoreceptor/ tactile hair	We observed two types of tactile hairs. Consistent with Slifer's (1966) analysis of the sensilla of the stick insect *Carausius morosus*, these sensilla have grooves on the surface of the hair with a raised cuticular ridge around the base of the sensillum: **sensilla trichodea** (Tr) are more curved and have a narrow tip, while the remaining tactile hairs are **sensilla chaetica** (Ch).	
Hygro-/thermo-receptor (sensory pores)	We observed small pores in which no moulting cap was visible at the surface. As the countersunk hair/peg of sensilla coeloconica is not visible above the surface of the pore opening in some insects, including bees (Hymenoptera) (Carvalho et al., 2017) and carabid beetles (Merivee et al., 2002), the pores we observe are likely to be **sensilla coeloconica**, which are generally considered to be thermo- and hygro-receptors (Drilling & Klass, 2010; Zacharuk, 1985). These pores are more numerous than the campaniform sensilla which is consistent with Weide's (1960) observation that campaniform sensilla are less abundant than coeloconic sensilla on the antennae of *C. morosus*; however it is difficult to know what Weide (1960) was identifying in the absence of accompanying descriptions or micrographs.	

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Table D2. Summary table of ordinary least squares models and planned pairwise comparisons for Lord Howe Island stick insect sensory organs for which morphology was significantly explained by source population. Statistics were obtained with ANOVA type III; partial eta squared is used to report effect size. Following the significant ANOVA *F* test for population, four planned pairwise comparisons were performed. CI = confidence interval.

Model/parameter	**Statistics**			
A. Compound eye surface area				n = 37
	df	***F ratio***	***p > F***	**η^2_p (95% CI)**
Intercept	1,31	5.033	0.032	
Population (LHI, BP, MZ gen 8–10, MZ gen 14)	3,31	3.354	0.031	0.25 (0.00, 0.45)
Sex (female, male)	1,31	4.132	0.051	0.12 (0.00, 0.35)
Femur length	1,31	1.05	0.314	0.03 (0.00, 0.22)
Residuals	31			
Planned pairwise comparisons for population			***t ratio***	***p > t***
LHI vs. BP			0.590	0.560
BP. vs. MZ gen 8–10			1.433	0.162
MZ gen 8–10 vs. MZ gen 14			-0.826	0.415
LHI vs. MZ gen 14			2.235	0.033
B. Compound eye ommatidia diameter				n = 38
	df	***F ratio***	***p > F***	**η^2_p (95% CI)**
Intercept	1,32	69.174	<0.001	
Population (LHI, BP, MZ gen 8–10, MZ gen 14)	3,32	4.491	0.0097	0.30 (0.03, 0.49)
Sex (female, male)	1,32	9.470	0.004	0.23 (0.03, 0.45)
Femur length	1,32	0.381	0.542	0.01 (0.00, 0.17)
Residuals	32			
Planned pairwise comparisons for population			***t ratio***	***p > t***
LHI vs. BP			2.931	0.006
BP. vs. MZ gen 8–10			-1.047	0.303
MZ gen 8–10 vs. MZ gen 14			-1.021	0.315
LHI vs. MZ gen 14			2.056	0.048
C. Apical antennomer chemoreceptor density				n = 36
	df	***F ratio***	***p > F***	**η^2_p (95% CI)**
Intercept	1,30	41.084	<0.001	
Population (LHI, BP, MZ gen 8–10, MZ gen 14)	3,30	2.984	0.047	0.23 (0.00, 0.44)
Sex (female, male)	1,30	6.344	0.017	0.17 (0.00, 0.41)
Femur length	1,30	13.26	0.001	0.31 (0.07, 0.53)
Residuals	30			
Planned pairwise comparisons for population			***t ratio***	***p > t***
LHI vs. BP			1.442	0.160
BP. vs. MZ gen 8–10			0.284	0.778
MZ gen 8–10 vs. MZ gen 14			0.862	0.395
LHI vs. MZ gen 14			2.925	0.007

D. Apical antennomer thick-walled chemoreceptor density				n = 36
	df	***F ratio***	***p > F***	**η^2_p (95% CI)**
Intercept	1,30	23.466	<0.0001	
Population (LHI, BP, MZ gen 8–10, MZ gen 14)	3,30	2.972	0.048	0.23 (0.00, 0.44)
Sex (female, male)	1,30	4.022	0.054	0.12 (0.00, 0.35)
Femur length	1,30	7.278	0.011	0.20 (0.01, 0.43)
Residuals	30			
Planned pairwise comparisons for population			***t ratio***	***p > t***
LHI vs. BP			1.230	0.228
BP. vs. MZ gen 8–10			0.607	0.549
MZ gen 8–10 vs. MZ gen 14			0.564	0.577
LHI vs. MZ gen 14			2.844	0.008