

Mating behaviour and genital morphology
of *Ciulfina* praying mantids
(Mantodea: Liturgusidae)

Gregory Ian Holwell, BSc (Hons) Grad. Dip. Ed.

Department of Biological Sciences
Macquarie University

Thesis submitted in fulfilment of the requirements for the
degree of Doctor of Philosophy

March 2006

For Dad, Mum, Jodie & Scott

And for Anne

Table of Contents

Summary	i
Declaration	iii
Acknowledgements	iv
Introduction	1
<i>Chapter One:</i>	Parapatric distributions of <i>Ciulfina</i> praying mantids (Mantodea: Liturgusidae) and descriptions of three new species. 21
<i>Chapter Two:</i>	Mirror-image genital dimorphism in <i>Ciulfina</i> praying mantids (Mantodea: Liturgusidae). 41
<i>Chapter Three:</i>	Geographic variation in genital morphology of <i>Ciulfina</i> praying mantids (Mantodea: Liturgusidae). 55
<i>Chapter Four:</i>	Mate location, antennal morphology and ecology in praying mantids (Insecta: Mantodea). 78
<i>Chapter Five:</i>	Spermatophore feeding in <i>Ciulfina</i> praying mantids (Mantodea: Liturgusidae). 100
<i>Chapter Six:</i>	Genital morphology influences copulation duration in a praying mantis <i>Ciulfina klassi</i> (Mantodea: Liturgusidae). 126
Conclusions	149
Appendices	161

Summary

The study of genital morphology is important to our understanding of evolutionary processes. Often the only reliable means by which to identify a species is by using male genitalic traits. Therefore the processes that lead to the diversification and elaboration of genitalia are of broad-ranging interest to evolutionary biologists. This study investigates male genital morphology in four species of praying mantid from the genus *Ciulfina* (Mantodea: Liturgusidae). The other major aim of this study is to document and investigate patterns of mating behaviour in *Ciulfina*, which differ greatly from those of other well-studied praying mantids. The topics covered are:

- (1) Species descriptions and geographic distributions of four praying mantids from the genus *Ciulfina* based on male genital morphology, and the identification of parapatric boundaries between species' ranges.
- (2) The description and analysis of a unique genital polymorphism exhibited by males of three species of *Ciulfina* in which individuals possess genitalia that are oriented to the left (sinistral) or the right (dextral), and are shown to be mirror-images of one-another.
- (3) The analysis of geographic variation in male genital morphology for *Ciulfina*. All species were found, using elliptic Fourier analyses, to vary geographically. The patterns of variation support the sexual selection hypothesis of genital evolution.

- (4) Mechanisms of mate-location are described for *Ciulfina biseriata* and an unrelated mantid *Pseudomantis albofimbriata*, based on the results of choice tests and scanning electron micrography of antennal morphology.
- (5) Mating behaviour is described for *Ciulfina*, with particular attention to spermatophore feeding and variation in the duration of copulation and spermatophore attachment. Spermatophore feeding is documented for the first time in the Mantodea.
- (6) The relationship between genital morphology and copulation duration is explored in *Ciulfina klassi*. Both the shape and size of one particular genital structure, the left epiphallus was found to influence the duration of copulation for this species.

The *Ciulfina* system is finally discussed as a potential model system for the investigation of a number of ecological and evolutionary phenomena including parapatry, genital polymorphism, chirality, spermatophore feeding, mate-searching and the effect of sexual selection on genitalia.

Declaration

I declare that the work presented in this thesis is my own and was undertaken during my PhD candidature. Wherever sources of information or the work of others has been used, I have acknowledged it in the text. This thesis has not been submitted for a higher degree to any other university or institution.

Gregory Ian Holwell

March 2006

Acknowledgements.

Firstly I would like to thank my supervisor Marie Herberstein. Her support and encouragement throughout my candidature has been extraordinary. She has taught me a great deal and provided me with many wonderful opportunities to develop my career. She has always had time for me. She trusted me to direct my research along a number of different paths. Being a part of her lab has made my postgraduate experience a wholly positive one.

Along with Mariella, the entire behavioural ecology lab group have provided a very supportive and congenial environment. Anne Gaskett, Ann Goth, Astrid Heiling, Matt Bruce, Dinesh Rao, Ajay Narendra, Anne Wignall, Nansi Richards, Jutta Eymann, Mike Kasumovic, Stefan Nessler, Aaron Harmer, Preethi Radhakrishnan, Kate Barry, Jacqueline Read and others have all contributed intellectually and socially to a wonderful time in the lab. I must particularly thank Matt for many fieldtrips (including many pie vans and Hawaiian packs), Dinesh for reading this thesis (and providing creationist arachnological literature) and Ann for sharing her office (and biscuits).

Academics and post-docs in the Department of Biological Sciences have contributed greatly to the development of this project and provided a stimulating environment in which to undertake research. Particularly I must thank Andy Beattie, Dave Briscoe, Mark Westoby, Leslie Hughes, Michael Gillings, Luciano Beheregaray, Dick Frankham, Noel Tait, Jane Williamson, Jean Joss, Malcolm Reed, Julia Raftos, Irina Pollard, Barbara Rice,

Mark Eldridge, Astrid Heiling, Ann Goth, Adam Stow, Tish Silberbauer Diana Perez, Sam Banks, Maxine Piggott, Angela Moles and Fiona Scarf. It is through interacting with these people on a daily basis, that ideas could develop, new skills could be learned and the project could take shape.

Working alongside other postgraduate students has provided both intellectual stimulation and much-needed distraction. Along with those from my lab group, I must thank all of the postgraduates students that have crossed over with me during the last few years. Thanks to: Dave Nipperess, Kevin Woo, Chris Longson, Robyn Sinclair, Noina Wattanatongchai, Samyuktha Krishnan, Garreth Kyle, Nigel Andrew, Huw Morgan, Margareta Sutija, Thalie Partridge, Richard Peters, Anthony Young, Sandra Tanz, Mark Broughton, Guy Williams, Peter Wilson, Ulysse Bove, Belinda Curley, Charlie Huveneers, Kerstin Bilgman, Chris Muller, Michael Joss, and Carolyn Michael.

A large number of people have given me technical assistance over the past few years. Specifically I must thank Debra Birch and Nicole Vella for their patience in teaching me scanning electron micrography and Marita Holley, Tonia Schwartz, Paul Worden, Luciano Beheregaray, Michael Gillings and Adam Stow for teaching me valuable skills in molecular genetics (although none of my results made the thesis). Ray Cameron and Craig Angus deserve a special mention for their continual willingness to help and for advice with fieldwork. Others who have also contributed, both to my project and the general sense of wellbeing in the Department are Alison Downing, Ray Duell, Libby Eyre, Jenny Minard, Margaret Mercieca, Elsa Mardones, Rekha Joshi, Rod Nurthern, Ron Oldfield, Frank Sharples, Peter Tung, Rod Bashford, Laura McMillan, Keith Maxwell, Jane Edgeson,

Wendy Southwell, Helma Neumann, Marie Howitt. Worth a special mention for assistance with all my computer issues are Rees Griffiths, Michael Bastion and Paul Rohozky.

Three weeks of my PhD were spent learning geometric morphometric techniques in the research laboratory of Assoc. Prof. Goran Arnqvist at Uppsala University in Sweden. I thank him particularly for the opportunity to spend time there and for what he taught me. I must also thank those who made me welcome there: Martin Edvardsson, Alex Maklakov, Natacha Kremer, Emma Rova and Urban Friberg. I have also had the opportunity to speak to many people at conferences over the last few years, who have all given me great advice regarding my project. Specifically I must thank Bill Eberhard, Tom Tregenza, Nina Wedell, Leigh Simmons, Geoff Parker, Joe Tomkins, Teresa Jones and Mike Bull for great advice and feedback on my work. I must also thank Luke Finley for teaching me geometric morphometric techniques and Stefan Nessler for capturing the well-time images of spermatophore feeding. Particularly, I need to thank David Rentz, John Balderson, Klaus-Dieter Klass and Graham Milledge for their assistance with taxonomic collections at ANIC, the Australian Museum and the Dresden Museum. The work of the late John Balderson was inspirational and his input confirmed my decision to study *Ciulfina*.

I have received a great deal of assistance in the field and in the laboratory from volunteers, friends and undergraduate students. Thanks go to Anne Gaskett, Scott Holwell, Matt Bruce, Chris Longson, Tom Joss, Scott Ginn, James O'Hanlon, Stefan Nessler, Alex Meguid, Matt Kovach, Simon Hardwick, Nathan Langelaar, Katie Robinson and Kate Barry.

My friends deserve my thanks for showing an interest in what I do (despite being a bit unsavoury in some of their minds). Thankyou Mick, Michael, Nick, Mat, Matt, Angela, Emily, Marty, Sandra, Paula, Richard, Lynda, Kit, Lisa, Lola, Kiki, Kate, Elaine, Anthony, Georgia, Bec, Justyna, and others. Your support means a lot to me. I must also especially thank my Uncle John, Auntie Myra, Kelly and Rod for their hospitality, stimulating conversation and port, on my way between Sydney and my field sites.

The support and encouragement that I have received from my family throughout my PhD has been extraordinary. From the day that I proclaimed as a six-year-old that I wanted to be a zoologist, they have always believed in my abilities, trusted in my decisions and shown enthusiasm for what is important to me. I could not have imagined a more loving and supportive family. Thankyou to my mother Terry, my father Ian, my sister Jodie, and my brother Scott.

Lastly, my partner Anne deserves special mention for her guidance and companionship over the past few years. She has provided loving support through some difficult moments and shared in my excitement when things were going well. I really could not have done this without her.

Introduction

Mating behaviour in praying mantids

The mating behaviour of praying mantids has received considerable attention from biologists in recent decades (reviewed in Maxwell, 1999a). The majority of recent observational and experimental studies have focussed on two major themes: (1) mate attraction and sex pheromones (Robinson & Robinson, 1979; Prete *et al.*, 2000; Perez, 2005; Hurd *et al.*, 2004; Edmunds, 1975) and (2) sexual cannibalism (Maxwell, 1998; Maxwell, 1999c; Maxwell, 2000; Lawrence, 1992; Kynaston *et al.*, 1994; Hurd *et al.*, 1994; Fagan & Odell, 1996). Within the Mantodea, males respond to airborne pheromones in a number of genera, including *Acanthops* (Robinson & Robinson, 1979), *Sphodromantis* (Hurd *et al.*, 2004), *Hierodula* (Perez, 2005) *Mantis* and *Empusa* (Gemeno *et al.*, 2005). While this research contributes to the vibrant field of insect chemical ecology, it also has potential implications for the attraction of praying mantids for use in pest control (Hurd *et al.*, 2004). Although chemical sexual signals have received more attention, visual cues can also be important in close-range mate-location and courtship (Roeder, 1935; Edmunds, 1979; Maxwell, 1999). For example, some mantids display characteristic behaviours that expose colourful patterns on the raptorial fore-legs, wings and thorax (Edmunds, 1975).

The majority of studies of praying mantid mating behaviour have focussed on male and female mating strategies with regards to sexual cannibalism. Four species that have been particularly well-studied are *Mantis religiosa* (Roeder, 1935; Prokop & Vaclav, 2005;

Lawrence, 1992), *Iris oratoria* (Maxwell, 2000; Maxwell, 1999c), *Stagmomantis limbata* (Maxwell, 1998) and *Tenodera aridifolia sinensis* (Liske & Davis, 1984; Liske & Davis, 1987) and this research has provided the basis for our current understanding of praying mantid behaviour, and contributed a great deal to our theoretical framework for sexual cannibalism. However, studies of mating behaviour within praying mantids from other families have received little scientific attention. In a recent review, Maxwell (1999) showed that mating behaviour has only been described in 33 species of praying mantid, representing less than 2% of currently described species. Of these, only 11 species have been observed in greater than ten encounters. Given the paucity of observations for the vast majority of the Mantodea, it may be that aspects of mating behaviour considered to be characteristic of all praying mantids, such as the production of airborne sex pheromones and sexual cannibalism are not in fact widespread.

The recent classification of the Mantodea into a larger number of families and sub-families (Ehrmann, 2002) and the development of a molecular phylogeny of the Mantodea (Svenson & Whiting, 2004), allows us to now view aspects of praying mantid behaviour in an evolutionary context. If data from a wider range of mantid species from different families becomes available, it will now be possible to determine the stages in Mantodean evolution at which aspects of mating behaviour such as airborne pheromone production and sexual cannibalism arose. Significantly, eight praying mantid families (Chaeteessidae, Mantoididae, Metallyticidae, Amorphoscelidae, Empusidae, Eremiaphilidae, Thespidae, and Toxoderidae) do not contain any species for which mating observations have been made, and some of these represent basal groups within the Mantodea (Klass, 1997). It is likely, therefore that mating behaviour is much more diverse within the Mantodea than we

currently recognise, and could rival the diversity observed within other insect Orders. Obviously, the solution to this is the investigation of mating behaviour in a wider variety of mantid species representing a greater diversity of families.

Genital evolution in insects

Sexual selection and insect genital evolution

Animal genitalia follow unusual yet strikingly consistent patterns of evolution. These are particularly obvious in male genitalia and when fertilization is internal and the mating system is promiscuous (Arnqvist, 1998). Particularly within the insects, most groups exhibit evidence of rapid and divergent evolution of male genitalic traits resulting in high levels of elaboration and morphological complexity (Eberhard, 1985; Hosken & Stockley, 2004; King Sirot, 2003). Even in closely related species with similar general morphology, genital morphology can be dramatically different, and is often the only reliable means of species identification (Eberhard, 1985). Considering the relatively simple task of transferring sperm, it is unlikely that such highly elaborate structures are required simply for passing on gametes. Despite the generality and wide spread occurrence of rapid genital divergence, our ignorance of the responsible mechanisms is 'truly perplexing' (Arnqvist, 1997).

Until relatively recently, two general, non-sexual selection mechanisms for genital evolution were proposed: (1) the species isolation or lock-and-key hypothesis, and (2) pleiotropy (neutral evolution). The species isolation hypothesis states that selection for reproductive isolation, in order to avoid costly hybridisation, resulted in divergence of

genitalia. Thus only the male of the right species with the correct 'key' can fit the female 'lock' (Shapiro & Porter, 1989). The pleiotropy hypothesis postulates that the divergence in genitalia results indirectly from the evolution of genetically correlated traits (Mayr, 1963). However, neither hypothesis is strongly supported, and it is now widely acknowledged that sexual selection is most likely to be driving rapid and divergent genital evolution (Hosken & Stockley, 2004).

Proposed by Eberhard (1985) and supported by comparative data collated from extensive surveys of the taxonomic literature, the sexual selection hypothesis has received recent support from both experimental studies and comparative analyses accounting for phylogeny. These studies have all used insects to test the various hypotheses. In particular, male genital morphology has been shown to influence fertilization success in the water striders *Gerris lateralis* (Arnqvist & Danielsson, 1999) and *Gerris lacustris* (Arnqvist & Danielsson, 1999), the dung beetle *Onthophagus taurus* (House & Simmons, 2003; House & Simmons, 2006; House & Simmons, 2005b), the seedbug *Lygaeus simulans* (Tadler, 1999) and the fly *Dryomyza anilis* (Otronen, 1998). Studies using quantitative genetics (House & Simmons, 2005a) and multivariate selection analysis (Bertin & Fairbairn, 2005) have also supported the sexual selection hypothesis using insect models.

The three main mechanisms of sexual selection that may operate genital evolution are: sperm competition, cryptic female choice and sexual conflict (for review see (Eberhard, 1985; Hosken & Stockley, 2004)). When the sperm of two or more males compete over the ova of a female, genital traits that assist the male in removing foreign sperm may be selected for (Simmons, 2001; Waage, 1979). Alternatively (but not exclusively), male genitalia may stimulate the female during copulation, and females may cryptically choose

the sperm of males whose genitalia are superior stimulators (Eberhard, 1977; Eberhard, 1996; Eberhard, 1985). Finally, male genitalia may evolve in a coevolutionary arms-race with conflicting female strategies for control over fertilisation (Arnqvist & Rowe, 2005).

Recent reviews (Eberhard, 2004a; Eberhard, 2004b; Eberhard, 1985; Hosken & Stockley, 2004; King Sirot, 2003) have highlighted the urgent need for studies investigating the role of sexual selection in the evolution of genitalia. The main challenges remain in providing detailed investigations of single species and in distinguishing between the mechanisms of sexual selection outlined above. It is likely that studies of insect genitalia will lead the way in developing our understanding of these mechanisms.

Other approaches to the study of genital evolution in insects

A number of other approaches have investigated insect genital evolution in recent decades and add to our growing understanding of the processes involved. Below is a brief summary of some of these approaches:

(a) Genitalic traits, although always valued taxonomically, have provided the basis of new phylogenies (Keffer, 2004; Stoks *et al.*, 2005) and have been transposed over molecular phylogenies to gain an understanding of patterns of diversification in genital morphology (Aldrich *et al.*, 2004; Eberhard, 1977; Kopp & True, 2002; Sota, 2002; Sota *et al.*, 2005).

(b) Functional morphology of genitalia has been investigated using detailed histological and SEM procedures, to provide some insight into the mechanics of insect copulation and copulatory courtship (Eberhard, 1993a; Eberhard, 1993b; Eberhard, 2001a; Eberhard, 2001b; Eberhard, 2001c; Eberhard & Huber, 1998; Fairbairn *et al.*, 2003). A few studies

have also managed to experimentally manipulate parts of the male genitalia (Eberhard, 2002; Takami, 2003) and sensilla (Acebes *et al.*, 2003) in order to explore function and mechanics.

(c) Allometry of genitalic traits has been explored as a means to test the various hypotheses about the evolution of genitalia (Eberhard *et al.*, 1998; Hosken *et al.*, 2005; Ohno *et al.*, 2003; Tatsuta *et al.*, 2001).

(d) A number of studies have highlighted the incidence of geographic variation in genitalic characters (Colless, 1983; Cordero Rivera *et al.*, 2004; Hribar, 1994; Tatsuta & Akimoto, 2000). The patterns observed are important due to the different predictions about geographic variation made by the different hypotheses of genital evolution.

(e) The study of male genitalia that cause harm to females (Blanckenhorn *et al.*, 2002; Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005; Reinhardt *et al.*, 2005; Reinhardt *et al.*, 2003; Stutt & Siva-Jothy, 2001) has contributed greatly to sexual conflict theory and support the sexual selection by sexual conflict hypothesis for rapid genital divergence. The significance of male genitalia that exploit female sensory biases (Cordoba-Aguilar, 1999; Cordoba-Aguilar, 2002) is also intriguing, lending support to models of sexual selection by cryptic female choice to explain genital evolution.

(f) Particularly intriguing is the recent documentation of a number of examples of genital polymorphism (Huber & Gonzalez, 2001; Mound *et al.*, 1998; Mutanen & Kaitala, 2006). Species exhibiting discontinuous variation in genitalic traits are likely to be important models to compare with species exhibiting intrasexual polymorphisms in external morphology. Genital polymorphism may also be much more common than the literature

suggests and, taxonomic procedures may in fact bias against their discovery (Huber, 2003; Jocque, 2002).

(g) Studies of the role of genitalic morphology in reproductive isolation provide convincing evidence that although the species isolation hypothesis is unlikely to account for the process of rapid and divergent evolution of genitalic traits, genitalia can certainly be important in the prevention of costly mismating for some species (Horton & Lewis, 2005; Shapiro, 2000; Sota & Kubota, 1998; Usami *et al.*, 2006).

The *Ciulfina* system

The remainder of this thesis presents a novel system which is ideal for the study of a number of evolutionary patterns. *Ciulfina* is a genus of praying mantids (Mantodea: Liturgusidae) found throughout northern Australia, and as much of this thesis attests to, *Ciulfina* mantids are unique among the Mantodea in a number of respects. Although related to other praying mantids that have been well studied, most aspects of ecology, behaviour and morphology of *Ciulfina* are unique. Particularly intriguing is the male genital morphology of this genus, and one of the aims of this thesis is to nominate *Ciulfina* as a model system for the study of the evolutionary processes leading to and maintaining genital divergence and elaboration. This thesis, which uncovers much of the basic biology of *Ciulfina* will hopefully provide a basis for further study of this remarkable group of insects.

Thesis Outline

Each of the chapters in this thesis is intended to be self-contained and is formatted for submission to particular scientific journals. Below is a brief synopsis of the contents of each chapter.

Chapter One: This paper covers the taxonomy and biogeography of the study system: four species of praying mantid within the genus *Ciulfina*. Three of these species are taxonomically described within, on the basis of male genital morphology. Scanning electron micrographs of genital morphology are included and the collection locations for the four species are presented in a map of Queensland. The parapatric boundaries between *Ciulfina* species distributions are described and the possible mechanisms for their maintenance are discussed. This paper has been submitted and is currently in review for publication in the *Australian Journal of Entomology*.

Chapter Two: This paper describes the unique male genital dimorphism present in three *Ciulfina* species, whereby males possess either dextrally- or sinistrally-oriented genitalia. The paper also uses geometric morphometrics and elliptic Fourier analyses to test whether dextral and sinistral genitalia are mirror-images of one-another and finds that they are for both *C. rentzi* and *C. baldersoni*. The significance of genital polymorphism is discussed and parallels are drawn with the chiral dimorphism of shells in a number of gastropod species. This paper has been submitted and is currently in review for publication in the journal *Naturwissenschaften*.

Chapter 3: This paper analyses geographic variation in genital morphology for four species of *Ciulfina* testing the predictions of the species-isolation and sexual selection hypotheses of genital evolution. Geometric morphometrics and elliptic Fourier analyses were once again used, and geographic variation was detected between all populations of all species supporting the sexual selection hypothesis. As morphological divergence was detected between populations that were not in contact with other species of *Ciulfina*, the species isolation hypothesis was rejected as a general explanation for genital divergence in *Ciulfina*. This paper has been submitted and is currently in review for publication in the journal *Evolutionary Ecology*.

Chapter 4: This paper compares mate location in male praying mantids from two quite different praying mantid species: *Ciulfina biseriata* and *Pseudomantis albofimbriata*. Predictions about the importance of chemical and visual cues for the two species are tested, based on differences in their ecology. Choice tests and scanning electron microrography (SEM) of antennal morphology are used to test these predictions. *Pseudomantis*, like many mantids, is shown to use airborne chemical cues, possibly relating to their widely dispersed and patchy distribution, lower population densities and male-biased dispersal by flight. *Ciulfina*, conversely rely on visual cues, which possibly relates to their homogeneous distribution, high population densities and lack of male flight. This paper has been submitted and is currently in review for publication in the *Biological Journal of the Linnean Society*.

Chapter 5: This paper describes the first incidence of spermatophore feeding known in the Mantodea. The general sequence of mating behaviour for *Ciulfina* praying mantids is described. Copulation duration and the spermatophore attachment duration are compared for the four *Ciulfina* species, and the relationship between these durations and male and female mass are explored. The significance of spermatophore feeding in this new non-Orthopteran system is discussed. This paper has been submitted and is currently in review for publication in the *Journal of Zoology (London)*.

Chapter 6: This paper describes the relationship between genital morphology and copulation duration in *Ciulfina klassi*. Landmark-based geometric morphometrics were used to quantify morphological variation in both the size and shape of two elements of the male genital complex. Multivariate regression analyses showed a relationship between male genital morphology and copulation duration, most strongly influenced by the size and shape of the left epiphallus genital structure. The issue of relative male and female influence over copulation duration is also explored using a cross-mating experiment. Males are shown to have the strongest influence over copulation duration and the significance of this for the relationship with male genital structure is discussed. This paper has been submitted and is currently in review for publication in the journal *Behavioral Ecology & Sociobiology*.

Appendices: Following the general conclusions to this thesis are two papers to which I contributed during my PhD candidature. The first relates to the thesis as the focus is

Ciulfina biseriata and patterns of habitat choice, and is published the journal *Acta Oecologica*. The second investigates patterns of food caching behaviour in *Nephila* orb-web spiders and is published in the *Australian Journal of Zoology*.

References

- Acebes, A., Cobb, M. & Ferveur, J. F. 2003. Species-specific effects of single sensillum ablation on mating position in *Drosophila*. *Journal Of Experimental Biology*, **206**, 3095-3100.
- Aldrich, B. T., Zolnerowich, G. & Kambhampati, S. 2004. Interspecific morphological variation in the wood-feeding cockroach, *Cryptocercus* (Dictyoptera: Cryptocercidae). *Arthropod Structure & Development*, **33**, 443-451.
- Arnqvist, G. 1997. The evolution of animal genitalia: distinguishing between hypotheses by single species studies. *Biological Journal of the Linnean Society*, **60**, 365-379.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature*, **393**, 784-786.
- Arnqvist, G. & Danielsson, I. 1999. Copulatory behavior, genital morphology, and male fertilization success in water striders. *Evolution*, **53**, 147-156.
- Arnqvist, G. & Rowe, L. 2005. *Sexual conflict*. Princeton: Princeton University Press.
- Bertin, A. & Fairbairn, D. J. 2005. One tool, many uses: precopulatory sexual selection on genital morphology in *Aquarius remigis*. *Journal of Evolutionary Biology*, **18**, 949-961.

- Blanckenhorn, W. U., Hosken, D. J., Martin, O. Y., Reim, C., Teuschl, Y. & Ward, P. I. 2002. The costs of copulating in the dung fly *Sepsis cynipsea*. *Behav. Ecol.* %R 10.1093/beheco/13.3.353, **13**, 353-358.
- Colless, D. H. 1983. Geographic variation in an Australian species of *Chaoborus* (Diptera: Culicidae). *Aust. J. Zool.*, **31**, 15-27.
- Cordero Rivera, A., Andres, J. A., Cordoba-Aguilar, A. & Utzeri, C. 2004. Postmating sexual selection: allopatric evolution of sperm competition mechanisms and genital morphology in calopterygid damselflies (Insecta: Odonata). *Evolution*, **58**, 349-359.
- Cordoba-Aguilar, A. 1999. Male copulatory sensory stimulation induces female ejection of rival sperm in a damselfly. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **266**, 779-784.
- Cordoba-Aguilar, A. 2002. Sensory trap as the mechanism of sexual selection in a damselfly genitalic trait (Insecta: Calopterygidae). *American Naturalist*, **160**, 594-601.
- Crudginton, H. S. & Siva-Jothy, M. T. 2000. Genital damage, kicking and early death - the battle of the sexes takes a sinister turn in the bean weevil. *Nature*, **407**, 855-856.
- Eberhard, W. G. 1985. *Sexual selection and animal genitalia*. Harvard University Press.
- Eberhard, W. G. 1993a. Copulatory Courtship And Genital Mechanics Of 3 Species Of *Macroductylus* (Coleoptera Scarabaeidae Melolonthinae). *Ethology Ecology & Evolution*, **5**, 19-63.

- Eberhard, W. G. 1993b. Copulatory Courtship And Morphology Of Genitalic Coupling In 7 *Phyllophaga* Species (Coleoptera, Melolonthidae). *Journal Of Natural History*, **27**, 683-717.
- Eberhard, W. G. 1996. *Female control: Sexual selection by cryptic female choice*. Princeton: Princeton University Press.
- Eberhard, W. G. 2001a. The functional morphology of species-specific clasping structures on the front legs of male *Archiseptis* and *Palaeoseptis* flies (Diptera, Sepsidae). *Zoological Journal Of The Linnean Society*, **133**, 335-368.
- Eberhard, W. G. 2001b. Genitalic behavior in *Hybosciara gigantea* (Diptera: Sciaridae) and the evolution of species-specific genitalia. *Journal Of The Kansas Entomological Society*, **74**, 1-9.
- Eberhard, W. G. 2001c. Species-specific genitalic copulatory courtship in sepsid flies (Diptera, Sepsidae, *Microsepsis*) and theories of genitalic evolution. *Evolution*, **55**, 93-102.
- Eberhard, W. G. 2002. Physical restraint or stimulation? The function(s) of the modified front legs of male *Archiseptis diversiformis* (Diptera, Sepsidae). *J. Insect. Behav.*, **15**, 831-850.
- Eberhard, W. G. 2004a. Male-female conflict and genitalia: failure to confirm predictions in insects and spiders. *Biological Reviews*, **79**, 121-186.
- Eberhard, W. G. 2004b. Rapid divergent evolution of sexual morphology: Comparative tests of antagonistic coevolution and traditional female choice. *Evolution*, **58**, 1947-1970.

- Eberhard, W. G. & Huber, B. A. 1998. Courtship, copulation and sperm transfer in *Leucauge marina* (Araneae, Tetragnathidae) with implications for higher classification. *J. Arachnol.*, **26**, 324-368.
- Eberhard, W. G., Huber, B. A., Rodriguez, R. L., Briceno, R. D., Salas, I. & Rodriguez, V. 1998. One size fits all - relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. *Evolution*, **52**, 415-431.
- Edmunds, M. 1975. Courtship, mating and possible sex pheromones in three species of Mantodea. *Ent. Month. Mag.*, **111**, 53-57.
- Edvardsson, M. & Tregenza, T. 2005. Why do male *Callosobruchus maculatus* harm their mates? *Behav. Ecol. %R 10.1093/beheco/ari055*, **16**, 788-793.
- Ehrmann, R. 2002. *Mantodea: Gottesanbeterinnen der Welt*. Natur und Tier Verlag.
- Fagan, W. F. & Odell, G. M. 1996. Size-dependent cannibalism in praying mantids: Using biomass flux to model size-structured populations. *American Naturalist*, **147**, 230-268.
- Fairbairn, D. J., Vermette, R., Kapoor, N. N. & Zahiri, N. 2003. Functional morphology of sexually selected genitalia in the water strider *Aquarius remigis*. *Canadian Journal Of Zoology-Revue Canadienne De Zoologie*, **81**, 400-413.
- Gemeno, C., Claramunt, J. & Dasca, J. 2005. Nocturnal calling behavior in mantids. *J. Insect. Behav.*, **18**, 389-403.
- Horton, D. R. & Lewis, T. M. 2005. Size and shape differences in genitalia of males from sympatric and reproductively isolated populations of *Anthocoris antevolens* White (Heteroptera: Anthocoridae) in the Yakima Valley, Washington. *Annals Of The Entomological Society Of America*, **98**, 527-535.

- Hosken, D. J., Minder, A. M. & Ward, P. I. 2005. Male genital allometry in Scathophagidae (Diptera). *Evolutionary Ecology*, **19**, 501-515.
- Hosken, D. J. & Stockley, P. 2004. Sexual selection and animal genitalia. *TREE*, **19**, 87-93.
- House, C. M. & Simmons, L. W. 2003. Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc. R. Soc. Lond. B*, **270**, 447-455.
- House, C. M. & Simmons, L. W. 2005a. The evolution of male genitalia: patterns of genetic variation and covariation in the genital sclerites of the dung beetle *Onthophagus taurus*. *Journal Of Evolutionary Biology*, **18**, 1281-1292.
- House, C. M. & Simmons, L. W. 2005b. Relative influence of male and female genital morphology on paternity in the dung beetle *Onthophagus taurus*. *Behav. Ecol. %R* *10.1093/beheco/ari066*, **16**, 889-897.
- House, C. M. & Simmons, L. W. 2006. Offensive and defensive sperm competition roles in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabeidae). *Behav. Ecol. Sociobiol.*
- Hribar, L. J. 1994. Geographic variation of male genitalia of *Anopheles nuneztovari* (Diptera: Culicidae). *Mosquito Systematics*, **26**, 132-144.
- Huber, B. A. 2003. Rapid evolution and species-specificity of arthropod genitalia: fact or artifact? *Organisms Diversity & Evolution*, **3**, 63-71.
- Huber, B. A. & Gonzalez, A. P. 2001. Female genital dimorphism in a spider - (Araneae: Pholcidae). *Journal Of Zoology*, **255**, 301-304.

- Hurd, L. E., Eisenberg, R. M., Fagan, W. F., Tilmon, K. J., Snyder, W. E., Vandersall, K. S., Datz, S. G. & Welch, J. D. 1994. Cannibalism Reverses Male-Biased Sex-Ratio in Adult Mantids - Female Strategy against Food Limitation. *Oikos*, **69**, 193-198.
- Hurd, L. E., Prete, F. R., Jones, T. H., Singh, T. B., Co, J. E. & Portman, R. T. 2004. First identification of a putative sex pheromone in a praying mantid. *Journal of Chemical Ecology*, **30**, 155-166.
- Jocque, R. 2002. Genitalic polymorphism - a challenge for taxonomy. *J. Arachnology.*, **30**, 298-306.
- Keffer, S. L. 2004. Morphology and evolution of waterscorpion male genitalia (Heteroptera: Nepidae). *Systematic Entomology*, **29**, 142-172.
- King Sirot, L. 2003. The evolution of insect mating structures through sexual selection. *Florida Entomologist*, **86**, 124-133.
- Klass, K. D. 1997. *The external male genitalia and the phylogeny of Blattaria and Mantodea*. Bonn: Zoologisches Forschungsinstitut und Museum Alexander Koenig.
- Kopp, A. & True, J. R. 2002. Evolution of male sexual characters in the Oriental *Drosophila melanogaster* species group. *Evolution & Development*, **4**, 278-291.
- Kynaston, S. E., McErlainward, P. & Mill, P. J. 1994. Courtship, Mating-Behavior And Sexual Cannibalism In The Praying-Mantis, *Sphodromantis-Lineola*. *Animal Behaviour*, **47**, 739-741.
- Lawrence, S. E. 1992. Sexual Cannibalism in the Praying Mantid, *Mantis-Religiosa* - a Field-Study. *Animal Behaviour*, **43**, 569-583.
- Liske, E. & Davis, W. J. 1984. Sexual behaviour of the Chinese praying mantis. *Animal Behaviour*, **32**, 916-917.

- Liske, E. & Davis, W. J. 1987. Courtship and mating behaviour of the Chinese praying mantis, *Tenodera aridifolia sinensis*. *Animal Behaviour*, **35**, 1524-37.
- Maxwell, M. R. 1998. Lifetime mating opportunities and male mating behaviour in sexually cannibalistic praying mantids. *Animal Behaviour*, **55**, 1011-1028.
- Maxwell, M. R. 1999a. Mating behavior. In: *The praying mantids* (Ed. by Prete, F. R., Wells, H., Wells, P. H. & Hurd, L. E.). Baltimore: The John Hopkins University Press.
- Maxwell, M. R. 1999b. The risk of cannibalism and male mating behavior in the Mediterranean praying Mantid, *Iris oratoria*. *Behaviour*, **136**, 205-219.
- Maxwell, M. R. 2000. Does a single meal affect female reproductive output in the sexually cannibalistic praying mantid *Iris oratoria*? *Ecological Entomology*, **25**, 54-62.
- Mayr, E. 1963. *Animal species and evolution*. Harvard University Press.
- Mound, L. A., Crespi, B. J. & Tucker, A. 1998. Polymorphism and kleptoparasitism in thrips (Thysanoptera: Phlaeothripidae) from woody galls on Casuarina trees. *Australian Journal Of Entomology*, 8-16.
- Mutanen, M. & Kaitala, A. 2006. Genital variation in a dimorphic moth *Selenia tetralunaria* (Lepidoptera, Geometridae). *Biological Journal Of The Linnean Society*, **87**, 297-307.
- Ohno, S., Hoshizaki, S., Ishikawa, Y., Tatsuki, S. & Akimoto, S. 2003. Allometry of male genitalia in a lepidopteran species, *Ostrinia latipennis* (Lepidoptera: Crambidae). *Applied Entomology And Zoology*, **38**, 313-319.
- Otronen, M. 1998. Male asymmetry and postcopulatory sexual selection in the fly *Dryomyza anilis*. *Behav. Ecol. Sociobiol.*, **42**, 185-191.

- Perez, B. 2005. Calling behaviour in the female praying mantis, *Hierodula patellifera*. *Physiological Entomology*, **30**, 42-47.
- Prete, F. R., Hurd, L. E., Jones, T., Portman, R., Singh, T. B. & Co, J. E. 2000. Scent of a woman: the first identification and assay of a mantid pheromone. *American Zoologist*, **40**, 1180-1180.
- Prokop, P. & Vaclav, R. 2005. Males respond to the risk of sperm competition in the sexually cannibalistic praying mantis, *Mantis religiosa*. *Ethology*, **111**, 836-848.
- Reinhardt, K., Naylor, R. A. & Siva-Jothy, M. T. 2003. Reducing the cost of traumatic insemination: female bedbugs evolve a unique organ. *Proc. Roy. Soc. Lond. B*, **270**, 2371-2375.
- Reinhardt, K., Naylor, R. A. & Siva-Jothy, M. T. 2005. Potential sexual transmission of environmental microbes in a traumatically inseminating insect. *Ecological Entomology*, **30**, 607-611.
- Robinson, M. H. & Robinson, B. 1979. By dawn's early light: matitudinal mating and sex attractants in a neotropical mantid. *Science*, **205**, 825-827.
- Roeder, K. D. 1935. An experimental analysis of the sexual behavior of the praying mantis (*Mantis religiosa*, L.). *Biol. Bull.*, **69**, 203-220.
- Shapiro, A. M. & Porter, A. H. 1989. The lock and key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *Ann. Rev. Ent.*, **34**, 231-245.
- Shapiro, L. H. 2000. Reproductive costs to heterospecific mating between two hybridizing katydids (Orthoptera: Tettigoniidae). *Ann. Ent. Soc. America.*, **93**, 440-446.
- Simmons, L. W. 2001. *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press.

- Sota, T. 2002. Radiation and reticulation: extensive introgressive hybridization in the carabid beetles *Ohomopterus* inferred from mitochondrial gene genealogy. *Popul. Ecol.*, **44**, 145-156.
- Sota, T. & Kubota, K. 1998. Genital lock-and-key as a selective agent against hybridization. *Evolution*, **52**, 1507-1513.
- Sota, T., Takami, Y., Monteith, G. B. & Moore, B. P. 2005. Phylogeny and character evolution of endemic Australian carabid beetles of the genus *Pamborus* based on mitochondrial and nuclear gene sequences. *Molecular Phylogenetics And Evolution*, **36**, 391-404.
- Stoks, R., Nystrom, J. L., May, M. L. & McPeck, M. A. 2005. Parallel evolution in ecological and reproductive traits to produce cryptic damselfly species across the Holarctic. *Evolution*, **59**, 1976-1988.
- Stutt, A. D. & Siva-Jothy, M. T. 2001. Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. *PNAS* %R 10.1073/pnas.101440698, **98**, 5683-5687.
- Svenson, G. J. & Whiting, M. F. 2004. Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology*, **29**, 359-370.
- Tadler, A. 1999. Selection of a conspicuous male genitalic trait in the seedbug *Lygaeus simulans*. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **266**, 1773-1777.
- Takami, Y. 2003. Experimental analysis of the effect of genital morphology on insemination success in the ground beetle *Carabus insulicola* (Coleoptera Carabidae). *Ethol. Ecol. Evol.*, **15**, 51-61.
- Tatsuta, H. & Akimoto, S. I. A. 2000. Variability in phenotypic covariance structure of female genitalia in the brachypterous grasshopper *Podisma sapporensis*

(Orthoptera: Acrididae: Podisminae). *Annals Of The Entomological Society Of America*, **93**, 127-132.

Tatsuta, H., Mizota, K. & Akimoto, S. I. 2001. Allometric patterns of heads and genitalia in the stag beetle *Lucanus maculifemoratus* (Coleoptera: Lucanidae). *Annals Of The Entomological Society Of America*, **94**, 462-466.

Usami, T., Yokoyama, J., Kubota, K. & Kawata, M. 2006. Genital lock-and-key system and premating isolation by mate preference in carabid beetles (*Carabus* subgenus *Ohomopterus*). *Biol. J. Linn. Soc.*, **87**, 145-154.

Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. *Science*, **203**, 916-918.

Chapter One

Parapatric distributions in the praying mantid genus *Ciulfina* (Mantodea: Liturgusidae) with descriptions of three new species.

Submitted to the *Australian Journal of Entomology*

**This chapter includes some results collected by Mr Scott Ginn
who is a co-author on the submitted paper.**

Abstract:

The genus *Ciulfina* (Mantodea: Liturgusidae) includes a number of small tree-trunk dwelling species of praying mantids that are found through eastern Queensland and northern Australia. Here we describe the biogeographic distribution of four species (including three newly described species), throughout eastern Queensland, Australia. No two species were ever collected in sympatry, but many distributional edges formed parapatric boundaries between species distributions. Parapatry and the potential mechanisms for the formation and maintenance of parapatric boundaries are discussed. The four species: *C. baldersoni* sp. nov., *C. biseriata* Westwood, *C. klassi* sp. nov. and *C. rentzi* sp. nov. are formally described on the basis of male genital morphology and scanning electron micrographs are presented to demonstrate these differences.

eucalypt woodland, urban parks and mangroves. *Ciulfina* are relatively small (3-4 cm), cryptic mantids that are cursorial generalist predators feeding on a variety of arthropod prey. Although found in a variety of wooded habitats, they show preference for smooth-barked trees (Hill *et al.*, 2004) and can be found in densities that are relatively high for praying mantids. Balderson (Balderson, 1978) reported 17 putative species of *Ciulfina* throughout Australia, but only *C. biseriata* Westwood and *C. liturgusa* Giglio-Tos have been formally described. The unusual phenomenon of phallic reversal has also been described for *Ciulfina*, whereby the orientation of the male genital complex varies between and in one case within, species (Balderson, 1978; Holwell *et al* in review). The 'normal' orientation described by Balderson (1978) is referred to herein as the sinistral orientation, and defined as a genital complex in which the caudal process of the left epiphallus is directed to the left. The 'reversed' orientation described by Balderson (1978) is described herein as the dextral orientation, and defined as a genital complex in which the caudal process of the left epiphallus is directed to the right. This paper aims to continue the work of Balderson and describes three new species on the basis of male genital morphology. External morphology and female genitalic morphology did not vary between the species described, and so were not taxonomically useful.

Methods:

Collection

Ciulfina were collected from 60 locations from eastern Queensland, Australia, between 2002 and 2005. Attempts were made to collect from locations that were spaced out by roughly 50-100 km, except in areas where the range of one species met the range of another species. In this situation, *Ciulfina* were sampled more intensively to determine

range boundaries. Latitudes and longitudes of collection locations were recorded using a Garmin® GPS 12XL personal navigator. Mantids were collected by hand from their tree trunk habitats and housed in the laboratory to observe behavioural interactions as part of other studies.

Specimen preparation

After their death, individuals were placed into vials of 70% ethanol. Genitalia were dissected out and placed in 10% KOH for one hour to dissolve unsclerotised tissue. Genital complexes were then viewed under a binocular microscope and described. Terminology for the genitalia descriptions follows that of La Greca (1954).

A small number of individuals were examined using scanning electron micrography (SEM): two males of each species (one of each genital orientation where relevant) and one female. These mantids were euthanased by freezing, after which the abdomens were fixed overnight in 4% paraformaldehyde 3% gluteraldehyde in 0.1M phosphate buffer. The specimens were then washed and left overnight in 0.1M phosphate buffer solution. Abdomens were then submerged in 1% osmium tetroxide for approximately 30 minutes. The specimens were washed several times with water, then with a graded series of ethanol baths (from 50-100% ethanol). Abdomens were removed, critical point dried, mounted on a 10 mm stub using carbon adhesive tabs, sputter-coated with gold, and viewed with a Jeol JSM 840 scanning electron microscope.

Results:

Distribution

Collection locations for the four species of *Ciulfina* are presented on Figure 1. *Ciulfina rentzi* n. sp. were collected between latitudes of 16°05' and 18°09' ranging approximately from Cape Tribulation to Murray Falls and with one isolated population near Little Crystal Creek (19°00'S, 146°15'E). *Ciulfina klassi* were collected between latitudes of 17°53' and 18°51', occurring through a very coastal distribution between Mission beach and the Paluma range. *Ciulfina biseriata* were collected between latitudes of 17°00' and 24° 22' and covered the largest range of the four species ranging between Mareeba in the north to Miriam Vale in the south, and extending inland around the distributions of both *C. klassi* and *C. rentzi*. *Ciulfina baldersoni* were collected from between latitudes of 24°52' and 25°33', approximately between Bundaberg and Maryborough. No more than one species of *Ciulfina* were ever collected at any location. Despite the more intensive sampling effort in regions where one species was replaced by another, no sympatry was detected. Some populations of *C. biseriata*, *C. klassi* and *C. rentzi* were located within 1km of one-another (e.g. near Mission Beach and near Cardwell).

Taxonomy

External morphology for all species as per original description of *Ciulfina* by Giglio-Tos (1915). (see Figure 2). *C. baldersoni*, *C. klassi* and *C. rentzi* were considered to be heterospecific to *C. biseriata* due to clear differences in male genitalic morphology.

Ciulfina baldersoni Ginn & Holwell sp. nov. (Figure 3 a (sinistral genitalia), b (dextral genitalia)).

Etymology. Named in honour of the late Mr John Balderson, in recognition of his extensive work on the genus *Ciulfina* and his remarkable contribution to the study of Australian Mantodea.

Type. Holotype male (here designated), Queen's Park, Bundaberg, Queensland, Australia, 24°52'S 152°20'E (AM).

Other material examined. 5♂♂ Great Sandy Region Botanic Gardens, Hervey Bay, Qld 21.ix.2003; 5♂♂ Queen's Park, Bundaberg, Qld 22.ix.2003

Description of genitalia. Right dorsal phallomere typical of genus, thumb-shaped, deeply pouched, orientated posteriorly, attached to ventral phallomere centrally by long arm that is wide basally and tapers apically; anterior apodeme typically spoon-shaped; phalloid apophysis heavily sclerotised, conical, tapering apically, distal one third at right angle to base of process angled toward dorsal surface; apical process of left phallomere with robust base, process slightly sinuate, gradually tapering apically, apex slightly bulbous and heavily sclerotised, process projects away from right phallomere parallel to base of ventral phallomere, process projects beyond pubescent triangular membrane at base of left dorsal phallomere; pubescent triangular membrane terminates in distinct tip: ventral sclerotised

process stout, heavily sclerotised, slightly recurved apically; distal process of ventral phallomere broad, widening apically, with vertical row of heavily sclerotised teeth along outer margin; lateral apex of ventral phallomere rounded, about as wide as distal process but produced well beyond distal process.

Description of subgenital plate (Fig. 2) Sternite widened medially with upper margin raised and tapering inwards medially; posterior margin visibly excised centrally with distinct crack extending anteriorly along plate, base stalks of styli long ~0.5-0.8mm; styli stout, pubescent, shorter than length of basal stalk.

Ciulfina biseriata (Westwood) (Figure 3 e,f)

Nanomants biseriata Westwood (1889), p32; holotype Australian-NW, Rockhampton (OXUM)

Ciulfina biseriata: Giglio-Tos, (1915), p. 64.

Type material examined. holotype Australian-NW, Rockhampton (OXUM).

Other material examined. 5♂♂ Bushland 5 km West of Yeppoon, Rockhampton-Yeppoon Rd, Qld 23.ix.2003; 5♂♂ Queen's Park, Mackay, Qld 24.ix.2003 3♂♂ Clifton Beach, Cairns, Qld Sept 2002. 5♂♂ Bushland 1km Sth of Mareeba, Qld 14.i.2005 5♂♂ Cardwell Forest, 3km West of Cardwell, Qld 16.i.2002.

Description of genitalia. Right dorsal phallomere typical (see Type1), thumb-shaped, anterior apodeme typically spoon-shaped; phalloid apophysis heavily sclerotised, basal one third stout, then bent downwards at right angle then tapering apically, apex slightly

bulbous but flattened at tip; apical process of left phallomere with robust base, moderately sclerotised, process slightly sinuate, gradually tapering apically, apex slightly bulbous and moderately sclerotised, process projects away from right phallomere angled acutely away from base of ventral phallomere, process not projecting beyond pubescent triangular membrane at base of left dorsal phallomere; pubescent triangular membrane slightly rounded without apical tip; ventral sclerotised process slender, heavily sclerotised, distinctly recurved apically, process much longer than in Type 1; distal process of ventral phallomere broad at base, gradually tapering apically, terminating in large upper tooth with 2-3 smaller teeth below, heavily sclerotised, apex slightly curved inwards; lateral apex of ventral phallomere somewhat quadrate, about three times as wide as distal process at widest point and terminating in line with apex of distal process.

Description of subgenital plate (Fig. 4). Sternite widened medially; posterior margin with narrow triangular excision centrally; base stalks of styli medium length 0.3-0.5 mm, sometimes asymmetrical; styli stout, pubescent, similar in length to basal stalk.

Ciulfina klassi Ginn & Holwell sp. nov. (Figure 3 c)

Etymology. Named in honour of Dr Klaus Dieter Klass, in recognition of his contribution to our understanding of the morphology, evolution and phylogeny of the Mantodea.

Type. Holotype male (here designated), bushland 2 km West of Mission Beach, Queensland, Australia, 17°48'S 146°01'E (AM).

Other material examined. 10♂♂ Bushland 2 km West of Mission Beach, Mission Beach Rd, Qld 23.ix.2003; 5♂♂ Bruce Hwy at Hinchinbrook channel lookout, 40km Sth of Cardwell, Qld 17.i.2004; 5♂♂ Bushland alongside Bruce Hwy at Conn Creek, 20km Sth of Cardwell, Qld 17.i.2004.

Description of genitalia. Right dorsal phallomere typical (see *C. baldersoni*), thumb-shaped, anterior apodeme typically spoon-shaped, with sclerotised bulbous region below arm; phalloid apophysis small, heavily sclerotised, essentially straight, tapering apically, apex slightly decumbent; apical process of left phallomere with robust base but shorter than in *C. baldersoni*, thus locating process somewhat closer to base of ventral phallomere, process slightly sinuate, gradually tapering apically with apical half very thin, apex slightly bulbous and heavily sclerotised, process projects away from right phallomere parallel to base of ventral phallomere, process projects beyond pubescent triangular membrane at base of left dorsal phallomere; pubescent triangular membrane terminates without distinct tip; ventral sclerotised process slender, heavily sclerotised, distinctly recurved apically, process much longer than in *C. baldersoni*; distal process of ventral phallomere broad at base, strongly curved inwards and anteriorly underneath lateral apex of ventral phallomere, gradually tapering apically, terminating in large, single, heavily sclerotised tooth; lateral apex of ventral phallomere rounded, twice as wide as distal process but produced as paddle-shaped plate well beyond apex of distal process.

Description of subgenital plate. Sternite widened medially with upper margin raised and tapering inwards medially; posterior margin visibly excised centrally with distinct crack extending anteriorly along plate, base stalks of styli long ~0.5-0.8mm; styli stout, pubescent, shorter than length of basal stalk.

Ciulfina rentzi Ginn & Holwell sp. nov. (Figure 3 d)

Etymology. Named in honour of Dr David Rentz, in recognition of his outstanding contribution to Australian entomology, and particularly to the study of the Australian Mantodea.

Type. Holotype male (here designated), Flecker Botanic Gardens, Edge Hill, Cairns, Queensland, Australia, 16°54'S 145°44'E (AM).

Material examined. Holotype? 6♂♂ Flecker Botanic Gardens, Edge Hill, Cairns, Qld 26.ix.2003; 3♂♂ Babinda Boulders, Qld Sept 2002; 1♂ Barron Falls, Qld Sept 2002; 5♂♂ Babinda Boulders, Wooroonooran N.P., Qld 15.i.2004; 5♂♂ Lacey Creek, Tam O' Shanter S.F., Qld 16.i.2004.

Description of genitalia. Right dorsal phallomere typical (see *C. baldersoni*), thumb-shaped but stouter and broader than other types, rounded tip of phallomere bent inwards, covered with long hairs; anterior apodeme typical spoon-shaped; phalloid apophysis heavily sclerotised, long and slender, approximately half the length of the right phallomere; process projects posteriorly, tapering apically with last one quarter bent outwards towards right phallomere; apical process of left phallomere with robust base, process slightly sinuate, process very slender throughout length, apex terminates in fine rounded tip, moderately sclerotised, process projects away from right phallomere more or less parallel to base of ventral phallomere, process projects as far as pubescent triangular

membrane at base of left dorsal phallomere; pubescent triangular membrane flattened at tip; ventral sclerotised process slender, medium length, heavily sclerotised, more or less straight; distal process of ventral phallomere moderately broad basally, tapering apically, gently curved inwards, terminating in 1-2 small heavily sclerotised teeth; inner lateral apex of ventral phallomere gently rounded, about 3-4 times wider than distal process, not produced beyond apex of distal process, outer lateral apex of ventral phallomere distinctly lobed.

Description of subgenital plate (Fig. 3). Sternite very broad, distinctly widened and angled medially on lateral margins; posterior margin strongly concave, base stalks vestigial, reduced to terminal angles; styli slender, pubescent, distinctly longer than length of basal stalk.

Discussion:

The parapatric distributions of *C. biseriata*, *C. rentzi* and *C. klassi* are particularly interesting because boundaries occur at a number of locations, and between all three combinations of these species. The majority of studies of parapatry have focussed on boundaries between two species, and often at one location. For the *Ciulfina* system however, the maintenance of parapatry may be driven by processes that operate over a broad range of taxa and geographical areas. Environmental gradients appear to be involved to some extent as each of these species is predominantly confined to a particular type of habitat: *C. rentzi* to primary rainforest, *C. klassi* to coastal woodland and *C. biseriata* to open eucalypt woodland. However, many exceptions occur and transitional regions between habitat types still contained only one *Ciulfina* species. For example, sites at El

Arish and Edmund Kennedy N. P. consisted of coastal *Melaleuca* woodland but contained populations of *C. rentzi*. Similarly, locations in Broadwater S.F.P. and 30km Sth of Cardwell consisted of open eucalypt woodland but contained *C. klassi*. The transition between habitat types is therefore not the only factor driving the maintenance of these boundaries.

Competitive interactions may explain parapatry in *Ciulfina* distributions, however not enough is known about the ecology of resource use in this group. It is also possible that competition and environmental factors combine to assist each species differentially in different habitats. Similarly, predation and parasites may also contribute to the distributional ecology of *Ciulfina* species. However, only two incidences of predation (one bird and one spider) and one parasite (a nematomorph worm) have been observed by the authors, so this suggestion is speculative. Distributions of prey species are unlikely to be influential, as all *Ciulfina* species are generalist predators feeding on a broad variety of small arthropod prey (Hill *et al.*, 2004).

The final possibility is that reproductive interference may play a role in shaping the distributions of *Ciulfina* species. The 'satyr effect' was suggested by Ribeiro and Spielmann (1986) to explain both parapatry and extinction in species where individuals mate indiscriminately with both conspecifics and heterospecifics. The reduced fitness suffered by individuals who mate with heterospecifics would lead to either the extinction of one species (if any asymmetry existed) or the formation of a stable parapatric boundary. Reproductive interference has been shown to influence the fitness of individuals in the narrow overlap zone of the parapatric boundary of the reptile ticks *Aponomma hydrosauri*, *Amblyomma albolimbatum* and *Ambliomma limbatum* (Andrews *et al.*, 1982). This has also been suggested to explain the existence of parapatric boundaries and narrow hybrid

zones for carabid beetles (Sota & Kubota, 1998) and mosquitoes (Spielman & Feinsod, 1979). There is certainly potential for reproductive interference to be important in the maintenance of *Ciulfina* parapatric boundaries. Males of all four *Ciulfina* species discussed showed no preference for conspecific females in choice tests, and regularly mated with and deposited spermatophores into heterospecific females in the laboratory (unpublished data). Although no hybrid offspring were produced, the time and energetic investment, and the gametes wasted by males (and possibly females) may represent a reduction in fitness great enough to influence the likelihood of range overlap between *Ciulfina* species. Also, the ‘satyr effect’ model predicts parapatry to occur when species have low vagility (Ribeiro & Spielman, 1986), relevant for *Ciulfina*, due to the flightlessness of both males and females (Holwell *et al.*, (in review)).

Much is needed to elucidate the processes maintaining parapatry in *Ciulfina*. The potential influence of competition, environmental factors and reproductive interference, however suggests hypotheses that can be tested. Therefore, the genus *Ciulfina* may be an ideal system with which to test current ideas about parapatry.

Acknowledgements:

We wish to thank Anne Gaskett, Scott Holwell, Tom Joss and Chris Longson for assistance with field collection, and Debra Birch and Nicole Vella for assistance with scanning electron microscopy. We also wish to thank David Rentz, John Balderson, and Laurence Mound for assistance with collections at the Australian National Insect Collection (ANIC), and Michelle Nissen for assistance with permits. Collection from a number of locations was conducted under permit ATH 05/006 from the Queensland Parks

and Wildlife Service and the Environmental Protection Authority. Financial support was received from Macquarie University and the Australian Research Council.

References:

- Andrews, R. H., Petney, T. N. & Bull, C. M. 1982. Reproductive interference between three parapatric species of reptile tick. *Oecologia*, **52**, 281-286.
- Balderson, J. 1978. Reversal of the phallic complex in the genera *Ciulfina* Giglio-Tos and *Stenomantis* Saussure (Mantodea: Mantidae: Iridopteryginae). *J. Aust. Ent. Soc.*, **17**, 235-239.
- Bergstrom, B. J. 1992. Parapatry and encounter competition between chipmunk (*Tamias*) species and the hypothesized role of parasitism. *Am. Mid. Nat.*, **128**, 168-179.
- Bull, C. M. 1991. Ecology of parapatric distributions. *Annu. Rev. Ecol. Syst.*, **22**, 19-36.
- Bull, C. M. & Burzacott, D. 2001. Temporal and spatial dynamics of a parapatric boundary between two Australian reptile ticks. *Mol. Ecol.*, **10**, 639-648.
- Burger, W. 1992. Parapatric close-congeners in Costa Rica: hypotheses for pathogen-mediated plant distribution and speciation. *Biotropica*, **24**, 567-570.
- Cimmaruta, R., Forti, G., Nascetti, G. & Bullini, L. 1999. Spatial distribution and competition in two parapatric sibling species of European plethodontid salamanders. *Ethology Ecology & Evolution*, **11**, 383-398.
- Flynn, M. F. & Hobbs, H. H. I. 1984. Parapatric crayfishes in southern Ohio: evidence of competitive exclusion? *J. Crustacean Biol.*, **4**, 382-389.
- Giglio-Tos, E. 1915. Mantidi esotici. generi e specie nuove. *Bull. Soc. Ent. Ital. Firenze*. **46**, 31-108.

- Hill, P. J. B., Holwell, G. I., Goth, A. & Herberstein, M. E. 2004. Preference for habitats with low structural complexity in the praying mantid *Ciulfina* sp (Mantidae). *Acta Oecologica*, **26**, 1-7.
- Holwell, G. I., Barry, K. & Herberstein, M. E. (in review). Mate location, antennal morphology and ecology in praying mantids. *Biol. J. Linn. Soc.*
- Huey, R. B. 1979. Parapatry and niche complimentarity of Peruvian Desert geckos (*Phyllodactylus*): the ambiguous role of competition. *Oecologia*, **38**, 249-259.
- Key, K. H. L. 1981. Species, parapatry and the Morabine grasshoppers. *Syst. Zool.*, **30**, 425-458.
- Reullier, J., Perez-Tris, J., Bensch, S. & Secondi, J. 2006. Diversity, distribution and exchange of blood parasites meeting at an avian moving contact zone. *Mol. Ecol.*, (online publication date: Jan 9, 2006).
- Ribeiro, J. M. C. & Spielman, A. 1986. The satyr effect: a model predicting parapatry and species extinction. *Am. Nat.*, **128**, 513-528.
- Settle, W. H. & Wilson, L. T. 1990. Invasion by the variegated leafhopper and biotic interactions: parasitism, competition and apparent competition. *Ecology*, **71**, 1461-1470.
- Sota, T. & Kubota, K. 1998. Genital lock-and-key as a selective agent against hybridization. *Evolution*, **52**, 1507-1513.
- Spielman, A. & Feinsod, F. M. 1979. Differential distribution of peridomestic *Aedes* mosquitoes on Grand Bahama Island. *Trans. R. Soc. Trop. Med. Hyg.*, **73**, 381-384.
- Taylor, C. M. & Lienesch, P. W. 1996. Regional parapatry of the congeneric Cyprinids *Lythrurus snelsoni* and *L. umbratilis*: species repacement along a complex environmental gradient. *Copeia*, **1996**, 493-497.

Westwood, J. O. 1889. Revisio insectorum familiae mantidarum, speciebus novis aut minus cognitis descriptis et delineatis. *Revisio Mantidarum*, Gurney & Jackson, 55 S., 14 Taf.; London

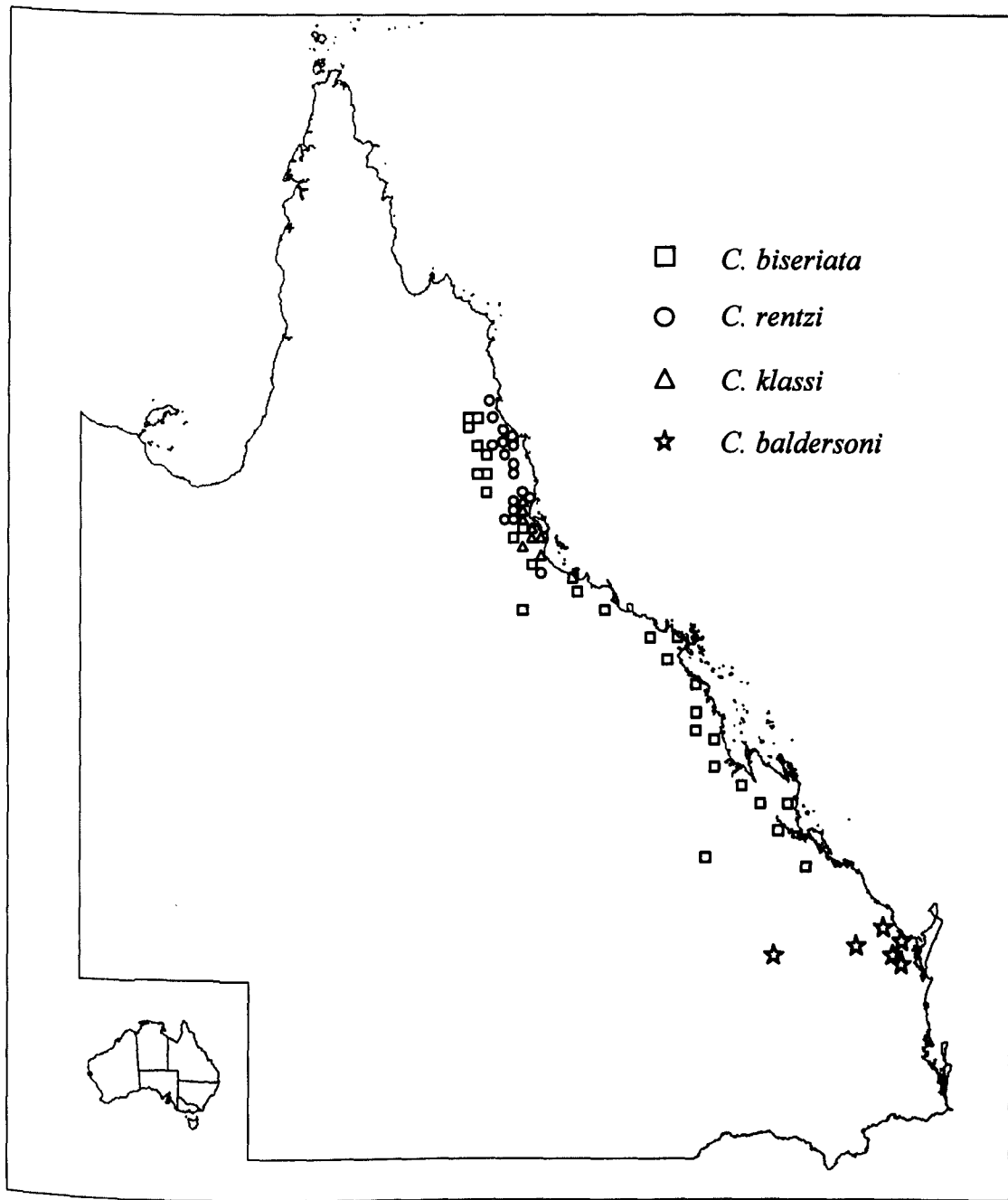
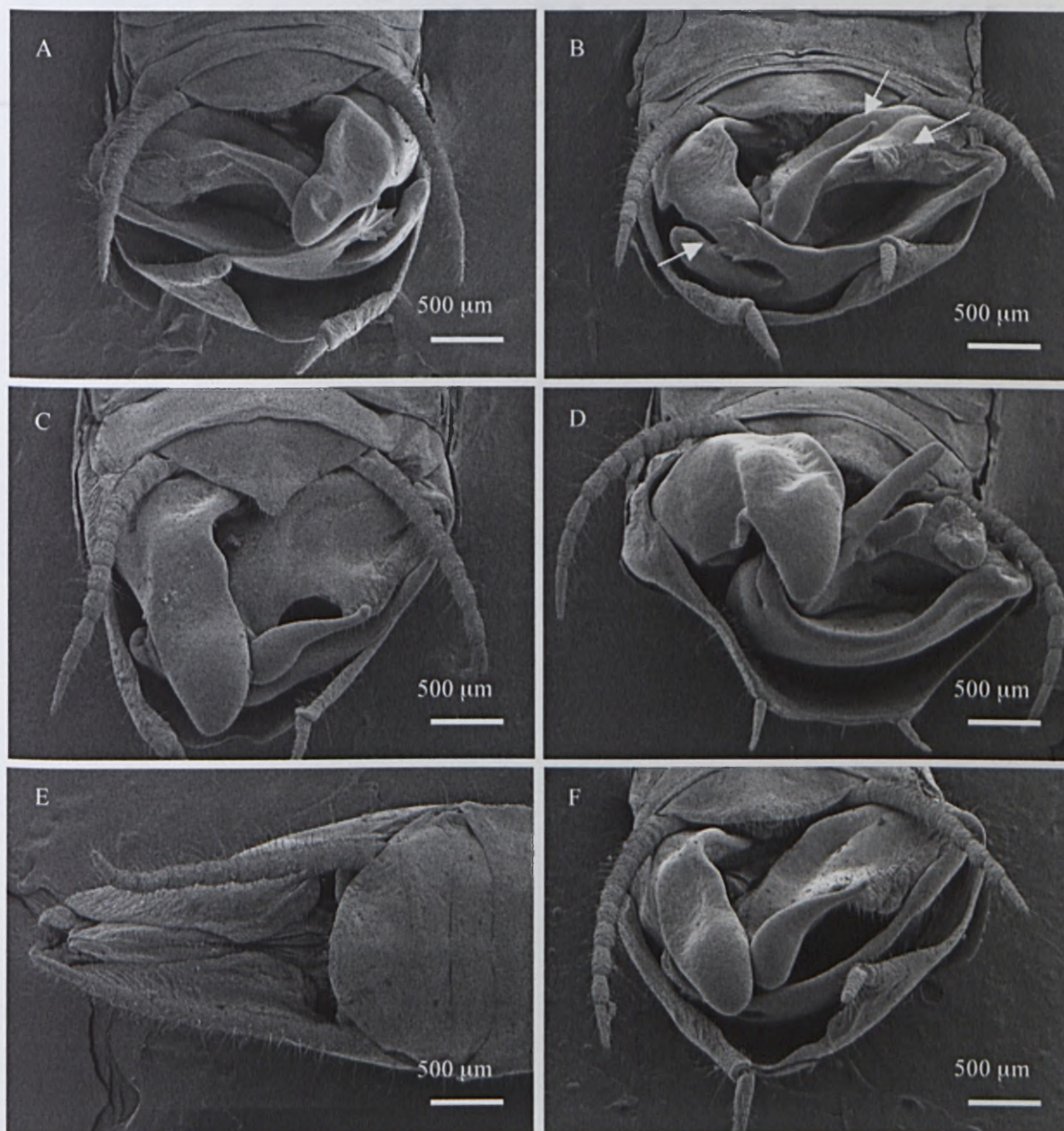


Figure 1. Collection locations for *C. baldersoni* sp. nov. (☆), *C. biseriata* (□), *C. klassi* sp. nov. (△) and *C. rentzi* sp. nov. (○) throughout Queensland, Australia.



Figure 2. Copulating pair of *Ciulfina rentzi*.

Figure 3. Scanning electron micrographs (SEM) of genital morphology in *Ciulfina*: (A) male *C. baldersoni* sp. nov. sinistral genital orientation, (B) male *C. baldersoni* sp nov. dextral genital orientation (arrows indicate structures of taxonomic significance), (C) male *C. klassi* sp. nov., (D) male *C. rentzi* sp. nov. (E) female *C. biseriata*, (F) male *C. biseriata*.



Chapter Two.

Mirror image genital dimorphism in *Ciulfina* praying mantids (Mantodea: Liturgusidae).

Submitted to the journal *Naturwissenschaften*

Abstract:

Although male polymorphisms occur widely in nature and have received considerable recent attention from studies of alternative mating strategies, male genital polymorphism has been described on only a few occasions. Here we describe a dimorphism in the orientation of the male genitalic complex of the praying mantid genus *Ciulfina*. Three species of *Ciulfina* contain males with dextral (right-oriented) and sinistral (left-oriented) genitalia within the same population. We used elliptic Fourier analysis (EFA) to quantify shape and size variation between the genitalia of dextral and sinistral males and determined that the two forms were mirror-images of one-another. Apart from orientation, no difference was detected in the shape or size of four genital structures between dextral and sinistral males of *C. baldersoni* and *C. rentzi*. Possible mechanisms for the maintenance of genital dimorphism in *Ciulfina* are discussed and parallels are drawn with studies of the chiral dimorphism of gastropods.

Introduction.

Polymorphisms represent patterns of discontinuous morphological variation within a species (Andersson, 1994) and can occur between or within sexes. Intrasexual polymorphisms can include differences in colour pattern (Roulin, 2004), body size (Shuster & Wade, 1991), dispersal ability (Zera & Denno, 1997) and structural morphology (Tomkins & Brown, 2004). Among the arthropods, male structural polymorphisms that have received attention include the horn dimorphism of the dung beetle *Onthophagus taurus* (Simmons *et al.*, 1999), the forcep dimorphism of the earwig *Forficula auricularia* (Tomkins & Brown, 2004) and the leg dimorphism of the mite *Sancassania berlesei* (Radwan *et al.*, 2002). These dimorphisms develop over a threshold with body size, and correspond with alternative mating strategies.

While polymorphisms in external morphology are receiving considerable attention from biologists, polymorphism in genitalia has been largely ignored or dismissed. However, although genital polymorphism is rarely described by taxonomists (Huber, 2003), it may be more common than the literature suggests. Taxonomic procedures may even bias against their discovery through the *a priori* assumption that genitalia are species specific (Huber, 2003). Recently, three intriguing examples of genital polymorphism have been described: dimorphism in the female epigynum of the pholcid spider *Ciboneya antraia* (Huber & Gonzalez, 2001), dimorphism in the length of the male genitalia of the thrips *Iotatubothrips kranzae* (Mound *et al.*, 1998) and discontinuous shape variation in the male genitalia of the moth *Selenia tetralunaria* (Mutanen & Kaitala, 2006). Alternative mating strategies have been implicated in maintaining genital polymorphism in *C. antraia* and *I. kranzae* (Huber & Gonzalez, 2001; Mound *et al.*, 1998) and in the latter, genital

dimorphism is linked to an external dimorphism in wing development and dispersal ability, lending support to this suggestion.

The present paper focuses on a remarkable male genital polymorphism whereby males of the praying mantid genus *Ciulfina* (Mantodea: Liturgusidae) possess genitalia exhibiting either a dextral (right) or sinistral (left) orientation. The Mantodea exhibit asymmetric male genitalia that generally develop in single orientation. Balderson (1978) described a number of putative species of *Ciulfina*, and documented the unusual 'phallic reversal' found within the genus. While some putative species of *Ciulfina* exhibit the 'normal' orientation, some exhibited a 'reversed' orientation, and one putative species included some normal and some reversed males, described as mirror images of one another (Balderson, 1978). The mechanism driving the evolution of two genitalic orientations within this species is however, uncertain. This study reports the presence of genital dimorphism in two additional species of *Ciulfina* and investigates the incidence of sinistral and dextral morphology for all three dimorphic species, collected from Queensland, Australia. It also tests the suggestion that dextral and sinistral genitalia develop as mirror-images of one-another, by evaluating size and shape variation in genitalic traits using elliptic fourier analysis (EFA).

Methods.

Field collection

Adult male mantids were collected from 10 locations throughout Queensland, Australia. *Ciulfina baldersoni* were collected from Queens park in Bundaberg and from the Great Sandy Bay Botanic Gardens in Hervey Bay. *Ciulfina rentzi* were collected from near

Babinda in Wooroonooran National Park, and Lacey Creek in the Tam O' Shanter State Forest. *Ciulfina biseriata* was collected at four locations due to the larger distribution of this species, from roadside vegetation 1km south of Mareeba, from Cardwell lookout in Cardwell State Forest, from Mt Inkerman and from roadside vegetation 3km west of Yeppoon.

Preparation of Genitalia

Genitalia were dissected out of the ethanol-preserved male mantids and submerged in 10% KOH for one hour to dissolve the muscle and fat bodies. The male genitalia of *Ciulfina* praying mantids consist of three major phallomeres: the hypophallus, left epiphallus and right epiphallus (Balderson, 1978). The hypophallus is the most ventral phallomere and possesses a latero-caudal process. The left epiphallus lies dorsally and possesses a medial and a caudal process. The right epiphallus is a pouch-shaped structure that also lies dorsally over the hypophallus (Balderson, 1978). Sinistral genitalia were defined as possessing a left epiphallus whose caudal process was directed to the left when viewed dorsally. Dextral genitalia were defined as possessing a left epiphallus whose caudal process was directed to the right when viewed dorsally (Fig. 1). Phallomeres were separated and mounted onto glass slides in glycerol. Individuals were recorded as possessing dextral or sinistral genital orientations. Digital images (mag. = x 5) were taken of each genital phallomere using a Moticam 480® microscope-mounted camera.

Digital photographs were imported into the tpsDig2 (Rohlf, 2004) digitization program. Images of sinistral morphs were reversed so as to remove the influence of orientation from the analysis. Outlines of the (a) latero-caudal process of the hypophallus, (b) caudal process of the left epiphallus, (c) medial process of the left epiphallus and (d) caudal

process of the right epiphallus, were digitized using tpsDig2 (Rohlf, 2004). The number of digitized points for each structure ranged between 25 and 50 (Table 1). One person (GIH) completed all digitizations.

Geometric morphometrics

Geometric morphometric analysis allows the quantitative analysis of fine-scale variation in morphological shape and is becoming increasingly popular in many fields of biological research. Two major types of analysis exist: (1) landmark-based methods which analyse variation in the relative position of assigned landmarks, and (2) outline-based methods which analyse variation in the shape of the outlines of structures. As useful landmarks were not apparent for all genital structures for all species in this study, elliptic Fourier analysis (EFA) was used to quantitatively describe the shape of genital structures based on variation in their digitised outlines. EFA has been utilized successfully to analyse shape variation in a number of studies of insect genitalia (e.g. Monti et al., 2001; Arnqvist, 1998). Fourier coefficients for each genital structure were computed from the X-Y coordinates of each digitized outline using EFAWin (Rohlf, 1993) and the analyses were made invariant to orientation, rotation, size and starting location. Size was removed from the analyses, so Fourier coefficients could be calculated based only on shape variation. However, the calculated areas within outlines were added to subsequent analyses to assess for any effect of genital size. Reconstructed outlines were observed to determine the number of harmonics necessary to accurately represent the outline of each genital structure for each species (see Table 1). The four coefficients describing each harmonic were combined and the resulting data matrix was reduced to a smaller number of descriptors using principal components analysis. Jolliffe cut-offs for the PCA eigenvalues were used to determine the

number of principal components that significantly contributed to the variation in genital phallomere shape (Table 1). We used multivariate analysis of variance (MANOVA) on these principal component scores to assess the effect of orientation on genital shape. A second series of analyses were conducted including the genital outline area, to determine if genital size had any influence on the analysis.

Results:

Both *C. baldersoni* and *C. rentzi* exhibited high frequencies of both dextral and sinistral male genitalia although the two forms were distributed more equally in *C. baldersoni*. *Ciulfina klassi* males exhibited only dextral genitalia and only one population of *C. biseriata* exhibited both orientations (see Table 2) so these species were removed from the elliptic Fourier analyses of genital orientation.

No significant difference was found between the fine-scale morphological shape of dextral and sinistral male genitalia for both *Ciulfina baldersoni* and *Ciulfina rentzi* (Table 2). Similarly, the addition of genital size (outline area) to the principal components analysis had no influence on the comparison.

Discussion:

Remarkably, our research has shown that when the effect of orientation is removed, there is no difference between the shape and size of dextral and sinistral male genitalia in both *C. baldersoni* and *C. rentzi*. The absence of fine-scale variation between dextral and

sinistral forms supports the original suggestion (Balderson, 1978) that the two genitalic orientations are mirror images of one another. Three hypotheses have been suggested to explain the rapid diversification of animal genitalia: sexual selection, species isolation and pleiotropy (Eberhard, 1985). However, it now seems clear that sexual selection, as proposed by Eberhard (1985) is the most plausible explanation (Hosken & Stockley, 2004). How sexual selection might maintain chiral dimorphism in *Ciulfina* is uncertain. Intriguingly, the dimorphism in genital orientation that we have observed in *Ciulfina* resembles the more well-studied example of coil dimorphism in gastropod shells (Schilthuizen & Davison, 2005). Individuals with dextral or sinistral shell coiling within the same population occur in a number of gastropod genera and shifts in chirality have repeatedly occurred through the evolutionary history of the gastropoda (Asami *et al*, 1998). However, *Ciulfina* differs from the gastropod examples in one important way: female genitalia are symmetrical and do not preclude males of either orientation from mating. Although many gastropod groups exhibit alternative chirality between populations or species, *Ciulfina* more closely resembles snail genera such as *Amphidromus*, in which both dextral and sinistral morphs are equally likely to mate with one-another (Schilthuizen & Davison, 2005). A number of possible explanations for snail chirality may be relevant for understanding the maintenance of dextral and sinistral genital orientations in *Ciulfina*. Firstly, frequency dependent selection may act on male genital orientation through female preference for the less common chiral form. In this scenario, female choice would be postcopulatory, as genital orientation would be unknown to the female prior to copulation. For this to be supported, it would be necessary to understand both the levels of polyandry in the field and the possible mechanism by which females may select for sperm from males of a particular orientation. An alternative, and perhaps more parsimonious explanation is

that both morphs are maintained through a genetically and developmentally stable dimorphism that is selectively neutral.

The study of alternative mating strategies has elucidated many well-studied male polymorphisms (Simmons *et al.*, 1999), and this appears to be a good candidate to explain genital dimorphism in the pholcid spider *Ciboneya antraia* (Huber & Gonzalez, 2001) and the thrips *Iotatubothrips kranzae* (Mound *et al.*, 1998). However, it seems unlikely to explain the different genital orientations observed in *Ciulfina* species, as no other differences are apparent. There is clearly much to learn about the evolution and maintenance of chiral dimorphism in the male genitalia of *Ciulfina*. This genus may represent an ideal opportunity to test ideas previously only applied to gastropod coil dimorphism, and also contribute significantly to current debate about genital evolution.

Acknowledgements:

We wish to thank Matthew Bruce, Anne Gaskett, Scott Ginn, Scott Holwell and James O'Hanlon for assistance with field collection of mantids. We also thank Matt Kovach for assistance with specimen preparation, and Ray Cameron, Peter Tung and Simon Hardwick for assistance with digital imaging. We particularly thank Goran Arnqvist and Luke Finley for morphometric advice. This research complies with current Australian legal requirements.

References

- Andersson, M. 1994. *Sexual selection*. Princeton: Princeton University Press.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature*, **393**, 784-786.
- Asami, T., Cowie, R. H. & Ohbayashi, K. 1998. Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. *Am. Nat.*, **152**, 225-236.
- Balderson, J. 1978. Reversal of the phallic complex in the genera *Ciulfina* Giglio-Tos and *Stenomantis* Saussure (Mantodea: Mantidae: Iridopteryginae). *J. Aust. Ent. Soc.*, **17**, 235-239.
- Eberhard, W. G. 1985. *Sexual selection and animal genitalia*. Harvard University Press.
- Hosken, D. J. & Stockley, P. 2004. Sexual selection and animal genitalia. *TREE*, **19**, 87-93.
- Huber, B. A. 2003. Rapid evolution and species-specificity of arthropod genitalia: fact or artifact? *Org. Div. Evol.*, **3**, 63-71.
- Huber, B. A. & Gonzalez, A. P. 2001. Female genital dimorphism in a spider - (Araneae: Pholcidae). *J. Zool. Lond.*, **255**, 301-304.
- Monti, L., Baylac, M. & Lalanne-Cassou, B. 2001. Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biol. J. Linn. Soc.*, **72**, 391-400.
- Mound, L. A., Crespi, B. J. & Tucker, A. 1998. Polymorphism and kleptoparasitism in thrips (Thysanoptera: Phlaeothripidae) from woody galls on Casuarina trees. *Aust. J. Entom.*, 8-16.
- Mutanen, M. & Kaitala, A. 2006. Genital variation in a dimorphic moth *Selenia tetralunaria* (Lepidoptera, Geometridae). *Biol. J. Linn. Soc.*, **87**, 297-307.

- Radwan, J., Unrug, J. & Tomkins, J. L. 2002. Status-dependence and morphological trade-offs in the expression of a sexually selected character in the mite, *Sancassania berlesei*. *J. Evol. Biol.*, **15**, 744-752.
- Rohlf, F. J. 1993. EFAWin. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F. J. 2004. TpsDig2. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.
- Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol. Rev.*, **79**, 815-848.
- Schilthuizen, M. & Davison, A. 2005. The convoluted evolution of snail chirality. *Naturwissenschaften*, **92**, 504-515.
- Shuster, S. M. & Wade, M. J. 1991. Equal mating success among male reproductive strategies in a marine isopod. *Nature*, **350**, 608-610.
- Simmons, L. W., Tomkins, J. L. & Hunt, J. 1999. Sperm competition games played by dimorphic male beetles. *Proc. Roy. Soc. Lond. B*, **266**, 145-150.
- Tomkins, J. L. & Brown, G. S. 2004. Population density drives the local evolution of a threshold dimorphism. *Nature*, **431**, 1099-1103.
- Zera, A. J. & Denno, R. F. 1997. Physiology and ecology of dispersal polymorphism in insects. *Ann. Rev. Ent.*, **42**, 207-230.

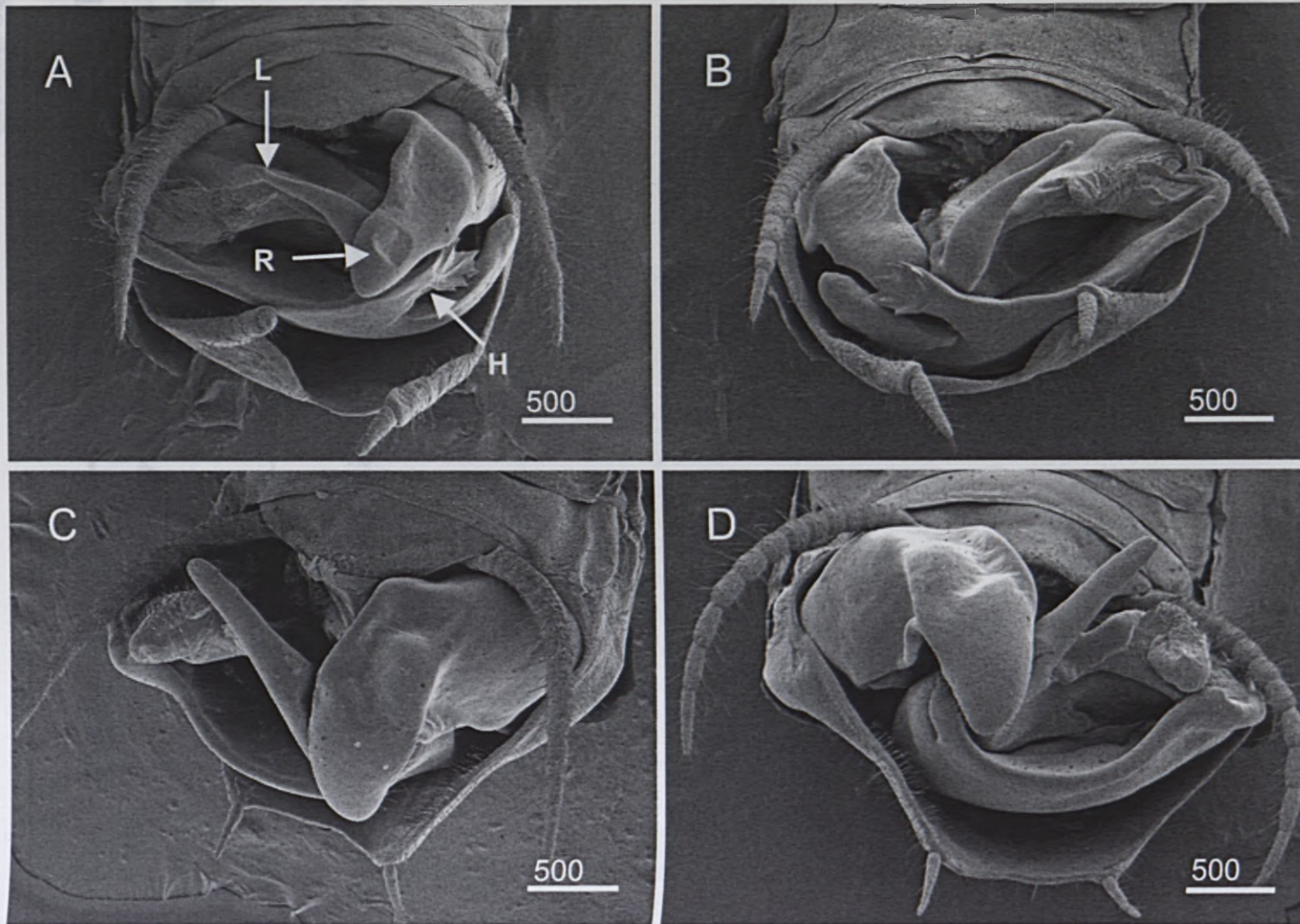
Table 1. Number of dextral and sinistral individuals for *C. rentzi* and *C. baldersoni*, with the number of digitized points (Pts), harmonics (H), Fourier coefficients (F.C) and principal components (P.C) used in statistical analyses for each genitalic phallomere.

Species	Dextral	Sinistral	Genitalic phallomere	Pts	H	F.C	P.C
<i>C. rentzi</i>	7	16	Hypophallus	30	15	60	8
			Caudal left epiphallus	45	20	80	7
			Right epiphallus	45	20	80	7
<i>C. baldersoni</i>	14	16	Hypophallus	40	20	80	11
			Caudal left epiphallus	40	20	80	8
			Medial left epiphallus	35	17	68	11
			Right epiphallus	50	15	60	5

Table 2. MANOVA statistics for the effect of orientation on morphological variation of genital phallomeres for *Ciulfina rentzi* and *Ciulfina baldersoni*. *P* values greater than 0.05 represent no significant difference between dextral and sinistral morphology.

Species	Genitalic phallomere	Orientation effect (shape only)	Orientation effect (shape and size)
<i>C. rentzi</i>	Hypophallus	<i>Wilk's</i> $\lambda = 0.6900$; $F_{22,183} = 0.618$; $P > 0.7$	<i>Wilk's</i> $\lambda = 0.4811$; $F_{22,206} = 1.198$; $P > 0.3$
	Caudal left epiphallus	<i>Wilk's</i> $\lambda = 0.6606$; $F_{22,160} = 0.991$; $P > 0.4$	<i>Wilk's</i> $\lambda = 0.6665$; $F_{22,183} = 0.813$; $P > 0.6$
	Right epiphallus	<i>Wilk's</i> $\lambda = 0.8296$; $F_{22,160} = 0.909$; $P > 0.5$	<i>Wilk's</i> $\lambda = 0.6357$; $F_{22,183} = 0.859$; $P > 0.5$
<i>C. baldersoni</i>	Hypophallus	<i>Wilk's</i> $\lambda = 0.6051$; $F_{29,329} = 1.068$; $P > 0.4$	<i>Wilk's</i> $\lambda = 0.5650$; $F_{11,359} = 1.005$; $P > 0.4$
	Caudal left epiphallus	<i>Wilk's</i> $\lambda = 0.7994$; $F_{29,239} = 0.335$; $P > 0.9$	<i>Wilk's</i> $\lambda = 0.7391$; $F_{29,269} = 0.407$; $P > 0.9$
	Medial left epiphallus	<i>Wilk's</i> $\lambda = 0.6541$; $F_{29,329} = 1.388$; $P > 0.2$	<i>Wilk's</i> $\lambda = 0.6109$; $F_{29,359} = 1.416$; $P > 0.2$
	Right epiphallus	<i>Wilk's</i> $\lambda = 0.8296$; $F_{29,149} = 0.986$; $P > 0.4$	<i>Wilk's</i> $\lambda = 0.7679$; $F_{29,179} = 1.159$; $P > 0.3$

Figure 1. Scanning electron micrographs (SEM) showing sinistral and dextral male genitalia of *Ciulfina baldersoni* (A = sinistral; B = dextral) and *Ciulfina rentzi* (C = sinistral; D = dextral). Genitalic phallomeres of importance are the hypophallus (H), left epiphallus (LE) and right epiphallus (RE).



Chapter Three.

Geographic variation in genital morphology of *Ciulfina* praying mantids (Mantodea: Liturgusidae).

Submitted to the journal *Evolutionary Ecology*

Abstract:

Geographic variation in morphological traits is widespread and important to our current understanding of evolutionary processes. Although male genitalia are perhaps the most divergent morphological traits in animals, geographic variation in genitalic traits has received little attention and the mechanism driving such variation is unclear. Both the sexual selection hypothesis and the species isolation hypothesis make explicit predictions about geographic variation in genitalia. While the species isolation hypothesis predicts patterns of genital divergence that reflect the risk of mating with related but incompatible species, the sexual selection hypothesis predicts general levels of geographic variation that reflect variation in female preferences. To test these predictions, we investigated geographic variation in genital morphology in the praying mantid genus *Ciulfina* using elliptic fourier analysis (EFA). We found significant levels of geographic variation in the genital morphology of four *Ciulfina* species irrespective of the relative proximity of different populations to contact zones with other species. These results support the growing amount of evidence in favour of the sexual selection hypothesis of genital evolution.

Introduction:

Geographic variation in morphological traits is widespread and important to our current understanding of evolutionary processes. Particularly important is the diversification of morphological traits between geographically separate populations, which is generally accepted as the forerunner of allopatric speciation. While evolutionary changes in external morphology over geographic space is well-studied, geographic variation in genitalia has received less attention, despite the importance of genital traits in species-level taxonomy (Eberhard, 1985). Genital traits can vary in size and shape between different geographic populations and has been demonstrated in a number of taxa including snakes (Inger & Marx, 1962), fish (Kelly *et al.*, 2000), mammals (Sullivan *et al.*, 1990), millipedes (Tanabe *et al.*, 2001), mosquitoes (Hribar, 1994) and snails (Madec & Guillar, 1994). However, the mechanism driving such variation is unclear. Three major hypotheses have been put forward to explain the rapid diversification and elaboration of genitalia between species: sexual selection, species isolation and pleiotropy (Eberhard, 1985; Hosken & Stockley, 2004). The sexual selection hypothesis has received the most recent attention and there is growing acceptance that sexual selection is the best general hypothesis to explain rapid genital evolution (Arnqvist, 1998; House & Simmons, 2003; Hosken & Stockley, 2004). Both the sexual selection hypothesis and the species isolation hypothesis make explicit predictions about geographic variation in genitalia (Eberhard, 1985).

If genitalia diverge under sexual selection, we may expect to see some degree of geographic variation. Other sexually selected male traits such as mating calls (Ryan *et al.*, 1996), exaggerated external morphologies (Moller, 1995) and colouration (Kwiatkowski &

Sullivan, 2002) exhibit variation throughout species distributions, relating to geographic variation in female preferences (Endler & Houde, 1995).

The species isolation hypothesis predicts that genitalic divergence would occur between populations in geographic contact with closely-related species and populations isolated from close relatives. Such divergence would reflect a function for genitalia in the prevention of mismating and the resulting reduction in fitness. Therefore, one prediction of this hypothesis is that it would be expected that populations found in sympatry with related species would possess divergent genitalia from populations that are isolated from related species. It would not be expected however, to observe variation between allopatric populations as they would not experience any differences in the risk of mismating (Eberhard, 1985).

The pleiotropy hypothesis has been difficult to test as it predicts an association between genitalic traits and other traits through genetic linkage (Mayr, 1963), but cannot be tested without knowledge of the genetic architecture of genitalic traits. A number of arguments produced by Eberhard (1985) have led to its general dissolution as a potential explanation for genitalic diversification and elaboration.

We have chosen to investigate geographic variation in genital morphology in the praying mantid genus *Ciulfina*. These mantids are cryptic cursorial predators that live on tree trunks in a variety of habitats throughout northern Australia. Species are distinguished solely on the basis of male genital morphology, and three species (*C. biseriata*, *C. rentzi* and *C. baldersoni*) exhibit a remarkable genital dimorphism in orientation (Holwell & Herberstein, in review). The distributions of *Ciulfina* species in north-eastern Australia are also intriguing in that they form abrupt parapatric boundaries, with no distributional overlap (Holwell *et al.* in review). Therefore, to test the predictions of the species-isolation

and sexual selection hypotheses of genital evolution, this study evaluates geographic variation in the size and shape of male genitalic traits in four *Ciulfina* species using elliptic fourier analysis (EFA).

Methods:

Field collection

Adult male mantids were collected from 10 locations throughout Queensland, Australia. *Ciulfina baldersoni* were collected from eucalypt woodland and mangroves in Queens Park, Bundaberg and from sub-tropical rainforest vegetation in the Great Sandy Bay Botanic Gardens, Hervey Bay. *Ciulfina rentzi* were collected from primary tropical rainforest, approximately 10 km west of Babinda, in Wooroonooran National Park, and from Lacey Creek in the Tam O' Shanter State Forest. *Ciulfina klassi* was collected from coastal *Melaleuca* woodland approximately 8km west of Mission beach, and from eucalypt woodland approximately 30km south of Cardwell. *Ciulfina biseriata* was collected from eucalypt woodland approximately 1km south of Mareeba, from Cardwell lookout in Cardwell State Forest, from Mt Inkerman and 5km west of Yeppoon (Figure 1). These sampling locations were chosen so as to represent some pairs of populations that differ in their relative risk of mismating, and some that do not differ in this respect. *C. rentzi* and *C. klassi* are represented by one population close to the contact zone and one population geographically distant to the contact zone. *C. biseriata* is represented by one population that is close to a contact zone and three populations that are allopatric. *C. baldersoni* is

represented by populations that are both allopatric, and some distance from the distributions of any other species.

Preparation of Genitalia

After collection, mantids were euthanased by freezing and stored in 70% ethanol. Genitalia were dissected out and submerged in 10% KOH for one hour to dissolve the muscle and fat bodies. The male genitalia of *Ciulfina* praying mantids consist of three major phallomeres: the hypophallus, left epiphallus and right epiphallus (Balderson, 1978). The hypophallus is the most ventral phallomere and possesses a latero-caudal process. The left epiphallus lies dorsally and possesses a medial and a caudal process. The right epiphallus is a pouch-shaped structure that also lies dorsally over the hypophallus (Balderson, 1978). The medial process of the left epiphallus is greatly reduced in *C. rentzi*, and was not used in further analyses. Phallomeres were separately mounted in glycerol onto glass slides and individuals were recorded as possessing dextral or sinistral genital orientations. Digital images of each genital phallomere were recorded using a Moticam 480[®] microscope-mounted camera.

Digital images were imported into the tpsDig2 (Rohlf, 2004) digitization program. Individuals were classified as possessing dextral (right-oriented) or sinistral (left-oriented) genitalia according to the dimorphism in genital orientation found within this genus (Holwell & Herbersein, in review). Images of sinistral morphs were reversed so as to remove the influence of orientation from the analysis. Outlines of (a) latero-caudal process of the hypophallus (referred to herein as the hypophallus), (b) the caudal process of the left epiphallus (c) the medial process of the left epiphallus and (d) the caudal process of the

right epiphallus (referred to herein as the right epiphallus), were digitized using tpsDig2 (Rohlf, 2004). The number of digitized points for each structure ranged between 25 and 50 (Table 1). One person (GIH) completed all digitizations.

Geometric morphometrics

Geometric morphometric analysis allows the quantitative analysis of fine-scale variation in morphological shape and is becoming increasingly popular in many fields of biological research. Two major types of analysis exist: (1) landmark-based methods, which analyse variation in the relative position of assigned landmarks, and (2) outline-based methods, which analyse variation in the shape of the outlines of structures. As useful landmarks were not apparent for all genital structures for all species, Elliptic Fourier Analysis (EFA) was used to quantitatively describe the shape of genital structures based on variation in their digitised outlines. In order to quantify variation, digitised outlines are converted to a series of harmonics, each of which is defined by four Fourier coefficients. The dataset resulting from this analysis can then be reduced using principal components analysis to a more manageable series of variables that summarise the shape variation within a sample. EFA has been utilized successfully to analyse shape variation in a number of studies of insect genitalia (Arnqvist, 1998; Arnqvist & Danielsson, 1999; Monti *et al.*, 2001; Horton & Lewis, 2005).

Fourier coefficients for each genital structure were computed from the X-Y coordinates of each digitised outline using EFAWin (Rohlf, 1993) and the analyses were made invariant to orientation, rotation, size and starting location. Size was removed from the analyses, so Fourier coefficients could be calculated based only on shape variation. However, the calculated areas within outlines were added to subsequent analyses to assess for an effect

of genital size. Reconstructed outlines were observed to determine the number of harmonics necessary to accurately represent the outline of each genital structure for each species (see Table 1). The four coefficients that described each harmonic were then combined and the resulting data matrix was reduced to a smaller number of descriptors using principal components analysis. Jolliffe cut-offs for the PCA eigenvalues were used to determine the number of principal components that significantly contributed to the variation in genital phallomere shape (listed in Table 1). We used multivariate analysis of variance (MANOVA) on these principal component scores to assess the effect of geographic location on genital shape. A second series of analyses were conducted including the genital outline area, to determine if genital size had any influence over the analysis.

Results:

Significant geographic variation was found in the genitalia of all species (Table 2). The extent of inter-population variation differed between species, and also differed between genital structures. *Ciulfina rentzi* showed geographic variation in the left and right epiphalli, but not in the hypophallus, except when size was included in the analysis. *Ciulfina klassi* showed variation in the hypophallus and both processes of the left epiphallus but none in the right epiphallus, except when size was included in the analysis. *Ciulfina baldersoni* only showed variation in the hypophallus. *Ciulfina biseriata* exhibited highly significant geographic variation in all four genitalic structures measured. Ordinations of the first two principal components for genital shape variation are presented

for the *C. biseriata* hypophallus (Fig. 2), caudal left epiphallus (Fig. 3), medial left epiphallus (Fig. 4) and right epiphallus (Fig. 5) along with the reconstructed outlines of each mean shape consensus for each geographic population.

The addition of a size variable (outline area) to the principal components analysis did affect the significance of the geographic variation for the *C. rentzi* hypophallus and the *C. klassi* right epiphallus. Analyses including size as a variable were significant for these two structures whereas analyses without size showed no significant variation between populations (Table 3).

Genital morphological variation was shown to exist between populations that differed in their risk of mismating (*C. rentzi* and *C. klassi*), and also between populations that had no risk of mismating (*C. biseriata* and *C. baldersoni*).

Discussion:

The results of this study indicate clear geographic variation in genital structure for all four *Ciulfina* species studied. This ranged from geographic variation only in the hypophallus for *C. baldersoni* (Table 2), through to geographic variation in all four genital phallomeres for *C. biseriata* (Table 2 & Figures 2-5). The addition of genital size (outline area) to the analyses revealed significant geographic differences in the hypophallus of *C. rentzi* and the right epiphallus of *C. klassi*, which were not apparent based on shape alone (Table 2). Therefore, when size was included, *C. rentzi*, *C. biseriata* and *C. klassi* all displayed

geographic variation for all phallomeres. This emphasizes that divergent selection acting on genital morphology in *Ciulfina* can influence both shape and size.

One of the main assumptions of the species isolation/lock and key hypothesis for genital evolution is that if variation within a species were to exist, it would occur between areas where the risk of costly mismating was high (sympatry) and those where it was low (allopatry). Through extensive collections of *Ciulfina*, no areas of sympatry have been discovered. Rather, species distributions reach abrupt parapatric boundaries where the presence of one species is replaced by the presence of another (Holwell *et al.*, in review). If any risk of mismating exists between *Ciulfina* species, it would be at these boundaries. The species isolation hypothesis would therefore predict that populations close to boundaries would exhibit divergence in genitalic traits from those distant to boundaries. If the species isolation hypothesis were to provide a general explanation for genital variation in *Ciulfina* however, allopatric populations should not vary from one another. The data presented have clearly rejected this hypothesis. Pairs (or groups) of populations in this study either (a) differed in their risk of mismating (i.e. closeness to contact zone) or (b) possessed the same low risk of mismating. However, geographic variation exists in the genital morphology of all *Ciulfina* species, irrespective of the relative proximity of different populations to contact zones with other species. Figures 2-5 clearly demonstrate that divergence has occurred between the genital morphology of the four populations of *C. biseriata* included in this study. It is also possible to visualize the differences between populations by comparing the consensus outline reconstructions of each genital phallomere, for each population (Figures 2-5). Three of these populations (Mareeba, Mt Inkerman & Yeppoon) are geographically isolated from all other species of *Ciulfina*, are at no risk of mismating, and yet still show morphological divergence. In fact, *C. biseriata* shows the most highly

significant differences of all species in this study (Table 3). The species isolation hypothesis therefore does not explain geographic genital divergence for *Ciulfina*.

The sexual selection hypothesis predicts that, as for other sexually selected traits, geographic variation in genitalia will occur (King Sirot, 2003). Sexually selected traits such as the coloration of lizards (Kwiatkowski & Sullivan, 2002) and fish (Boughman, 2001; Endler & Houde, 1995), tail ornaments of birds (Moller, 1995), cuticular hydrocarbons of grasshoppers (Tregenza *et al.*, 2000) and flies (Rundle *et al.*, 2005), and the mating calls of frogs (Ryan *et al.*, 1996; Smith *et al.*, 2003) and crickets (Gray & Cade, 2000; Simmons *et al.*, 2001) often show geographic variation (Andersson, 1994). Although geographic differences in male traits and female preferences can reflect variation in predation risk (Stoner & Breden, 1988) or the sensory environment (Boughman, 2001), they can also potentially represent divergence through Fisherian runaway selection (Lande, 1982). Due to the apparent arbitrary nature of many such traits and preferences, geographic variation would almost be expected.

If sexual selection is responsible for genital diversification and elaboration through a similar runaway process, then variation in the degree and direction of selection may result in a general trend of geographic variation in genitalic traits. This seems likely as geographic variation in genitalia has been shown for many taxa (Sullivan *et al.*, 1990; Hribar, 1994; Kelly *et al.*, 2000; Tatsuta & Akimoto, 2000; but see Zeh & Zeh, 1994 and Bond *et al.*, 2001).

Overall, sexual selection appears to be responsible for genitalic diversification in *Ciulfina*. The lack of support for the species isolation hypothesis and the general level of geographic variation in genital morphology for *Ciulfina* species lends support to the increasing number of studies demonstrating the influence of sexual selection on genitalia

(Eberhard, 1985; Arnqvist, 1998; Arnqvist & Danielsson, 1999; Cordoba-Aguilar, 1999; Tadler, 1999; Hosken & Stockley, 2004; Bertin & Fairbairn, 2005; House & Simmons, 2005). Genitalic shape and size have been shown to exhibit intraspecific geographic variation. However, the influence of these traits on male reproductive success in *Ciulfina* remains to be tested.

Acknowledgements:

We wish to thank Matthew Bruce, Anne Gaskett, Scott Ginn, Scott Holwell and James O'Hanlon for assistance with field collection of mantids. We also thank Scott Ginn for advice on preparation of samples and Matt Kovach for assistance with preparation, and Ray Cameron, Peter Tung and Simon Hardwick for assistance with digital imaging. We particularly thank Goran Arnqvist and Luke Finley for morphometric advice.

References.

- Andersson, M. 1994. *Sexual selection*. Princeton: Princeton University Press.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature*, **393**, 784-786.
- Arnqvist, G. & Danielsson, I. 1999. Copulatory behavior, genital morphology and male fertilization success in water striders. *Evolution*, **53**, 147-156.
- Balderson, J. 1978. Reversal of the phallic complex in the genera *Ciulfina* Giglio-Tos and *Stenomantis* Saussure (Mantodea: Mantidae: Iridopteryginae). *J. Aust. Ent. Soc.*, **17**, 235-239.

- Bertin, A. & Fairbairn, D. J. 2005. One tool, many uses: precopulatory sexual selection on genital morphology in *Aquarius remigis*. *J. Evol. Biol.*, **18**, 949-961.
- Bond, J. E., Hedin, M. C., Ramirez, M. G. & Opell, B. D. 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. *Mol. Ecol.*, **10**, 899-910.
- Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, **411**, 944-948.
- Cordoba-Aguilar, A. 1999. Male copulatory sensory stimulation induces female ejection of rival sperm in a damselfly. *Proc. Roy. Soc. Lond. B*, **266**, 779-784.
- Eberhard, W. G. 1985. *Sexual selection and animal genitalia*. Harvard University Press.
- Endler, J. A. & Houde, A. E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456-468.
- Gray, D. A. & Cade, W. H. 2000. Sexual selection and speciation in field crickets. *PNAS* **97**, 14449-14454.
- Holwell, G. I., Ginn, S., Milledge, G. & Herberstein, M. E. Parapatric distributions in *Ciulfina* praying mantids (Mantodea: Liturgusidae) and descriptions of three new species (in review).
- Holwell, G. I. & Herberstein, M. E. Mirror-image genital dimorphism in *Ciulfina* praying mantids (Mantodea: Liturgusidae) (in review).
- Horton, D. R. & Lewis, T. M. 2005. Size and shape differences in genitalia of males from sympatric and reproductively isolated populations of *Anthocoris antevolens* White (Heteroptera: Anthocoridae) in the Yakima Valley, Washington. *Ann. Entom. Soc. Am.*, **98**, 527-535.

- Hosken, D. J. & Stockley, P. 2004. Sexual selection and animal genitalia. *TREE*, **19**, 87-93.
- House, C. M. & Simmons, L. W. 2003. Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc. R. Soc. Lond. B*, **270**, 447-455.
- House, C. M. & Simmons, L. W. 2005. The evolution of male genitalia: patterns of genetic variation and covariation in the genital sclerites of the dung beetle *Onthophagus taurus*. *J. Evol. Biol.*, **18**, 1281-1292.
- Hribar, L. J. 1994. Geographic variation of male genitalia of *Anopheles nuneztovari* (Diptera: Culicidae). *Mosquito Systematics*, **26**, 132-144.
- Inger, R. F. & Marx, H. 1962. Variation of hemipenis and cloaca in the colubrid snake *Calamaria lumbricoidea*. *Syst. Zool.*, **11**, 32-38.
- Kelly, C. D., Godin, J. G. J. & Abdallah, G. 2000. Geographical variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*). *Can. J. Zool.*, **78**, 1674-1680.
- King Sirot, L. 2003. The evolution of insect mating structures through sexual selection. *Florida Entomologist*, **86**, 124-133.
- Kwiatkowski, M. A. & Sullivan, B. K. 2002. Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus*. *Evolution*, **56**, 2039-2051.
- Lande, R. 1982. Rapid origin of sexual isolation and character divergence in a cline. *Evolution*, **36**, 213-223.
- Madec, L. & Guillar, A. 1994. Geographic variation of distal genitalia in the landsnail *Helix aspersa* (Mollusca: Gastropoda). *J. Zool. Lond.*, **233**, 215-231.

- Mayr, E. 1963. *Animal species and evolution*. Harvard University Press.
- Moller, A. P. 1995. Sexual selection in the barn swallow (*Hirundo rustica*). V. Geographic variation in ornament size. *J. Evol. Biol*, **8**, 3-19.
- Monti, L., Baylac, M. & Lalanne-Cassou, B. 2001. Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biological Journal Of The Linnean Society*, **72**, 391-400.
- Rohlf, F. J. 1993. EFAWin. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F. J. 2004. TpsDig2. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rundle, H. D., Chenoweth, S. F., Doughty, P. & Blows, M. W. 2005. Divergent Selection and the Evolution of Signal Traits and Mating Preferences. *PLoS Biology*, **3**.
- Ryan, M. J., Rand, S. & Weigt, L. A. 1996. Allozyme and advertisement call variation in the tungara frog, *Physalaemus pustulosus*. *Evolution*, **50**, 2435-2453.
- Simmons, L. W., Zuk, M. & Rotenberry, J. T. 2001. Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus*. *Evolution*, **55**, 1386-1394.
- Smith, M. J., Roberts, J. D., Hammond, T. J. & Davis, R. A. 2003. Intraspecific variation in the advertisement call of the sunset frog *Spicospina flammocaerulea* (Anura: Myobatrachidae): a frog with a limited geographic distribution. *J. Herpetol.*, **37**, 285-291.
- Stoner, G. & Breden, F. 1988. Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.*, **22**, 285-292.

- Sullivan, R. M., Calhoun, S. W. & Greenbaum, I. F. 1990. Geographic variation in genital morphology among insular and mainland populations of *Peromyscus maniculatus* and *Peromyscus oreas*. *J. Mammalogy*, **71**, 48-58.
- Tadler, A. 1999. Selection of a conspicuous male genitalic trait in the seedbug *Lygaeus simulans*. *Proc. Roy. Soc. Lond. B*, **266**, 1773-1777.
- Tanabe, T., Katakura, H. & Mawatari, S. F. 2001. Morphological difference and reproductive isolation: morphometrics in the millipede *Parafontaria tonominea* and its allied forms. *Biol. J. Linn. Soc.*, **72**, 249-264.
- Tatsuta, H. & Akimoto, S. I. A. 2000. Variability in phenotypic covariance structure of female genitalia in the brachypterous grasshopper *Podisma sapporensis* (Orthoptera: Acrididae: Podisminae). *Ann. Ent. Soc. Am.*, **93**, 127-132.
- Tregenza, T., Pritchard, V. L. & Butlin, R. K. 2000. Patterns of trait divergence between populations of the meadow grasshopper, *Chorthippus parallelus*. *Evolution*, **54**, 574-585.
- Zeh, D. W. & Zeh, J. A. 1994. When morphology misleads: interpopulation uniformity in sexual selection masks genetic divergence in harlequin beetle-riding pseudoscorpion populations. *Evolution*, **48**, 1168-1182.

Table 1. Number of digitized points (Pts), harmonics (H), Fourier coefficients (C) and principal components (PC) used in statistical analyses for each genitalic phallomere.

Species	Genitalia	Pts.	H.	C.	PC
<i>C. rentzi</i>	Hypophallus	30	15	60	8
	Left epiphallus	45	20	80	7
	Right epiphallus	45	20	80	7
<i>C. klassi</i>	Hypophallus	40	20	80	8
	Left epiphallus	40	20	80	9
	Left epiphallus 2	25	12	48	7
	Right epiphallus	40	20	80	5
<i>C. biseriata</i>	Hypophallus	50	20	80	8
	Left epiphallus	40	20	80	7
	Left epiphallus 2	35	17	68	6
	Right epiphallus	40	20	80	7
<i>C. baldersoni</i>	Hypophallus	40	20	80	11
	Left epiphallus	40	20	80	8
	Left epiphallus 2	35	17	68	11
	Right epiphallus	50	15	60	5

Table 2. MANOVA statistics for the effect of geographic population on morphological variation of genital phallomeres for *Ciulfina rentzi* and *Ciulfina baldersoni*. *P* values less than 0.05 represent a significant difference between the genital morphology of mantids from different geographic populations.

Species	Genital phallomere	Population effect (shape only)	Population effect (shape & size)
<i>C. rentzi</i>	Hypophallus	Wilk's $\lambda = 0.3995$; $F_{22,183} = 2.067$; $p > 0.1$	Wilk's $\lambda = 0.1926$; $F_{22,206} = 4.658$; $p < 0.05$
	Caudal left epiphallus	Wilk's $\lambda = 0.3021$; $F_{22,160} = 4.612$; $p < 0.01$	Wilk's $\lambda = 0.3023$; $F_{22,183} = 3.750$; $p < 0.05$
	Right epiphallus	Wilk's $\lambda = 0.3373$; $F_{22,160} = 3.849$; $p < 0.05$	Wilk's $\lambda = 0.3338$; $F_{22,183} = 2.993$; $p < 0.05$
<i>C. klassi</i>	Hypophallus	Wilk's $\lambda = 0.3039$; $F_{25,207} = 4.867$; $p < 0.005$	Wilk's $\lambda = 0.2697$; $F_{25,233} = 4.815$; $p < 0.005$
	Caudal left epiphallus	Wilk's $\lambda = 0.2326$; $F_{25,233} = 5.133$; $p < 0.005$	Wilk's $\lambda = 0.1999$; $F_{25,259} = 5.204$; $p < 0.005$
	Medial left epiphallus	Wilk's $\lambda = 0.3862$; $F_{25,181} = 3.633$; $p < 0.05$	Wilk's $\lambda = 0.2379$; $F_{25,207} = 6.008$; $p < 0.005$
	Right epiphallus	Wilk's $\lambda = 0.8598$; $F_{25,129} = 0.554$; $p > 0.7$	Wilk's $\lambda = 0.2986$; $F_{25,155} = 6.263$; $p < 0.005$
<i>C. biseriata</i>	Hypophallus	Wilk's $\lambda = 0.0106$; $F_{32,263} = 9.239$; $p < 0.0001$	Wilk's $\lambda = 0.0051$; $F_{32,263} = 10.57$; $p < 0.0001$
	Caudal left epiphallus	Wilk's $\lambda = 0.0309$; $F_{32,230} = 7.029$; $p < 0.0001$	Wilk's $\lambda = 0.0193$; $F_{32,230} = 7.780$; $p < 0.0001$
	Medial left epiphallus	Wilk's $\lambda = 0.2027$; $F_{32,197} = 13.20$; $p < 0.0001$	Wilk's $\lambda = 0.0069$; $F_{32,197} = 12.82$; $p < 0.0001$
	Right epiphallus	Wilk's $\lambda = 0.1094$; $F_{32,230} = 3.364$; $p < 0.0005$	Wilk's $\lambda = 0.0889$; $F_{32,230} = 3.183$; $p < 0.0005$
<i>C. baldersoni</i>	Hypophallus	Wilk's $\lambda = 0.3572$; $F_{29,329} = 2.945$; $p < 0.05$	Wilk's $\lambda = 0.3069$; $F_{29,359} = 3.199$; $p < 0.05$
	Caudal left epiphallus	Wilk's $\lambda = 0.5002$; $F_{29,239} = 1.332$; $p > 0.2$	Wilk's $\lambda = 0.3868$; $F_{29,269} = 1.829$; $p > 0.1$
	Medial left epiphallus	Wilk's $\lambda = 0.6543$; $F_{29,329} = 1.387$; $p > 0.2$	Wilk's $\lambda = 0.6541$; $F_{29,359} = 1.175$; $p > 0.3$
	Right epiphallus	Wilk's $\lambda = 0.7995$; $F_{29,149} = 1.203$; $p > 0.3$	Wilk's $\lambda = 0.7416$; $F_{29,179} = 1.336$; $p > 0.2$

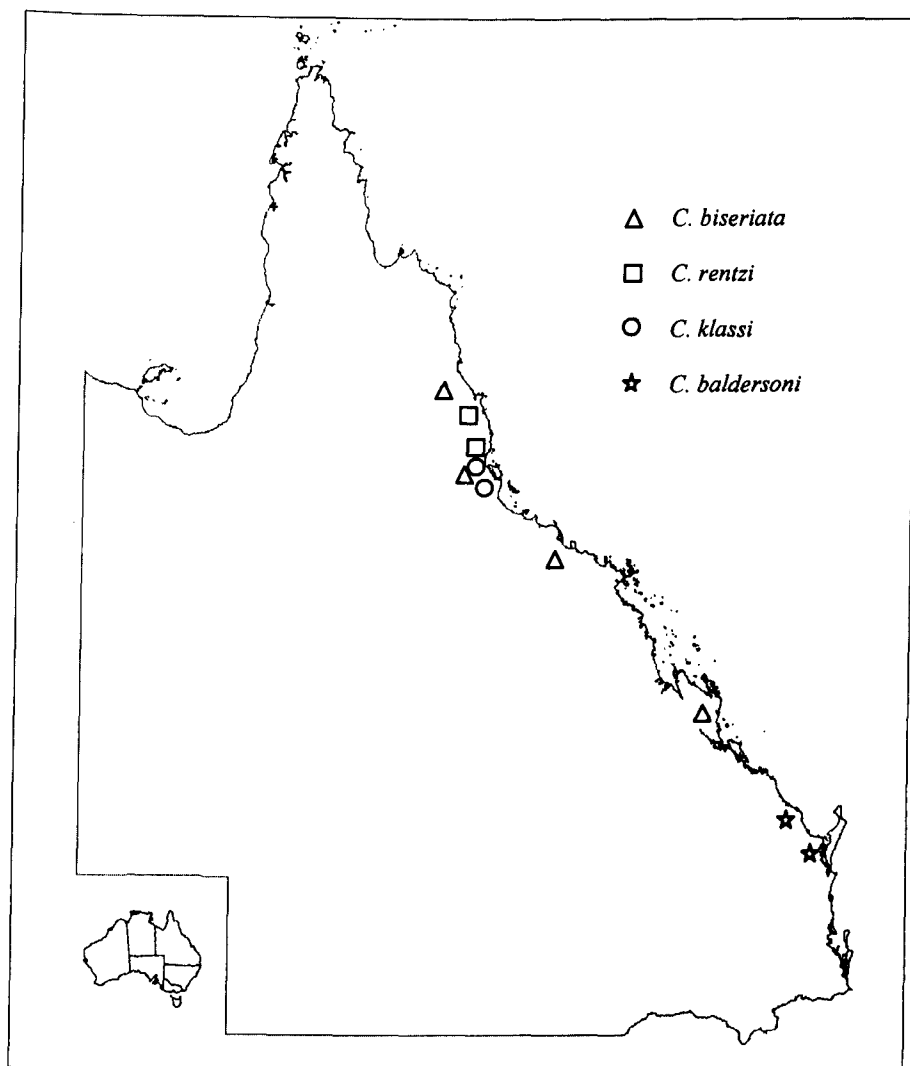


Figure 1. Sampling locations for *C. baldersoni* sp. nov. (☆), *C. biseriata* (Δ), *C. klassi* sp. nov. (○) and *C. rentzi* sp. nov. (□) throughout Queensland, Australia.

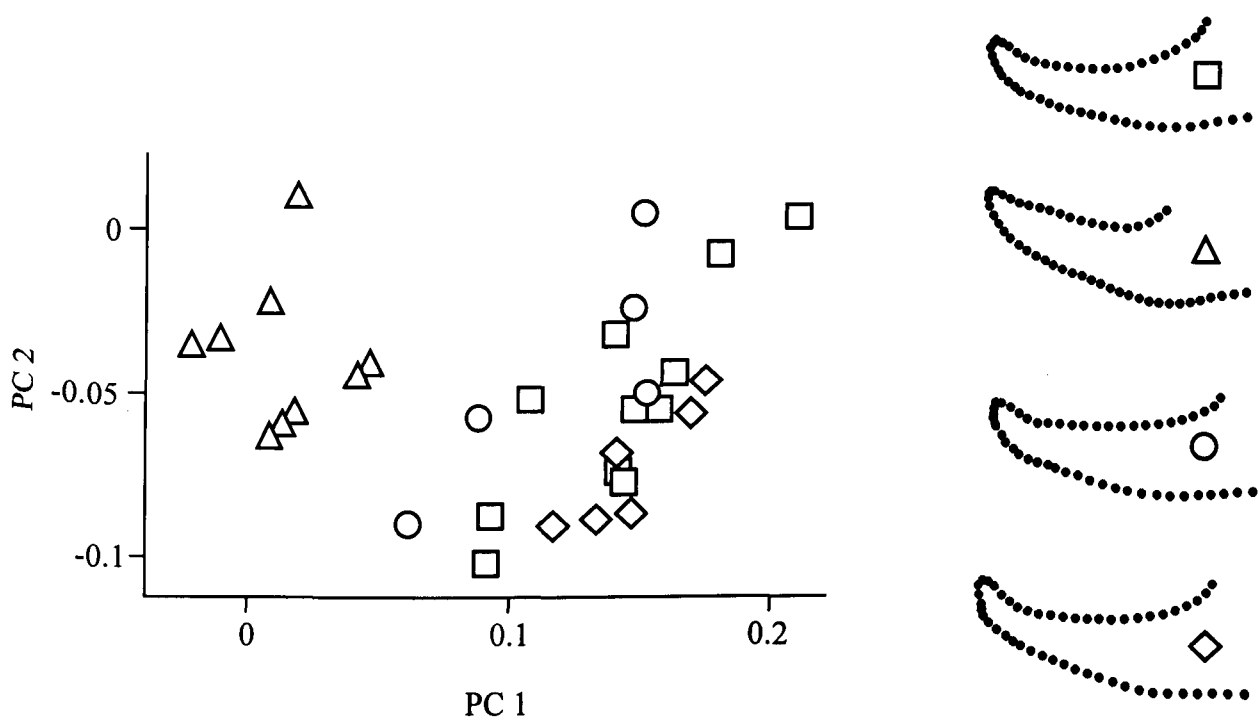


Figure 2: Ordination of first two principal components representing shape variation in the hypophallus for four populations of *Ciulfina biseriata* separated geographically (Yeppoon = \triangle ; Cardwell = \square ; Mareeba = \diamond ; Mt Inkerman = \circ) with consensus mean outlines for each population.

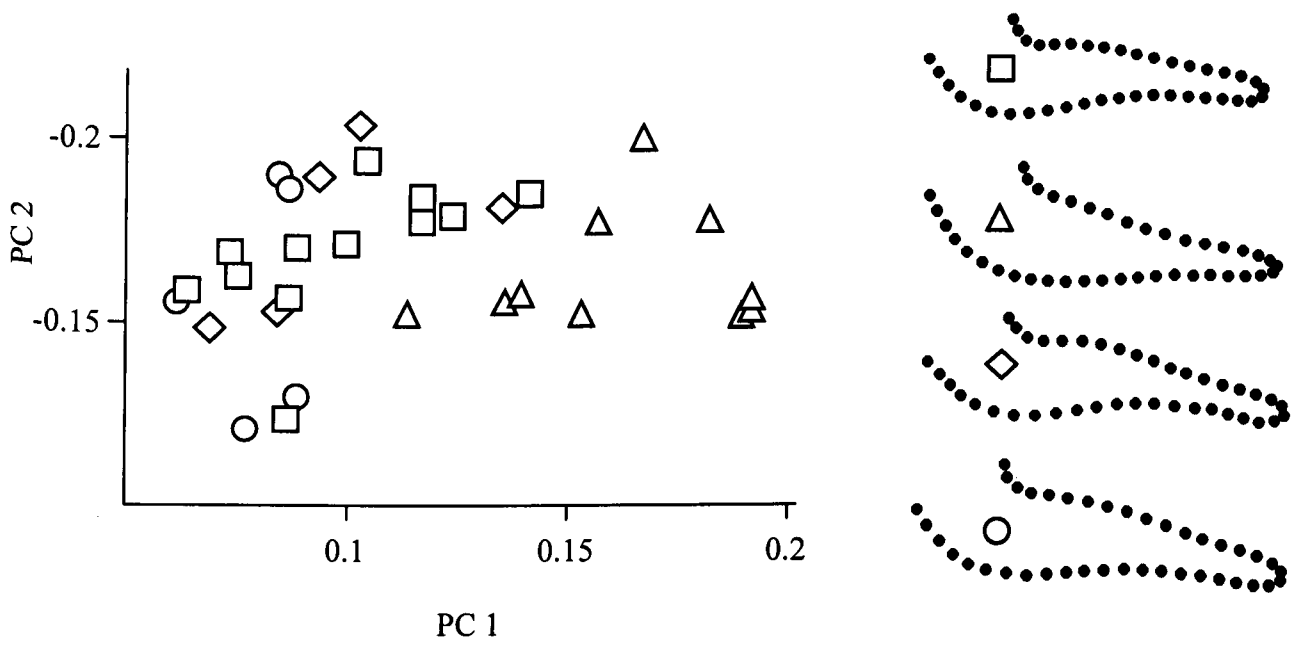


Figure 3. Ordination of first two principal components representing shape variation in the caudal left epiphallus for four populations of *Ciulfina biseriata* separated geographically (Cardwell = \square ; Yeppoon = \triangle ; Mareeba = \diamond ; Mt Inkerman = \circ) showing consensus mean outlines for each population.

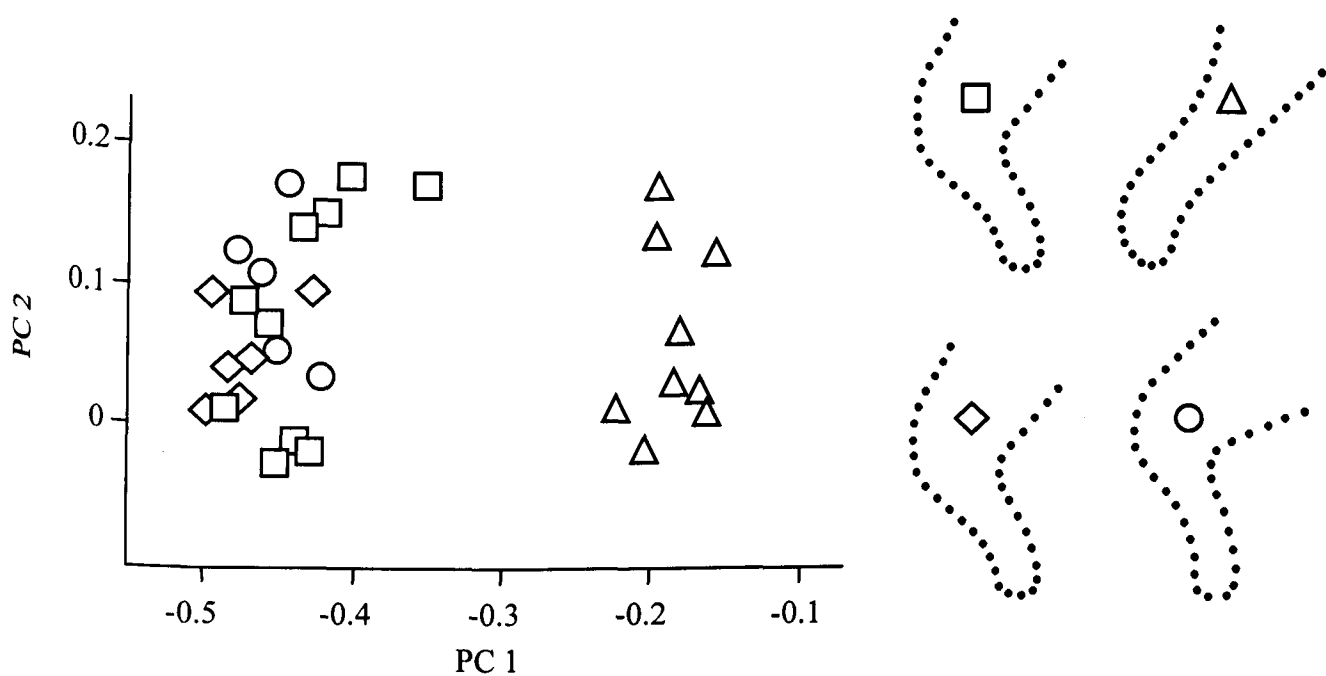


Figure 4. Ordination of first two principal components representing shape variation in the medial left epiphallus for four populations of *Ciulfina biseriata* separated geographically (Yelloon = \triangle ; Cardwell = \square ; Mareeba = \diamond ; Mt Inkerman = \circ) showing consensus mean outlines for each population.

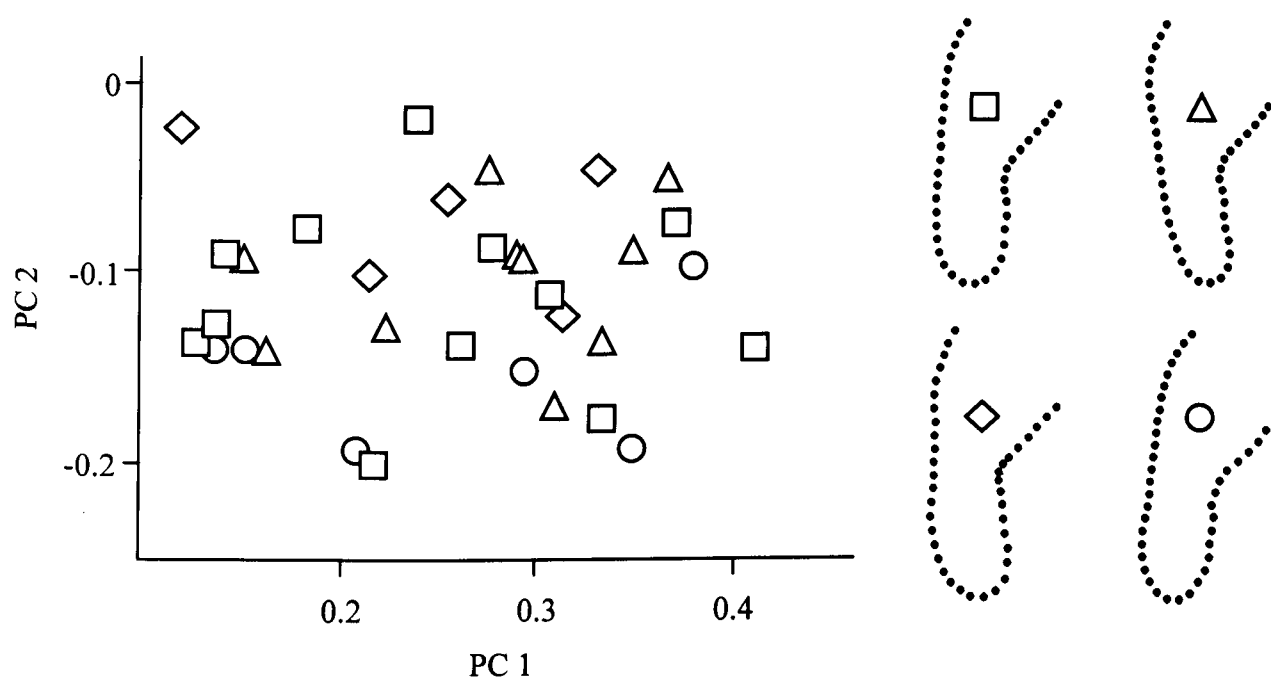


Figure 5. Ordination of first two principal components representing shape variation in the right epiphallus for four populations of *Ciulfina biseriata* separated geographically (Yeppoon = \triangle ; Cardwell = \square ; Mareeba = \diamond ; Mt Inkerman = \circ) showing consensus mean outlines for each population.

Chapter Four.

Mate location, antennal morphology and ecology in praying mantids (Insecta: Mantodea).

Submitted to the *Biological Journal of the Linnean Society*

**This chapter contains some results collected by Ms Kate Barry,
who is a co-author on the submitted paper.**

Abstract

The sensory systems employed by animals to locate potential mates are diverse. Among insects, chemical and acoustic cues are commonly used in long-distance signaling, with visual cues playing a role in close-range orientation and courtship. Within groups that exhibit a scramble competition mating system, selection on mate searching ability will be particularly strong. Species ecology can influence the type of sexual signal used. Praying mantids exhibit both chemical and visual sexual signaling behaviour, and also vary widely in their ecology. This study employs scanning electron microscopy (SEM) of antennal sensory morphology and behavioural assays to investigate the relative importance of chemical and visual signaling in two Australian praying mantid species: *Pseudomantis albofimbriata* and *Ciulfina biseriata*. As we predicted, the high level of habitat complexity, low population density and strong male dispersal capability of *P. albofimbriata* corresponded to the use of airborne sex pheromones. Conversely, the open habitat, high population density, and poor dispersal of *C. biseriata* corresponded to a greater reliance on visual cues for mate location. Praying mantids, as scramble competitors with diverse ecology, may be ideal models for studying the effect of ecology on mate location and sexual signals.

Introduction

Animals use diverse sensory mechanisms to locate potential mates. These sensory systems may be specialised for the task of mate location, but are often also used to locate other resources such as food, oviposition sites and suitable habitats, or to detect predators (Andersson, 1994). Within the insects, potential mates are located by the detection of a diverse range of signals. However, chemical and acoustic cues appear to be most commonly used over long distances, with visual cues playing a role once individuals are within courtship range (Cade, 1985; Bailey, 1991). Insect species that do not rely on any sort of signal to bring the sexes together are rarely described in the literature, perhaps due to the greater scientific interest in signalling. Sexual selection on signals and mate searching ability is predicted to be particularly strong in species exhibiting a scramble competition mating system (Able, 1999), where male-male competition is essentially restricted to the race for females and does not involve fighting or female defence (Emlen & Oring, 1977; Thornhill & Alcock, 2000). In scrambling species, males that can most efficiently detect female cues and locate potential mates will be more likely to successfully achieve fertilization (Parker, 1978; Schwagmeyer, 1988; Brown & Weatherhead, 1999).

The strategies used to locate potential mates, including the type and strength of signals produced will often be under phylogenetic constraints (Bradbury & Vehrencamp, 1998), but can also be greatly influenced by ecological constraints (Cade, 1985). Daily activity patterns, population density and the differing dispersal patterns of males and females could potentially play roles in selecting for specific mate location/signalling systems

within a species, irrespective of the patterns observed in related species. Comparative approaches permit insight to the ecological factors that may constrain mate location mechanisms, and the generation of hypotheses to explain their evolution. For example, nocturnal species are expected to be less reliant on visual cues during mate searching than related diurnal species (Bailey, 1991; Bradbury & Vehrencamp, 1998). By comparing the sexual signals of related species with different activity patterns, this idea can be tested. Similarly, it is expected that habitat complexity, population density/dispersion of potential mates, and dispersal capabilities will influence a species' mating system and mechanism of mate location (Emlen & Oring, 1977; Greenwood, 1980; Bell, 1990; Bailey, 1991; Bradbury & Vehrencamp, 1998; Shuster & Wade, 2003). A high degree of habitat complexity, low population density and high dispersal capabilities would promote the use of long-distance multidirectional signals, such as acoustic calls and airborne pheromones. Conversely, open habitats, high population density and poor dispersal may favour close-range visual cues, or even favour the absence of signals.

Praying mantids are an ideal group in which to investigate these questions. They vary considerably in their ecology, ranging from cursorial hunters to sit-and-wait predators (Svenson & Whiting, 2004) and live in a wide variety of environments. Within the Mantodea, both chemical and visual cues are important (Maxwell, 1999). Males respond to airborne pheromones in a number of genera, including *Acanthops* (Robinson & Robinson, 1980), *Sphodromantis* (Hurd *et al*, 2004), *Hierodula* (Perez, 2005) *Mantis* and *Empusa* (Gemeno *et al*, 2005). Visual cues can also be important in close-range mate-location and courtship (Roeder, 1935; Edmonds, 1979; Maxwell, 1999). Some mantids

exhibit characteristic behavioural displays involving colourful patterns on the raptorial fore-legs, wings and thorax e.g. *Oxyphilus* (Edmunds, 1979), *Paraoxyphilus*, *Gyromantis* and *Calofulcinia* (Holwell, pers. obs.). Some emphasis has been placed on the role of visual cues to ensure that females recognise males to avoid precopulatory cannibalism (Maxwell, 1999)

We have chosen two Australian praying mantid species with marked ecological differences. *Pseudomantis albofimbriata* Stal (Mantidae) is a medium-sized sit-and-wait predator found amongst foliage of trees, shrubs and grasses throughout Eastern Australia. The study population was found entirely on the flower spikes of grassy *Lomandra* bushes, a relatively complex habitat, within parkland in suburban Sydney. The population density was relatively low, with mantids found on only 6.7% of bushes (unpublished data) and patchily distributed. *Pseudomantis albofimbriata* exhibits obvious sexual dimorphism in dispersal capabilities. Males are fully-winged and capable of long-distance flight, whereas females are flightless. The frequency of sexual cannibalism is high (33%, Barry *et al*, in prep.).

Ciulfina biseriata Westwood (Liturgusidae) is a small cursorial predator found on the lower reaches of tree trunks, a relatively low-complexity habitat, in central Queensland, Australia. Population density was relatively high, with mantids located on 41.3% of preferred habitat trees (Hill *et al*, 2004), and distributed evenly throughout the study site. Dispersal of both male and female *C. biseriata* is limited by lack of true flight. Individuals move between tree trunks by running across the ground, jumping between branches, or using their hindwings to glide short distances. *Ciulfina biseriata* exhibits no sexual cannibalistic behaviour.

This study tests the prediction that airborne pheromones will be important for mate location in *P. albofimbriata*, found at low densities in complex habitats, and exhibiting strong male dispersal capabilities. In contrast, we predict that *C. biseriata*, will be more reliant on short range visual cues for mate location as it is found in less complex open environments, at higher densities, and exhibits comparatively poor dispersal.

Methods

Antennal morphology

We examined the antennae of two male and two female *P. albofimbriata* and *C. biseriata* using scanning electron microscopy (SEM) to determine whether males bear the appropriate morphological structures (basiconic sensilla) for receiving chemical signals from females, and to assess general differences in antennal morphology between the sexes, and between species. Mantids were immobilised with carbon dioxide vapour after which the heads (with antennae intact) were fixed overnight in 4% paraformaldehyde 3% glutaraldehyde in 0.1M phosphate buffer. The specimens were then washed and left overnight in 0.1M phosphate buffer solution. Heads were then submerged in 1% osmium tetroxide for approximately 30 minutes. The specimens were washed several times with water, then with a graded series of ethanol baths (from 50-100% ethanol). Antennae were removed, critical point dried, mounted on a 10 mm stub using carbon adhesive tabs, sputter-coated with gold, and viewed with a Jeol JSM 840 scanning electron microscope. The SEM images were examined to identify the type of antennal sensilla present on male and female mantids of both species. Antennal length was measured with electronic callipers and two-tailed t-tests were used to compare the length of antennae between the sexes.

Choice experiments: olfactory

To determine if *P. albofimbriata* and *C. biseriata* males respond to airborne chemicals emitted by conspecific females, we used a glass Y-maze olfactometer, which allows males to make an active choice between two stimuli. Each of the glass Y-maze tubes had a diameter of 2.3 cm and a length of 17 cm. During choice experiments, *C. biseriata* were kept on an *ad libitum* diet of *Drosophila melanogaster* and water daily, whereas *P. albofimbriata* were kept on a diet of two small house crickets (*Acheta domestica*) three times per week and water daily. For both species, virgin females (*C. biseriata*: $n = 15$; *P. albofimbriata*: $n = 10$) were chosen from the laboratory population and placed into one of two Perspex® boxes while the alternate box remained empty. Prior to each choice experiment, females were randomly allocated to a Perspex box and boxes were randomly allocated to the left or right position. Males ($n = 10$) were subsequently placed into the entry arm of the maze and an air pump connected to the rear of each Perspex box via plastic tubing allowed air flow to be directed towards the male. Air was pumped past both boxes for approximately one minute prior to the addition of the male so that any air-borne pheromones would be detectable. The anterior surface of each box was covered so that males could not use visual cues when making a choice. Males were given one hour to move within the Y-maze and a choice was recorded when they moved at least half way down one of the choice arms. Between each individual experiment, both Perspex boxes and the Y-maze tubing were washed with 100% ethanol so that the previous male and female scents did not affect the result of subsequent choices. Binomial statistics were used to test whether males moved towards females significantly more often than an empty box, suggesting the use of olfactory cues.

Choice experiments: visual

To determine if *C. biseriata* males ($n = 15$) respond visually to conspecific females, we firstly repeated the methods described above, but with the visual barriers removed from the Perspex boxes. Secondly, we used open-arena choice tests, and allowed males ($n = 20$) to choose between a tree trunk bearing a conspecific female and an empty tree trunk. This was to place males in a more realistic mate location scenario and to determine if males can locate females on a background that would render females more cryptic. Prior to these experiments, the two tree trunks used were pre-tested for uniformity of appeal to male mantids in a similar choice test but with females absent from both tree trunks. The left/right position for the two tree trunks were randomly allocated in the pre-test and experiment, and preference for left/right position was also tested in the pre-test. Tree trunks in both the pre-tests and experiments were positioned one metre apart, and one metre from the mantid. The pre-test revealed no preference for either tree trunk used in the experiment (Binomial: $p > 0.7$, $n = 24$) and no preference for either the left or right trunk position in the arena (Binomial: $p > 0.3$, $n = 24$). The results of the mate location experiment were therefore deemed to be unbiased by either trunk or positional preference. Binomial statistics were used to test whether males moved towards females significantly more often than an empty tree trunk, suggesting male ability to locate females visually.

Results

Mate attraction and recognition

Antennal morphology

In *C. biseriata*, there is no significant difference in antennal length between males and females (t-test: $t_5 = -1.032$, $p > 0.32$; Table 1). For *P. albofimbriata*, male antennae are significantly longer than female antennae (t-test: $t_5 = -14.545$, $P < 0.0001$; Table 1). Furthermore, male and female *P. albofimbriata* differ in the type of sensilla their antennae possess. The female antennae are primarily surfaced with large and medium trichoid sensilla (trichodea), corrugated by longitudinal ridges, and extending from relatively shallow antennal pores (Figure 1f). Trichoid sensilla are generally considered to be contact chemoreceptors. Also, each large trichoid sensilla grows from a cuticular protuberance, suggesting an additional mechanosensory function (Zacharuk, 1985). By contrast, male antennae are covered in relatively small basiconic sensilla with grooves down their length (Figure 1e). Grooved basiconic sensilla are olfactory receptors with a variety of functions in insects, including long distance pheromone reception (Zacharuk, 1985). Male antennae also have a small number of large trichodea.

The type of sensilla found on the antennae of male and female *C. biseriata* do not greatly differ, except that females appear to have more long trichoid sensilla, and males bear more short trichoid sensilla. In addition, female antennae of both species were much cleaner than male antennae, probably as a result of more frequent grooming performed by females (Holwell & Barry, pers. obs.).

Choice experiments

P. albofimbriata males quickly moved along the entry arm of the Y-maze while oscillating the antennae. Mantids spent the majority of their time at the fork of the maze, and it was not uncommon to see one antenna in each of the upper tubes. Males then proceeded to move more slowly down one of the tubes, making a choice between the contents of the

two Perspex boxes. Ten of the twelve male *P. albofimbriata* chose the box containing the female, which was significantly more often than the empty box (Binomial test: $P = 0.019$). *C. biseriata* males did not make any clear choice between the empty box and that containing a female, with the majority of males remaining in the entry arm of the Y-maze (9/15) and only half of those making a choice, chose the box containing a female (Binomial: $x = 4$, $n = 8$, $P = 0.6367$). When the visual barrier was removed from the entrances to the boxes, this significantly altered the choice behaviour of the male mantids. All males subsequently chose between test boxes, and they significantly chose boxes containing females over empty boxes (Binomial: $x = 12$, $n = 15$, $P = 0.0176$). In the open arena choice tests, given the choice between a tree trunk bearing a female and an empty tree trunk, male *C. biseriata* chose tree trunks bearing females significantly more often (Binomial: $x = 18$, $n = 20$, $P = 0.0002$).

Discussion

Mate location and the ecology of C. biseriata and P. albofimbriata

We have uncovered distinct differences in the way that male praying mantids locate females. While *P. albofimbriata* uses olfactory cues during mate searching, the mechanisms used by male *C. biseriata* are entirely visual, and very acute. The ability of males to visually detect motionless and cryptic females from a considerable distance demonstrates an impressive level of visual acuity (see Prete, 1999), supporting the results of previous studies on the ability of *C. biseriata* to visually discriminate between the bark-types of habitat trees (Hill *et al*, 2004). Interpretation of the antennal morphology of both species using SEM provides us with a proximate explanation for the different sensory

systems used by the two species. Basiconic sensilla, which were only found on male *P. albofimbriata* in this study, are generally associated with olfaction in insects, whereas trichodea, identified from all individuals in this study, are associated with a mechanosensory or gustatory function (Zacharuk, 1985; Keil, 1999). The lack of basiconic sensilla in male *C. biseriata* makes chemoreception of airborne pheromones unlikely. Sexual dimorphism in antennal length was also detected for *P. albofimbriata* but not for *C. biseriata* (Table 1). Such dimorphism in antennal length or structure is prevalent in species that use airborne pheromones for mate location (Keil, 1999). These data help to explain why *C. biseriata* could not detect females in the Y-choice olfactometer test. A more ultimate explanation for the difference in mate location mechanisms between these two species comes from a comparison of their ecology. In this regard, our initial predictions were supported by the results.

Habitat complexity.

Habitat is markedly different between the two species studied. In the relatively complex environment of *Lomandra* shrubs, male *P. albofimbriata* cannot rely on visual cues to locate females, as they may be visually obscured by vegetation in all directions. The use of airborne pheromones to attract males in *P. albofimbriata* may therefore be adaptive to their environment. *C. biseriata* is found in open eucalypt woodlands on the bark of tree trunks. Despite being cryptically coloured, *C. biseriata* can effectively use visual cues when locating preferred habitat trees (Hill *et al*, 2004) or mates (present study) without obstruction from vegetation. Chemical signals may be unnecessary in this environment.

Population density and dispersal.

The density and patchiness of the two study populations also relate to the observed results. For *P.albofimbriata*, which exhibits a low-density, patchy distribution, signals would be advantageous for locating mates (Bell, 1990). *C. biseriata* is found evenly distributed throughout its woodland at relatively high density where the location of females by males would not require a mate-attracting signal. Visual searching of a small number of trees would be likely to yield some success for an active male *C. biseriata*. Chemical signals would not only be unnecessary in this environment, but may over-saturate the receiver in a high density of close-proximity signallers (Wyatt, 2003).

Pseudomantis albofimbriata and *C. biseriata* differ in dispersal capability. Male *P. albofimbriata* are fully-winged and capable of long-distance flight, whereas females are flightless. The use of airborne pheromones has been linked to long-distance dispersal, and insects producing airborne sex pheromones can attract mates from large distances (Cade, 1985). Conversely, the dispersal capabilities of both male and female *C. biseriata* are limited by a lack of flight. In species lacking long-distance dispersal, such as *C. biseriata*, we might intuitively expect that populations exhibit relatively high densities, and that individuals would not require long-distance signals to locate mates.

Cannibalism.

Pseudomantis albofimbriata and *C. biseriata* differ in mating behaviour with *P.albofimbriata* exhibiting a high frequency of sexual cannibalism (Barry *et al.*, in prep.) and *C. biseriata* exhibiting no cannibalistic behaviour. This is potentially important for the results of the present study. Cannibalistic *P. albofimbriata* females, which produce airborne pheromones, could attract mates and gain substantial nutrition from each mating encounter. An effective long-distance sexual signal may be particularly advantageous for

sexually cannibalistic species and it has been suggested that food-limited female mantids may attract males as a foraging strategy (Hurd *et al.*, 1994). The detection of females through chemical stimuli may also be particularly beneficial to males of sexually cannibalistic species due to the risk of being cannibalised before mating (Maxwell, 1999). Precopulatory cannibalism occurs frequently in *P. albofimbriata* (Barry *et al. in prep*), demonstrating a real advantage for males that can detect females using multi-sensory cues.

Praying mantids and scramble-competition

Ecological factors are important in the evolution of different mating systems (Emlen & Oring, 1977). The dispersal capabilities of males, as well as the spatial and temporal distribution of females and resources, will all interplay to determine the strategies males will use to maximise their reproductive output (Emlen & Oring, 1977; Thornhill & Alcock, 2000). Praying mantids exhibit the characteristics of a scramble competition mating system. High male mate-searching efficiency, low possibility of female defence, low operational sex ratio and long periods of female receptivity would benefit 'roaming' males (Sandell & Liberg, 1992). In such a scramble mating system, indirect sexual selection may strongly influence male mate searching ability and their perception of female signals (Wiley & Poston, 1996; Legrand & Morse, 2000). For mantids, sexual cannibalism would further negate the possibility of a defence-based mating system in some species.

Scramble competition mating systems are probably much more common than the literature would suggest (Able, 1999). A small number of studies of vertebrate systems have shown that traits associated with mate searching are related to mating success (Schwagmeyer, 1988; Able, 1999; Brown & Weatherhead, 1999; Shine *et al.*, 2005; Spritzer *et al.*, 2005).

Invertebrate scramble competition mating systems have received even less attention. Some studies have shown links between antennal morphology and mating success (McLain, 1982; Bertin & Cezilly, 2003) suggesting strong sexual selection on mate locating ability. Other studies have simply detected the existence of scramble-competition amongst diverse insect species including bees (Seidelmann, 1999), beetles (Nahrung & Allen, 2004), damselflies and wasps (Thornhill & Alcock, 2000). Unfortunately there does not appear to be any direct evidence for a link between mate locating ability and mating success in an insect scramble mating system. In the present study, we have revealed two distinct mechanisms of scramble competition within the Mantodea: (a) scrambling by males in response to the sexual advertisement of a female, and (b) scramble competition between males through their efficiency in searching for females at likely encounter-sites. These two mechanisms have been highlighted previously for insect scramble competitors (Thornhill and Alcock, 2000). Although Emlen and Oring's (1977) classic treatment of the relationship between ecology and mating system describes factors that favour scramble competition, factors affecting the type of scramble system have received no attention.

Scramble competition and indirect mate choice

Also related to this study is the role of indirect mate choice in scramble competition mating systems. In chemically signalling species, the minute amounts of airborne chemicals produced by the signalling sex indirectly select for more sensitive and finely-tuned chemosensory abilities in the searching sex (Wyatt, 2003). If such traits were heritable, offspring of the searching sex would also be good searchers. Conversely, females that do not advertise their presence through sexual signals may be indirectly selecting for male ability to locate them at preferred encounter sites (Legrand & Morse,

2000). Similarly, females that actively evade males, may be selecting for the stealthiest males via indirect mate choice (Wiley & Poston, 1998). Legrand & Morse (2000) suggest that female crab spiders that indirectly select for males that can locate good predation sites (where females are more likely to occur), will ensure that offspring of both sexes will benefit from these traits. We have shown that female *C. biseriata* do not advertise themselves to roaming males (present study) and actively evade males when they become aware of them (Holwell & Herberstein, in prep), which may indirectly select for good visual acuity, efficient searching behaviour and stealth in male approach. For *C. biseriata*, which is a cursorial predator, any heritable component of these traits would provide advantages to offspring of both sexes through improved predatory efficiency. Praying mantids may therefore be useful models for the investigation of indirect sexual selection on male mate searching.

Conclusions

Although, single-species studies are required to deduce the relative importance of various ecological influences, the comparison of *P. albofimbriata* and *C. biseriata* we have presented highlights components of species' ecology that may influence whether females produce sexual signals, and also the modality of those signals. We suggest that long-distance chemical signalling would be favoured in species with (a) high-complexity habitats, (b) sparse and patchy distributions and (c) high dispersal capabilities in the searching sex. Among praying mantids, sexually cannibalistic species would also favour such signals. Praying mantids as a group exhibit both scramble strategies and may prove to be ideal for the study of scramble competition in insects. The differences in dispersal abilities of males, dispersion of females, range of feeding modes (cursorial through to sit-

and-wait predation) and the differing sensory systems used to locate mates (chemical and visual), within the Mantodea, provide a unique opportunity in which to study the effects of these factors on the scramble competition mating system.

Acknowledgements

We kindly thank Anne Gaskett for designing the Y-choice olfactometer and for assistance with the choice tests. Debra Birch and Nicole Vella assisted greatly with scanning electron microscopy. Anne Gaskett, Matthew Bruce, Katie Robinson, Kim Shaddick and Phoebe Hill assisted with collection of mantids in the field. We also thank David Briscoe for helpful comments on the manuscript.

References.

- Able DJ. 1999. Scramble competition selects for greater tailfin size in male red-spotted newts (Amphibia: Salamandridae). *Behavioral Ecology and Sociobiology* 46: 423-428.
- Andersson M. 1994. *Sexual selection*. Princeton University Press, Princeton.
- Bailey WJ. 1991. Mate finding: selection on sensory cues. In: Bailey WJ and Ridsdill-Smith J, eds. *Reproductive behaviour of insects: individuals and populations*. Melbourne: Chapman & Hall.
- Bell W. 1990. Searching behavior patterns in insects. *Annual Review of Entomology* 35: 447-467.
- Bertin A, and Cezilly F. 2003. Sexual selection, antennae length and the mating advantage of larger males in *Asellus aquaticus*. *Journal of Evolutionary Biology* 16: 491-500.
- Bradbury JW, and Vehrencamp SL. 1998. *Principles of animal communication*. Sinauer associates, Inc., Sunderland.
- Brown GP, and Weatherhead PJ. 1999. Female distribution affects mate searching and sexual selection in male northern water snakes (*Nerodia sipedon*). *Behavioral Ecology and Sociobiology* 47: 9-16.
- Cade WH. 1985. Insect mating and courtship behaviour. In: Kerkut GA and Gilbert LI, eds. *Comprehensive insect physiology, biochemistry and pharmacology*. Oxford: Pergamon press.

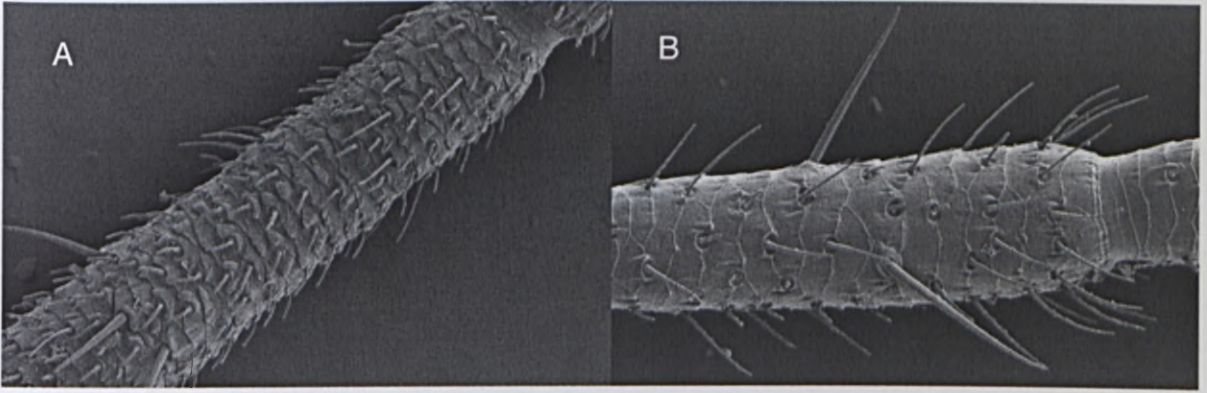
- Emlen ST, and Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197: 215-223.
- Gemeno C, Claramunt J, and Dasca J. 2005. Nocturnal calling behavior in mantids. *Journal of Insect Behavior* 18: 389-403.
- Greenwood P. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* 28: 1140-1162.
- Hill PJB, Holwell GI, Goth A, and Herberstein ME. 2004. Preference for habitats with low structural complexity in the praying mantid *Ciulfina* sp (Mantidae). *Acta Oecologica-International Journal of Ecology* 26: 1-7.
- Hurd LE, Eisenberg RM, Fagan WF, Tilmon KJ, Snyder WE, Vandersall KS, Datz SG, and Welch JD. 1994. Cannibalism Reverses Male-Biased Sex-Ratio in Adult Mantids - Female Strategy against Food Limitation. *Oikos* 69: 193-198.
- Hurd LE, Prete FR, Jones TH, Singh TB, Co JE, and Portman RT. 2004. First identification of a putative sex pheromone in a praying mantid. *Journal of Chemical Ecology* 30: 155-166.
- Keil TA. 1999. Morphology and development of the peripheral olfactory organs. In: Hansson BS, ed. *Insect olfaction*. Berlin: Springer-Verlag. 5-47.
- Legrand R, and Morse D. 2000. Factors driving extreme sexual size dimorphism of a sit-and-wait predator under low density. *Biological Journal of the Linnean Society* 71: 643-664.

- Maxwell MR. 1999. Mating behavior. In: Prete FR, Wells H, Wells PH and Hurd LE, eds. *The praying mantids*. Baltimore: The John Hopkins University Press.
- McLain DK. 1982. Density dependent sexual selection and positive phenotypic assortative mating in natural populations of the soldier beetle, *Chauliognathus pennsylvanicus*. *Evolution* 36: 1227-1235.
- Nahrung HF, and Allen GR. 2004. Sexual selection under scramble competition: mate location and mate choice in the eucalypt leaf beetle *Chrysophtharta agricola* (Chapuis) in the field. *Journal of Insect Behavior* 17: 353-366.
- Parker GA. 1978. Evolution of competitive mate searching. *Annual Review of Entomology* 23: 173-196.
- Perez B. 2005. Calling behaviour in the female praying mantis, *Hierodula patellifera*. *Physiological Entomology* 30: 42-47.
- Roeder KD. 1935. An experimental analysis of the sexual behavior of the praying mantis (*Mantis religiosa*, L.). *Biological Bulletin* 69: 203-220.
- Sandell M, and Liberg O. 1992. Roamers and stayers: a model on male mating tactics and mating systems. *American Naturalist* 139: 177-189.
- Schwagmeyer PL. 1988. Scramble-competition polygyny in an asocial mammal: male mobility and mating success. *American Naturalist* 131: 885-892.
- Seidelmann K. 1999. The race for females: the mating system of the red mason bee, *Osmia rufa* (L.) (Hymenoptera: Megachilidae). *Journal of Insect Behavior* 12: 13-25.

- Shine R, O'Donnell RP, Langkilde T, Wall M, and Mason R. 2005. Snakes in search of sex: the relation between mate-locating ability and mating success in male garter snakes. *Animal Behaviour* 69: 1251-1258.
- Shuster SM, and Wade MJ. 2003. *Mating systems and strategies*. Princeton University Press, Princeton.
- Spritzer MD, Solomon NG, and Meikle DB. 2005. Influence of scramble competition for mates upon the spatial ability of male meadow voles. *Animal Behaviour* 69: 375-386.
- Svenson GJ, and Whiting MF. 2004. Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology* 29: 359-370.
- Thornhill R, and Alcock J. 2000. *The evolution of insect mating systems*. iUniverse.com, Inc., Lincoln.
- Wiley R, and Poston J. 1996. Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution* 50: 1371-1381.
- Wyatt TD. 2003. *Pheromones and animal behaviour: communication by smell and taste*. Cambridge University Press, Cambridge.
- Zacharuk RY. 1985. Antennae and sensilla. In: Kerkut GA and Gilbert LI, eds. *Comprehensive insect physiology, biochemistry and pharmacology*. Oxford: Pergamon Press.

Table 1: Antennal morphology for males and females of *C. biseriata* and *P. albofimbriata*.
The density of sensilla was assessed qualitatively only (see Figure 1).

	Mean length (mm)	Type of Sensilla
<i>C. biseriata</i> male	20.14 ± 0.32	Short trichoid Long trichoid
<i>C. biseriata</i> female	19.62 ± 0.33	Long trichoid Short trichoid
<i>P. albofimbriata</i> male	27.67 ± 0.85	Basiconic Short trichoid
<i>P. albofimbriata</i> female	12.91 ± 0.56	Long trichoid Medium trichoid



Submitted to the *Journal of Zoology (London)*

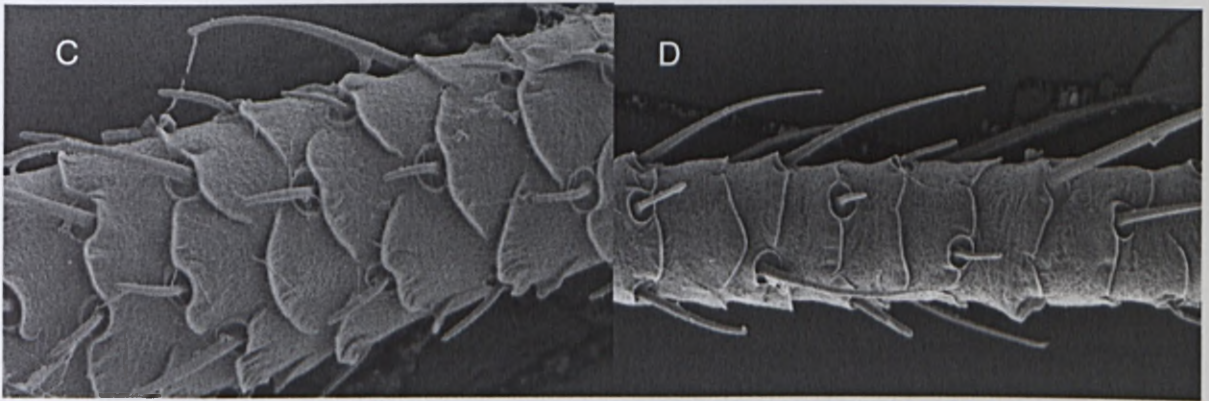


Figure 1 Scanning electron micrographs showing median segments of male (A) and female (B) *P. albofimbriata* antennae, male (C) and female (D) *C. biseriata* antennae, and higher magnification images of basiconic (E) and trichoid (F) sensillae of *P. albofimbriata* antennae.

Chapter Five.

**Spermatophore feeding and mating behaviour in
Ciulfina praying mantids (Mantodea:
Liturgusidae).**

Submitted to the *Journal of Zoology (London)*

Abstract.

In a number of Orthopteran insects, males transfer sperm to females via an externally attached spermatophore, which females subsequently remove and consume. Here I report the first known example of spermatophore feeding in a praying mantid. While studies of praying mantid mating behaviour have largely focussed on sexual cannibalism, the genus *Ciulfina* is not cannibalistic. The general patterns of mating behaviour and spermatophore feeding are described for four species: *C. rentzi*, *C. klassi*, *C. biseriata* and *C. baldersoni*. Copulation duration and postcopulatory spermatophore attachment duration were found to vary both inter- and intraspecifically. *Ciulfina rentzi* exhibited a considerably longer mean copulation duration and considerably shorter mean spermatophore attachment duration than the other *Ciulfina* species. Smaller males copulated for longer durations in *C. rentzi* and *C. klassi*, whereas the spermatophores of smaller males remained attached to females for longer durations in *C. biseriata*. For *C. baldersoni*, both copulation duration and spermatophore attachment duration increased with female mass. The genus *Ciulfina* is highlighted as an intriguing new system in which to test hypotheses surrounding the function of spermatophore feeding and the significance of copulation duration.

Introduction.

The mating behaviour of praying mantids has received considerable attention from biologists in recent decades (Maxwell, 1999a). Observational and experimental studies have investigated mate attraction and sex pheromones (Edmunds, 1975; Robinson and Robinson, 1979; Prete et al., 2000; Hurd et al., 2004; Perez, 2005), male approach (Kynaston, McErlainward and Mill, 1994), variation in mate quality (Lawrence, 1992), sex-ratio (Moran and Hurd, 1994), and the response of males to increased risk of sperm competition (Prokop and Vaclav, 2005). The majority of studies however, have focussed on male and female mating strategies with regards to sexual cannibalism. Four species that have received most of this attention are within the family Mantidae: *Mantis religiosa* (Roeder, 1935; Lawrence, 1992; Prokop and Vaclav, 2005), *Iris oratoria* (Maxwell, 1999b; Maxwell, 2000), *Stagmomantis limbata* (Maxwell, 1998) and *Tenodera aridifolia sinensis* (Liske and Davis, 1984; Liske and Davis, 1987). This may be because they represent some of the most charismatic and commonly-found praying mantids in Europe (e.g. *M. religiosa* and *I. oratoria*), North America (e.g., *S. limbata* and introduced *I. oratoria*) and Asia (e.g. *T. a. sinensis*). However, the Mantidae represent less than half (1137/2300) of the praying mantid species currently described (Ehrmann, 2002). Studies of mating behaviour within praying mantids from other families have received little scientific attention. In a recent review, Maxwell (1999) showed that only six species outside the Mantidae have been observed in greater than two encounters and half of those (*Acanthops falcata*, *Acontiothespis multicolor* and *Euantissa ornata*) did not display any cannibalism (Mathur, 1946; Quesnel, 1967; Robinson and Robinson, 1979). It may be that sexual cannibalism is not widespread among the Mantodea. Eight praying mantid families

(Chaeteessidae, Mantoididae, Metallyticidae, Amorphoscelidae, Empusidae, Eremiaphilidae, Thespidae, and Toxoderidae) do not contain any species for which mating observations have been made, so it is likely that mating behaviour is much more diverse within the Mantodea than we currently recognise, and could rival the diversity observed within other insect Orders.

This study investigates patterns of mating behaviour in four species of the praying mantid genus *Ciulfina* (Liturgusidae: Mantodea). *Ciulfina* are small cryptic mantids that live on the bark of trees throughout northern Australia, preferring trees possessing smooth bark (Hill et al., 2004). They are fast-moving, cursorial predators and males locate females visually without the use of airborne pheromones (Holwell, Barry and Herberstein, in review). Like many insects, male *Ciulfina* deliver sperm into the female reproductive tract via an externally-attached spermatophore. Most intriguingly, *Ciulfina* females are not cannibalistic, but remove and eat male spermatophores after mating, a behaviour which has previously never been observed in praying mantids.

Female spermatophore feeding is common among Orthopteran insects (crickets, katydids and their kin) but even within the Orthoptera, the function of spermatophore feeding varies between species (Gwynne, 1997). Females have been shown to influence male fertilization success by prematurely removing spermatophores prior to complete sperm transfer in the crickets: *Gryllus bimaculatus* (Simmons, 1987) and *Gryllodes sigillatus* (Sakaluk and Eggert, 1996). This suggests a role for postcopulatory cryptic female choice in spermatophore removal and feeding (Eberhard, 1996). In some species such as the sagebrush cricket *Gryllodes sigillatus* and many tettigoniids, a large edible attachment to the spermatophore, or spermatophylax, is consumed by females before the ampulla of the

spermatophore itself is removed (Will and Sakaluk, 1994; Gwynne, 1997). In many species, the spermatophylax acts as an ejaculate protection device, allowing complete sperm transfer (Wedell, 1993). However, the spermatophylax can also contribute significantly to female reproductive output (Gwynne, 1988; Reinhold, 1999). One extreme example of this is found in the mormon cricket, *Anabrus simplex*. When food is limited, females compete for access to males due to the nutritional benefits of feeding, leading to increased male choosiness and role-reversed sexual selection (Gwynne, 1985).

The functional significance of spermatophore feeding can therefore be variable, both within and between species. The main objective of this study is to investigate spermatophore feeding within the genus *Ciulfina* and to document interspecific variation in mating behaviour. Specifically, the duration of copulation and spermatophore attachment is compared between the four species and the relationship between body mass and the duration of copulation and spermatophore attachment are investigated.

Methods.

Collection and housing

Populations of four *Ciulfina* species were identified and collection took place in January, 2005. The four species do not share differences in external morphology and differ from one another only in male genital morphology and in their distributional ranges which do not overlap (Holwell *et al*, in review). *Ciulfina biseriata* were collected from dry eucalypt-dominated woodland approximately 3km west of Yeppoon (Queensland, Australia, 23°09'S 156°41'E). *Ciulfina rentzi* were collected from tropical rainforest at Lacey Creek, near Mission Beach (Queensland, Australia, 17°51'S 146°03'E) *Ciulfina klassi* were

collected from coastal *Melaleuca* woodland, approximately 8 km west of Mission Beach (Queensland, Australia, 17°48'S 146°01'E). *Ciulfina baldersoni* were collected from eucalypt and *Casuarina* woodland in Queens Park, Bundaberg (Queensland, Australia, 24°52'S 152°20'E). Twenty male and twenty female mantids were collected from each location as sub-adult nymphs and housed in the laboratory in inverted plastic cups (10cm diameter) containing bark for the mantid to perch on and a fabric window to allow airflow. They were fed *Drosophila melanogaster* three times per week and watered daily. All mantids were housed individually in the laboratory. Mantids were collected from Lacey creek under permit ATH 05/006 from the Queensland Parks and Wildlife Service and the Environmental Protection Authority.

Mating observations

For each of the four *Ciulfina* species, observations were made of mating behaviour in a semi-natural laboratory environment. The mating arena consisted of a tree trunk, sawn in half and screwed onto a Perspex® board to prevent mantids from moving out of the observer's range of view. Males and females were weighed using a Mettler Toledo PB303-5 DeltaRange® electronic scale immediately prior to each mating trial. Females were introduced to the tree trunk ten minutes before males to allow them to adjust to the novel environment. The time from introduction until mating commenced, copulation duration and the attachment duration of the spermatophore prior to female removal were recorded using a stopwatch. Observations of behaviours including male approach, interactions, grooming, copulatory and postcopulatory behaviour were recorded throughout the observational period.

Statistical analyses

Data were checked for normal distribution and homogeneity of variance before the analysis. Regression analyses were used to assess any relationships between the mass of males and females and the duration of copulation and spermatophore attachment. ANOVA was used to assess differences between the duration of copulation and spermatophore attachment between the four species. These analyses were conducted using SPSS 11 for Mac OS X.

Results.

Mating Behaviour

All *Ciulfina* species showed essentially the same repertoire of mating behaviours prior to, during and after copulation. Males became aware of females very shortly (less than 30 seconds) after being introduced to the mating arena. This was apparent, as males would turn their heads to face females and peer from side to side, a behaviour commonly associated with depth perception in praying mantids (Kral, 1999). Males would then alter their posture such that their thorax and abdomen were pressed against the tree trunk, and stealthily approach the female. If the male faced the front of the female, he would dart quickly around the tree trunk and move into position behind her. The male approach was generally very slow and almost imperceptible but was punctuated by rapid lunges forward. These lunges were usually attempted when the female was grooming or otherwise distracted. Once the male's head was within approximately 5-10 mm of the female's

abdomen, he would leap onto the female's back, gripping her thorax or mid-legs with his fore-legs and insert his genitalia into her genital opening. If the female became aware of the male before a mating attempt she would rapidly turn to face him or run away, after which the male's stealthy approach would resume. During copulation, both males and females remained motionless, except for the pulsation of the male abdomen and genitalia. Females positioned their antennae facing forwards, and males positioned their antennae facing backwards. Copulation was terminated when males removed their genitalia from the female and leaped back onto the tree trunk. Males always moved away from females and did not interact with them in any way. Some males would also attempt to leave the mating arena. After copulation, a small white spermatophore was visible in the female genital opening, and female genitalia appeared splayed out. After a variable period of time, females would groom themselves thoroughly. They would begin by using their mouthparts to groom their antennae, which they brought to their mouth using their fore-legs. Then each mid-leg and hind-leg would be groomed in a similar fashion. Finally, the female used a hind-leg to twist her abdomen around forming a 180° U-shape, and use her mouth-parts to remove the spermatophore and consume it (Figures 1 & 2). She then groomed her genital opening and it would gain its original appearance. One additional behaviour of note is the waving of fore-legs undertaken by both males and females, a behaviour noted for a number of mantid species (Maxwell, 1999a). This behaviour was only observed for a small number of pairings. The fore-legs would be stretched out in front of the head, and then moved laterally away from each other and circle around to rest in the original posture. This would be repeated either rapidly or more slowly. The behaviour was observed in all species, however *C. biseriata* was observed more frequently. Fore-leg waving was sometimes performed by females before males were introduced to the mating arena, but it

was more frequently observed as a behaviour that both males and females performed when facing each other, and by females after copulation.

Copulation duration and spermatophore attachment duration

Mean copulation duration and spermatophore attachment time was variable between species (Figure 3). Copulation duration varied significantly between species ($F_{3,48} = 37.807$; $p < 0.001$). Postcopulatory spermatophore attachment duration also varied significantly between species ($F_{3,48} = 6.635$; $p < 0.005$). Post-hoc tests revealed that the data for *C. rentzi* had the most significant influence over these results for both copulation duration ($p < 0.001$) and spermatophore attachment duration ($p < 0.02$). The copulation duration of *C. rentzi* was approximately three times that of *C. biseriata*, *C. baldersoni* and *C. klassi*. Spermatophore attachment time however was approximately three times shorter than for the other *Ciulfina* species (Figure 3).

Influence of body mass

To identify possible factors that may influence the variation found in the duration of copulation and spermatophore attachment, I used regression analyses. Specifically I looked at the effect of male and female mass (Table 1). Significant correlations were found between male mass and copulation duration for *C. rentzi* (Figure 4) and *C. klassi* (Figure 5), between male mass and spermatophore attachment duration for *C. biseriata* (Figure 6), and between female mass and both copulation duration and spermatophore attachment duration for *C. baldersoni* (Figure 7).

Discussion.

Spermatophore feeding

Female consumption of male spermatophores has been intensively studied in the Orthoptera, but this paper presents the first data on spermatophore feeding in a praying mantid. Although spermatophore feeding has not been documented for the Mantodea before, due to the absence of behavioural data for many mantid families, this behaviour may extend outside the genus *Ciulfina*. Amongst the Orthoptera, female spermatophore feeding is widespread and its functional significance appears to vary between species (Gwynne, 1997). The *Ciulfina* spermatophore is very small in comparison to those of the Orthoptera (see Fig 2), and does not possess a spermatophylax. Although the female consumes it after removal, it is unlikely that it is nutritionally rewarding. Therefore, the variation in removal time is likely to be a consequence of either (a) sperm flow duration or (b) postcopulatory female choice (Eberhard, 1996). First, females may only remove the spermatophore when all of the sperm have been released from it as a grooming behaviour. Female mantids are fastidious groomers, and spermatophore removal is usually preceded by grooming of antennae and legs, and followed by grooming of the genital opening. If spermatophore attachment duration simply relates to the amount of sperm transferred by the male, variation may represent relative male investment or cryptic male choice (Bonduriansky, 2001)

Secondly, if sperm flow continues from the spermatophore after copulation is terminated, premature spermatophore removal may represent a mechanism of postcopulatory female choice (Eberhard, 1996) which has been demonstrated for a number of Orthopteran species (Simmons, 1987; Wedell, 1991; Sakaluk and Eggert, 1996; Calos and Sakuluk, 1998; Fleischman and Sakuluk, 2004). Distinguishing between these hypotheses may be possible via detailed study of patterns of sperm transfer in *Ciulfina*.

The variation observed between the postcopulatory attachment duration of the spermatophore in *C. rentzi* and the other *Ciulfina* species (Figure 3) is also intriguing. *Ciulfina rentzi* spermatophores were removed by females approximately three times faster than in other species (Figure 3). This may be because copulation duration is much greater for *C. rentzi* and sperm transfer may be complete when copulation ends, limiting the potential for female manipulation.

Copulation duration

Copulation duration was variable both within and between *Ciulfina* species and was significantly higher in *C. rentzi* (Figure 1). Such variation is common (Simmons, 2001) and the factors affecting copulation duration in insects are diverse. They include male and female mating history (Jones and Elgar, 2004), male and female body size (Krebs, 1991), alternative mating tactics (Siva-Jothy and Tsubaki, 1989), time of day (Michiels, 1992), predation risk (Krupa and Sih, 1992), the size of nuptial gifts (Engqvist and Sauer, 2001), diet (Perez-Staples and Aluja, 2004), operational sex ratio (OSR) (Alonso-Pimentel and Papaj, 1999) and male density (Andres and Rivera, 2000). Some factors which could vary between closely-related species (e.g. male and female density, OSR and predation risk) could also influence the variation observed in copulation duration between closely-related

species. Alternatively, sexual selection may favour different copulation durations in different species, depending on the importance of copulatory courtship (Eberhard, 1996) or conflicts of interest between the sexes over optimal duration (Arnqvist and Rowe, 2005). Although a number of explanations exist, experimental approaches may assist us to understand the functional significance of copulation duration in this group.

Influence of mass

Ciulfina rentzi and *C. klassi* display a negative association between male body mass and copulation duration (Fig. 4 & 5) and *C. biseriata* displayed a similar negative relationship between male body mass and spermatophore attachment time (Fig. 6). As both copulation duration and spermatophore attachment duration both represent potential periods of sperm transfer, these relationships may be interpreted in the same way. Firstly, larger males may deposit sufficient sperm for successful fertilization in a shorter period of time. Secondly, in the context of sperm competition, larger males may be superior sperm competitors and influence fertilization success more successfully than smaller males (Simmons, 2001). Thirdly, individuals may copulate for a duration that depends on the likelihood of encountering another mate. Larger males may copulate for shorter durations because they can cover larger ranges in search of females and therefore have a higher probability of future mating success (Schwagmeyer, 1988). Smaller males may have such a low chance of finding another mate that investing more in their current mate may be a better strategy (Andrade, 2003).

A positive relationship between female body mass and both copulation duration and spermatophore attachment time (Figure 5), is intriguingly found only for *C. baldersoni*. A possible explanation for this pattern is that heavier females are more fecund or are closer to

oviposition. Heavier females may mate for longer to ensure fertilization of eggs when oviposition is imminent. An alternative and perhaps more interesting explanation for the positive relationship between female mass and copulation duration is that males copulate for longer and invest more into spermatophores when mating with heavier females, which may represent cryptic male choice (Bonduriansky, 2001). Populations of *C. baldersoni* are generally more dense than those of the other species (unpublished data) so males may be more choosy about how they allocate their sperm and preferentially mate for longer with higher quality females. This may also explain the comparatively shorter copulation duration and spermatophore attachment time of *C. baldersoni* (Figure 1).

To conclude, many biological factors may shape copulation duration and spermatophore attachment time in the genus *Ciulfina*. Experimental manipulation of copulation duration, spermatophore attachment duration and factors such as male density and male/female condition may help to explain the intraspecific variation observed in mating durations for *Ciulfina*. If comparative data can be obtained for a larger number of *Ciulfina* species, this may also help to explain the factors responsible for shaping the range of copulation durations and spermatophore attachment durations observed for individual species. The genus *Ciulfina* is an intriguing new system in which it will be possible to test hypotheses about the function of spermatophore feeding and the significance of copulation duration.

Acknowledgements.

We wish to thank Matthew Bruce, Anne Gaskett, Scott Ginn, Scott Holwell, James O'Hanlon, Kate Barry, Nathan Langelaar and Matt Kovach for assistance in collection, rearing and observation of mantids.

References.

- Alonso-Pimentel, H. and Papaj, D. R. (1999). Resource presence and operational sex ratio as determinants of copulation duration in the fly *Rhagoletis juglandis*. *Anim. Behav.* **57** (5): 1063-1069.
- Andrade, M. C. B. (2003). Risky male mate search and male self-sacrifice in redback spiders. *Behav. Ecol.* **14**(4): 531-538.
- Andres, J. A. and Rivera, A. C. (2000). Copulation duration and fertilization success in a damselfly: an example of cryptic female choice? *Anim. Behav.* **59** (4): 695-703.
- Arnqvist, G. and Rowe, L. (2005). *Sexual conflict*. Princeton: Princeton University Press.
- Bonduriansky, R. (2001). The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* **76**: 305-339.
- Calos, J. B. and Sakuluk, S. K. (1998). Paternity of offspring in multiply-mated female crickets: the effect of nuptial food gifts and the advantage of mating first. *Proc. Roy. Soc. B* **265**: 2191-2195.
- Eberhard, W. G. (1996). *Female control: sexual selection by cryptic female choice*. Princeton: Princeton University Press.
- Edmunds, M. (1975). Courtship, mating and possible sex pheromones in three species of Mantodea. *Ent. Month. Mag.* **111**: 53-57.
- Ehrmann, R. (2002). *Mantodea: Gottesanbeterinnen der Welt*. Natur und Tier Verlag.
- Engqvist, L. and Sauer, K. P. (2001). Strategic male mating effort and cryptic male choice in a scorpionfly. *Proc. Roy. Soc. B* **268**: 729-735.
- Fleischman, R. R. and Sakuluk, S. K. (2004). No direct or indirect benefits to cryptic female choice in house crickets (*Acheta domesticus*). *Behav. Ecol.* **15**(5): 793-798.

- Gwynne, D. T. (1985). Role-reversal in katydids: habitat influences reproductive behaviour (Orthoptera: Tettigoniidae, *Metaballus sp.*). *Behav. Ecol. Sociobiol.* **16**(4): 355-361.
- Gwynne, D. T. (1988). Courtship feeding and the fitness of female katydids (Orthoptera: Tettigoniidae). *Evolution* **42**(3): 545-555.
- Gwynne, D. T. (1997). The evolution of edible sperm sacs and other forms of courtship feeding in crickets, katydids and their kin (Orthoptera: Ensifera). In *The evolution of mating systems in insects and arachnids*: 110-129. Choe, J. C. and Crespi, B. J. (Eds.). Cambridge: Cambridge University Press.
- Hill, P. J. B., Holwell, G. I., Goth, A. and Herberstein, M. E. (2004). Preference for habitats with low structural complexity in the praying mantid *Ciulfina sp.* (Mantidae). *Acta Oecol.* **26**(1): 1-7.
- Holwell, G. I., Barry, K. and Herberstein, M. E. (in review). Mate location, antennal morphology and ecology in praying mantids.
- Hurd, L. E., Prete, F. R., Jones, T. H., Singh, T. B., Co, J. E. and Portman, R. T. (2004). First identification of a putative sex pheromone in a praying mantid. *J. Chem. Ecol.* **30**(1): 155-166.
- Jones, T. M. and Elgar, M. A. (2004). The role of male age, sperm age and mating history on fecundity and fertilization success in the hide beetle. *Proc. Roy. Soc. B* **271**: 1311-1318.
- Kral, K. (1999). Binocular vision and distance estimation. In *The praying mantids*: 114-140. Prete, F. R., Wells, H., Wells, P. H. and Hurd, L. E. (Eds.). Baltimore: The John Hopkins University Press.

- Krebs, R. A. (1991). Function and genetics of long versus short copulations in the cactophilic fruit fly, *Drosophila mojavensis* (Diptera: Drosophilidae). *J. Insect Behav.* **4**(2): 221-233.
- Krupa, J. J. and Sih, A. (1992). Fishing spiders, green sunfish, and a stream-dwelling water strider: male-female conflict and prey responses to single versus multiple predator environments. *Oecologia*: 258-265.
- Kynaston, S. E., McErlainward, P. and Mill, P. J. (1994). Courtship, mating behavior and sexual cannibalism in the praying mantis, *Sphodromantis lineola*. *Anim. Behav.* **47**(3): 739-741.
- Lawrence, S. E. (1992). Sexual cannibalism in the praying mantid, *Mantis religiosa* - a field study. *Anim. Behav.* **43**(4): 569-583.
- Liske, E. and Davis, W. J. (1984). Sexual behaviour of the Chinese praying mantis. *Anim. Behav.* **32**: 916-917.
- Liske, E. and Davis, W. J. (1987). Courtship and mating behaviour of the Chinese praying mantis, *Tenodera aridifolia sinensis*. *Anim. Behav.* **35**: 1524-1537.
- Mathur, R. N. (1946). Notes on the biology of some Mantidae. *Indian J. Ent.* **8**: 89-106.
- Maxwell, M. R. (1998). Lifetime mating opportunities and male mating behaviour in sexually cannibalistic praying mantids. *Anim. Behav.* **55**: 1011-1028.
- Maxwell, M. R. (1999a). Mating behavior. In *The praying mantids*. Prete, F. R., Wells, H., Wells, P. H. and Hurd, L. E. (Eds.). Baltimore: The John Hopkins University Press.
- Maxwell, M. R. (1999b). The risk of cannibalism and male mating behavior in the Mediterranean praying mantid, *Iris oratoria*. *Behaviour* **136**: 205-219.
- Maxwell, M. R. (2000). Does a single meal affect female reproductive output in the sexually cannibalistic praying mantid *Iris oratoria*? *Ecol. Entomol.* **25**(1): 54-62.

- Michiels, N. K. (1992). Consequences and adaptive significance of variation in copulation duration in the dragonfly *Sympetrum danae*. *Behav. Ecol. Sociobiol.* **29**(6): 429-435.
- Moran, M. D. and Hurd, L. E. (1994). Environmentally determined male-biased sex ratio in a praying mantid. *Am. Mid. Nat.* **132**(1): 205-208.
- Perez, B. (2005). Calling behaviour in the female praying mantis, *Hierodula patellifera*. *Physiol. Entomol.* **30**(1): 42-47.
- Perez-Staples, D. and Aluja, M. (2004). *Anastrepha striata* (Diptera: Tephritidae) females that mate with virgin males live longer. *Ann. Ent. Soc. Am.* **97** (6) 1336-1341.
- Prete, F. R., Hurd, L. E., Jones, T., Portman, R., Singh, T. B. and Co, J. E. (2000). Scent of a woman: the first identification and assay of a mantid pheromone. *Am. Zool.* **40**(6): 1180-1180.
- Prokop, P. and Vaclav, R. (2005). Males respond to the risk of sperm competition in the sexually cannibalistic praying mantis, *Mantis religiosa*. *Ethology* **111**(9): 836-848.
- Quesnel, V. C. (1967). Observations on the reproductive behaviour of the mantis, *Acontiothespis multicolor*. *J. Trinidad Field Nat. Club*: 53-56.
- Reinhold, K. (1999). Paternal investment in *Poecilimon veluchianus* bushcrickets: beneficial effects of nuptial feeding on offspring viability. *Behav. Ecol. Sociobiol.* **45**(3-4): 293-299.
- Robinson, M. H. and Robinson, B. (1979). By dawn's early light: matitudinal mating and sex attractants in a neotropical mantid. *Science* **205**(4408): 825-827.
- Roeder, K. D. (1935). An experimental analysis of the sexual behavior of the praying mantis (*Mantis religiosa*, L.). *Biol. Bull.* **69**: 203-220.

- Sakaluk, S. K. and Eggert, A. K. (1996). Female control of sperm transfer and intraspecific variation in sperm precedence: Antecedents to the evolution of a courtship food gift. *Evolution* **50**(2): 694-703.
- Schwagmeyer, P. L. (1988). Scramble-competition polygyny in an asocial mammal: male mobility and mating success. *Am. Nat.* **131**(6): 885-892.
- Simmons, L. W. (1987). Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.* **21**(3): 197-202.
- Simmons, L. W. (2001). *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press.
- Siva-Jothy, M. T. and Tsubaki, Y. (1989). Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae). 1. Alternative mate-securing tactics and sperm precedence. *Behav. Ecol. Sociobiol.* **24**: 39-45.
- Wedell, N. (1991). Sperm competition selects for nuptial feeding in a bushcricket. *Evolution* **145**: 1975-1978.
- Wedell, N. (1993). Spermatophore size in bushcrickets - comparative evidence for nuptial gifts as a sperm protection device. *Evolution* **47**(4): 1203-1212.
- Will, M. W. and Sakaluk, S. K. (1994). Courtship feeding in decorated crickets - is the spermatophylax a sham. *Anim. Behav.* **48**(6): 1309-1315.

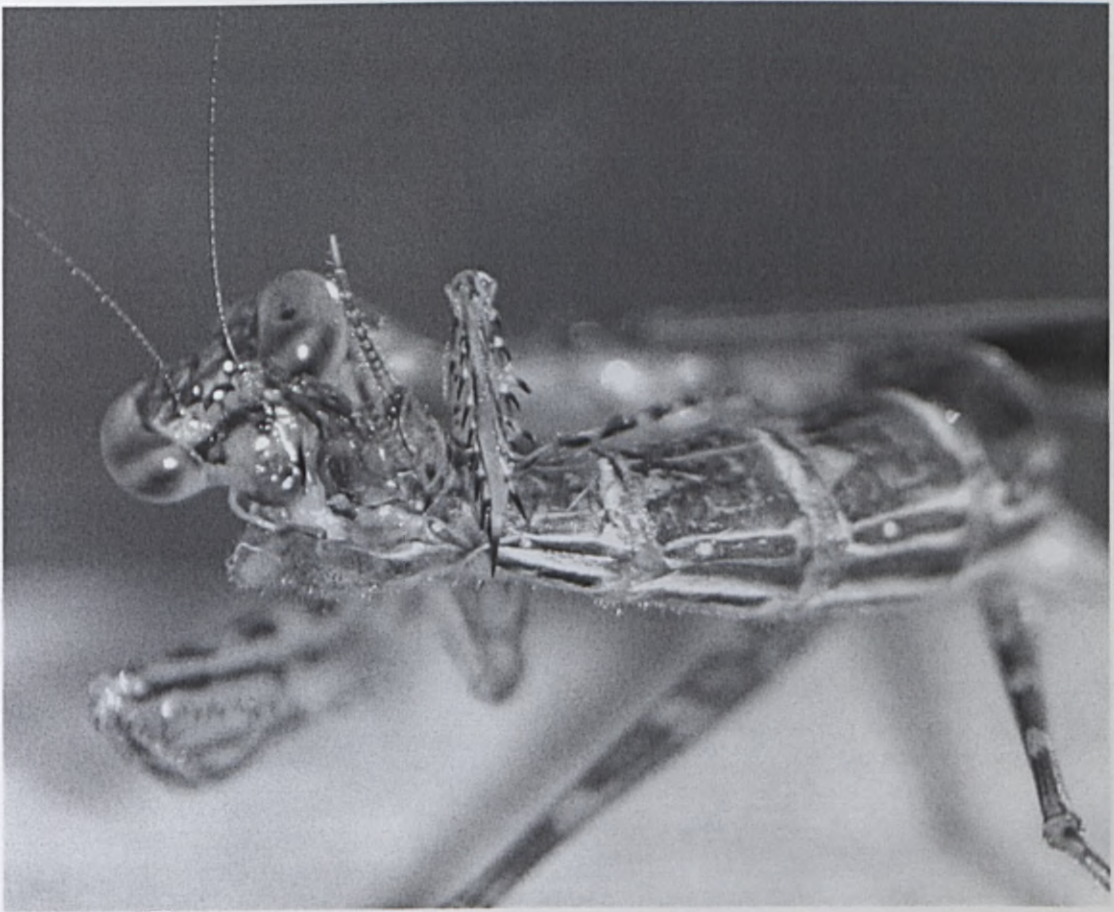


Figure 1: Female *Ciulfina rentzi* removing a spermatophore from her genital opening.

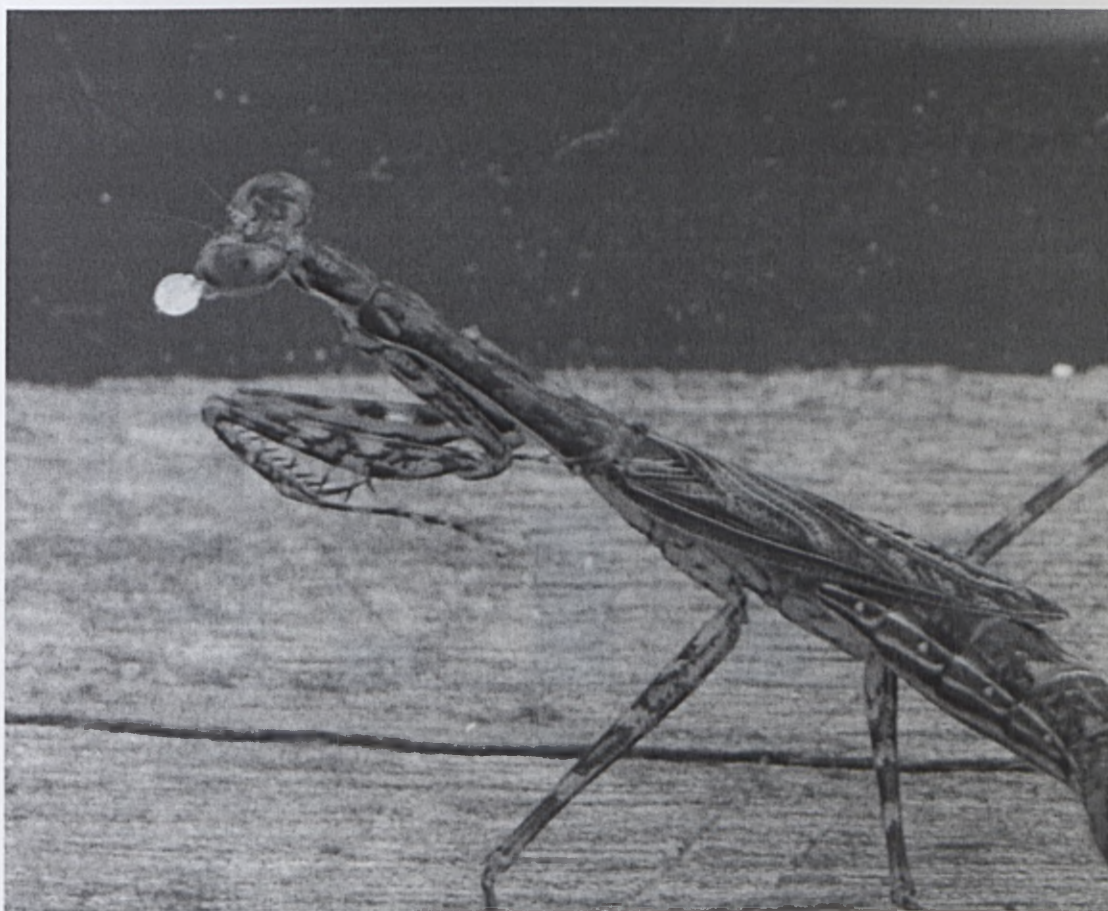


Figure 2: Female *Ciulfina rentzi* feeding on a spermatophore.

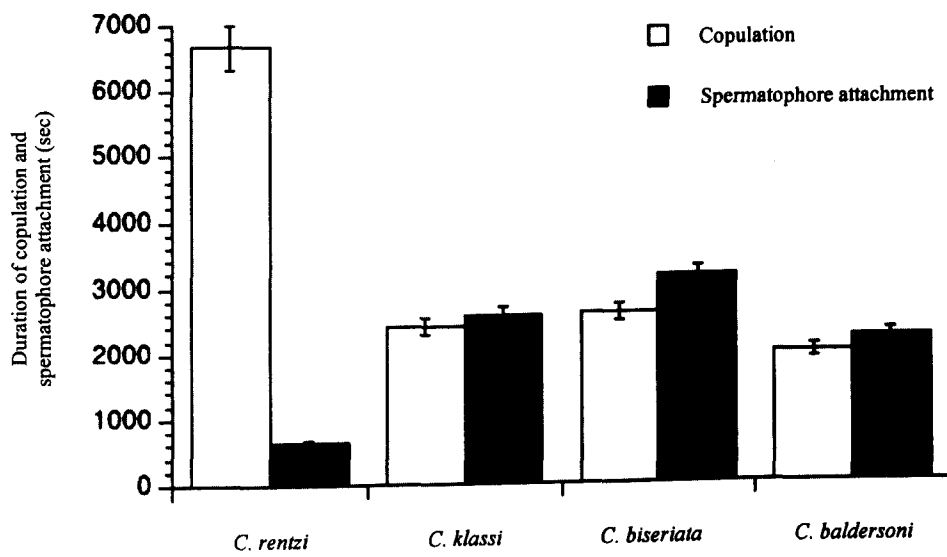


Figure 3: Duration of copulation and spermatophore attachment for four *Ciulfina* species.

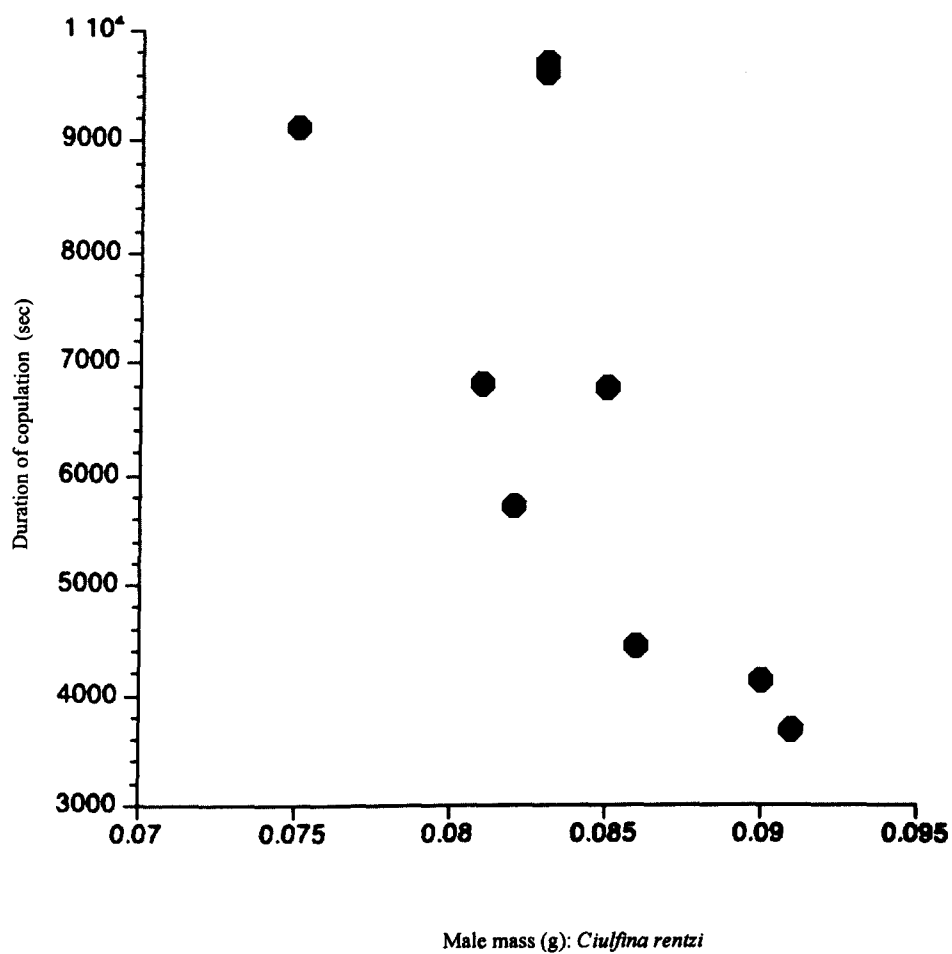


Figure 4: Relationship between male mass (g) and copulation duration (sec) in *Ciulfina rentzi* (R^2 0.537; $F_{1,9}$ 8.128; p = 0.025).

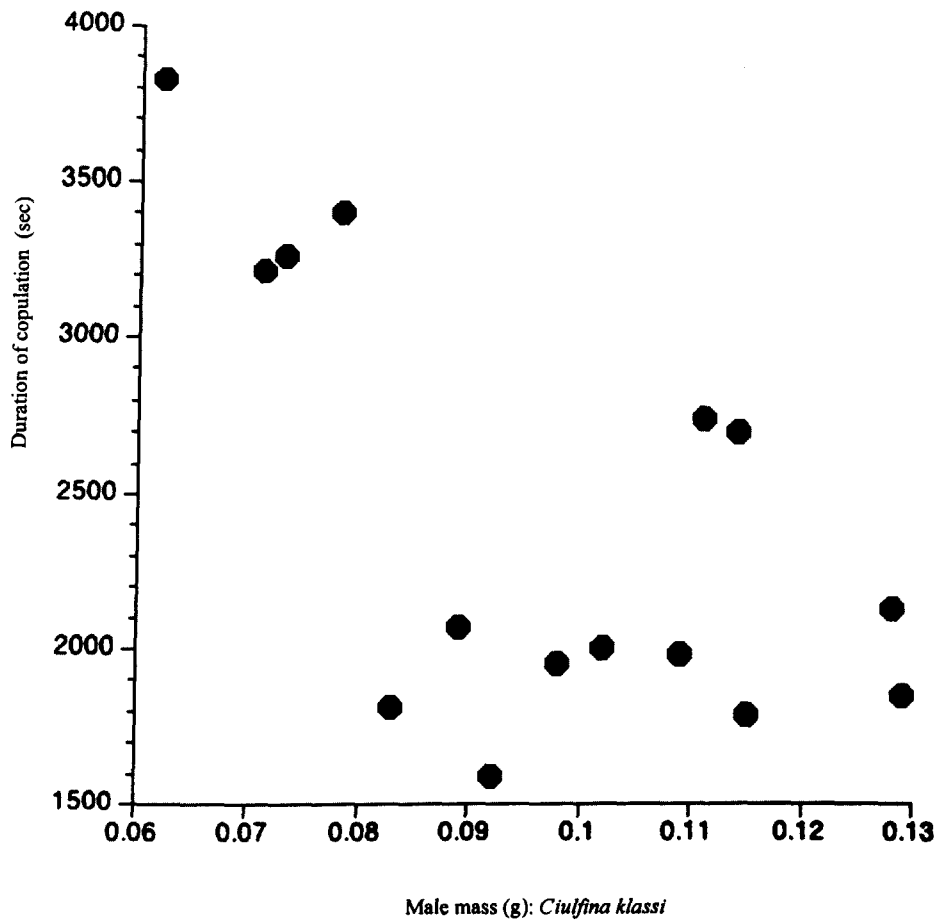


Figure 5: Relationship between male mass (g) and copulation duration (sec) in *Ciulfina klassi* (R^2 0.401; $F_{1,15}$ 8.710; $p = 0.011$).

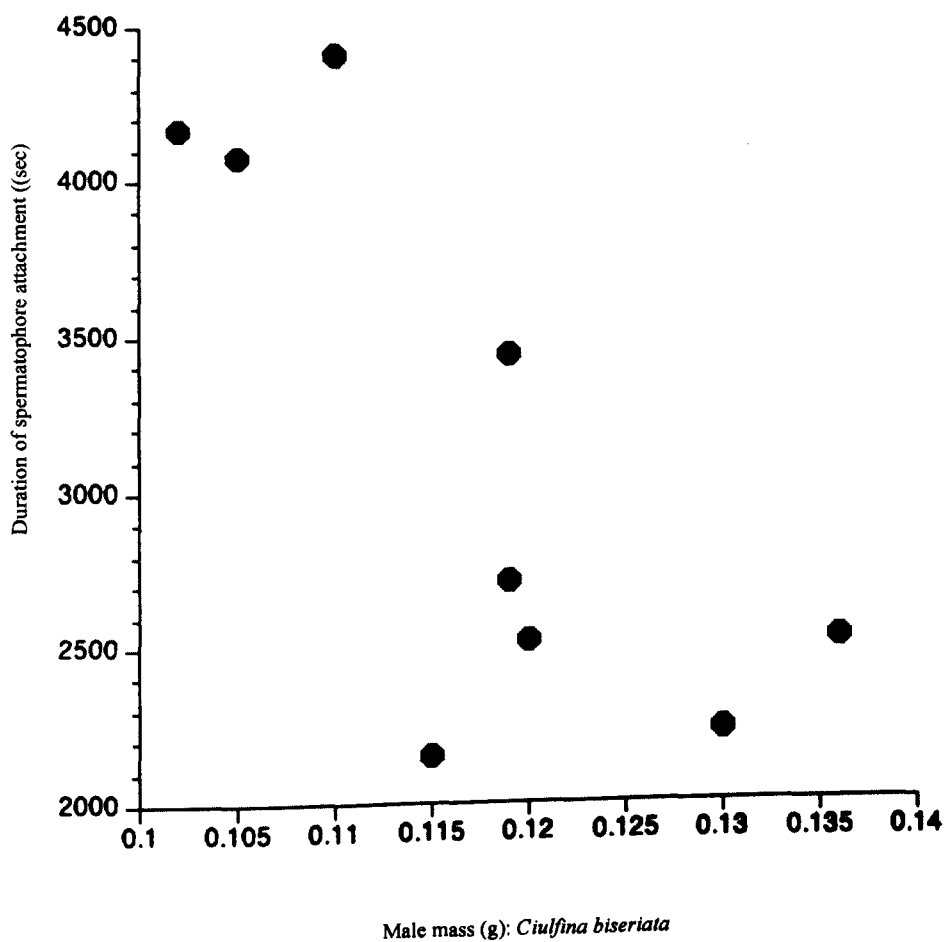


Figure 6: Relationship between male mass (g) and spermatophore attachment duration (sec) in *Ciulfina biseriata* (R^2 0.568; $F_{1,9}$ 9.222; $p = 0.019$).

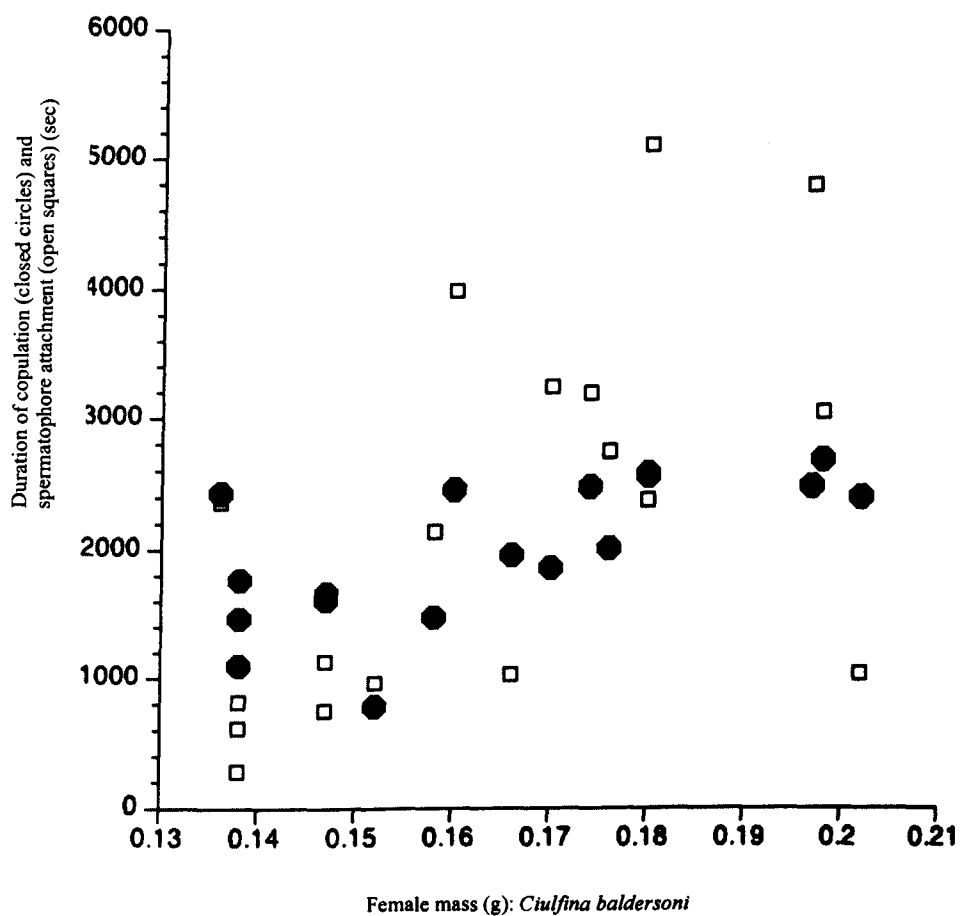


Figure 7: Relationship between female mass (g) and both copulation duration (sec) (filled circles) (R^2 0.429; $F_{1,19}$ 12.033; $p = 0.003$) and spermatophore attachment duration (sec) (unfilled squares) (R^2 0.327; $F_{1,19}$ 7.781; $p = 0.013$) in *Ciulfina baldersoni*.

Table 1: Regression statistics for analyses of the influence of male and female mass on copulation duration and spermatophore attachment duration.

		Copulation duration	Spermatophore attachment
<i>C. rentzi</i>	Male mass	R² 0.537; F_{1,9} 8.128; p = 0.025	R ² 0.007; F _{1,9} 0.049; p = 0.831
	Female mass	R ² 0.155; F _{1,9} 1.288; p = 0.294	R ² 0.013; F _{1,9} 0.089; p = 0.774
<i>C. klassi</i>	Male mass	R² 0.401; F_{1,15} 8.710; p = 0.011	R ² 0.001; F _{1,15} 0.010; p = 0.921
	Female mass	R ² 0.036; F _{1,15} 0.481; p = 0.500	R ² 0.112; F _{1,15} 1.643; p = 0.222
<i>C. biseriata</i>	Male mass	R ² 0.137; F _{1,9} 1.114; p = 0.326	R² 0.568; F_{1,9} 9.222; p = 0.019
	Female mass	R ² 0.069; F _{1,9} 0.523; p = 0.493	R ² 0.100; F _{1,9} 0.777; p = 0.407
<i>C. baldersoni</i>	Male mass	R ² 0.105; F _{1,19} 1.875; p = 0.190	R ² 0.017; F _{1,19} 0.270; p = 0.610
	Female mass	R² 0.429; F_{1,19} 12.033; p = 0.003	R² 0.327; F_{1,19} 7.781; p = 0.013

Chapter Six.

Genital morphology influences copulation duration in a praying mantis *Ciulfina klassi* (Mantodea: Liturgusidae).

Submitted to the journal *Behavioral Ecology & Sociobiology*

Abstract:

The rapid divergence of male genitalia is a widely observed evolutionary pattern. Although sexual selection is currently acknowledged as providing the most likely driving force behind genital diversification, the exact mechanisms responsible are still currently debated. Here we investigate the relationship between male genital morphology and copulation duration in the praying mantid *Ciulfina klassi*. We also address the issue of relative male and female influence over copulation duration using a cross-mating experiment between *C. klassi* and the congeneric *C. rentzi*. Both the size and shape of male genitalia in *C. klassi* were found to influence copulation duration, suggesting that genital morphology is under sexual selection for this species. Males species identity was shown to significantly influence copulation duration in heterospecific crosses, whereas female species identity had no such influence. These results show males to exhibit greater influence over copulation duration than females in *Ciulfina* praying mantids.

Introduction:

The morphology of male genitalia can be elaborate and diverse, even among closely related species. This is one of the most widely observed evolutionary patterns for animals with internal fertilization and promiscuous mating systems (Arnqvist, 1998; Arnqvist, 1997). Three general hypotheses to explain the rapid divergent evolution of genitalia have been debated: the species isolation, pleiotropy and sexual selection hypotheses (Eberhard, 1985). Today it is widely supported that sexual selection is responsible for the general pattern of rapid genital evolution (Arnqvist, 1998; Hosken & Stockley, 2004; House & Simmons, 2003), although the role of genitalia in species isolation is acknowledged for some species (Sota & Kubota, 1998; Usami *et al.*, 2006). The mechanisms involved in sexual selection on genital traits, however is unclear (Hosken & Stockley, 2004). Among the possible mechanisms are (1) sperm competition (Simmons, 2001; Waage, 1979), (2) female choice, either through Fisherian or good genes models of selection (Eberhard, 1993), and (3) sexual conflict generated by opposing selection between male and female reproductive strategies (Arnqvist & Rowe, 2005; Crudgington & Siva-Jothy, 2000). Distinguishing between the potential mechanisms is difficult (Hosken & Stockley, 2004), reflecting current difficulties in distinguishing between mechanisms of sexual selection in a range of contexts. Recent models (Gavrilets, 2000) and comparative data accounting for phylogeny (Arnqvist *et al.*, 2000) support higher rates of speciation driven by sexual conflict. As the majority of animal taxa are distinguished on the basis of genital morphology, these studies support sexual conflict as the driving mechanism behind genital divergence. Conversely, some large-scale comparative surveys (Eberhard, 2004a; Eberhard, 2004b; Eberhard, 2006) dismiss sexual conflict, supporting models of female choice.

One possible direction towards understanding the mechanisms involved is to establish the degree of influence that males and females have over behaviours associated with reproductive success, such as mating success, mate rejection, sperm transfer, copulation duration, mate-guarding and sperm removal or ejection. Although many of these involve interactions between the sexes, sometimes a greater degree of control can be accredited to either males or females (Krebs, 1991; Vepsäläinen & Savolainen, 1995). If, for example, females have complete control over mate acceptance, then traits associated with male mating success are more likely to be influenced by sexual selection through female choice. Demonstrating relative male and female influences on copulation duration and spermatophore attachment time however, can be difficult (Simmons, 2001). While male control of copulation duration is often inferred from behavioural observations, the extent to which females are compliant is rarely discussed. Previously, cues known to influence copulation duration and postcopulatory mate guarding have been differentially manipulated to determine relative male and female contributions (Vepsäläinen & Savolainen, 1995). Additionally, an alternative approach used different population strains with known copulation duration differences and determined the relative male and female influence through a cross-mating study (Krebs, 1991a). Although this approach is rarely utilized to explore variation in copulation duration, addressing the issue of control through cross-mating studies provides convincing data on male and female influences on mating behaviour.

Ciulfina praying mantids are small fast-moving mantids found on the bark of trees in northern Australia. Males stealthily approach females, mount from behind and leave an external spermatophore attached to the female genital opening after copulating. Females later remove the spermatophore with their mouthparts and consume it. Copulation duration

is variable between and within species and can potentially influence male reproductive success by (a) influencing the number of sperm transferred, (b) increasing copulatory mate-guarding and reducing the opportunity for other males to mate with the female, or (c) increasing the duration of copulatory courtship.

The present study tests the hypothesis that male genital morphology influences copulation duration in *C. klassi*, and uses a between-species cross-mating approach to assess the relative male and female contributions to copulation duration in two species: *C. klassi* and *C. rentzi*.

Methods:

Collection and housing

Populations of *Ciulfina klassi* and *Ciulfina rentzi* were identified and collection took place in January, 2005. *Ciulfina klassi* (35 male, 35 female) were collected from eucalypt woodland, approximately 30 km south of Cardwell (Queensland, Australia, 18°31'S 146°11'E). *Ciulfina rentzi* (25 male, 25 female) were collected from tropical rainforest at approximately 10km west of Babinda (Queensland, Australia, 17°20'S 146°07'E). *C. rentzi* appeared to exist at much lower densities and fewer were collected. All mantids were collected as sub-adult nymphs and housed individually in the laboratory in inverted plastic cups (10cm diameter) containing bark to perch on and a fabric window to allow airflow. They were fed *Drosophila melanogaster* three times per week and watered daily. Mantids were collected under permit ATH 05/006 from the Queensland Parks and Wildlife Service and the Environmental Protection Authority.

Mating observations

Observations were made of mating behaviour in a semi-natural laboratory environment. The mating arena consisted of a tree trunk, sawn in half and screwed onto a Perspex® board. This prevented mantids from moving out of the observer's range of view. Females were introduced to the tree trunk ten minutes before males to allow them to adjust to the novel environment. Mantids were observed throughout any proceeding interactions and copulation duration / attachment duration of the spermatophore prior to female removal were recorded using a stopwatch. Copulation duration was defined as the time when genitalic contact between the pair was made to the time when the male removed his genitalia from the female genital opening and dismounted. Postcopulatory spermatophore attachment duration was defined as the time when copulation ended to the time when the female removed the spermatophore from her genital opening.

Control of copulation duration

To assess relative male and female influence over copulation duration, individual *Ciulfina* were randomly paired into the following groups:

Conspecific pairings:

Male *Ciulfina rentzi* × female *Ciulfina rentzi* (n = 10)

Male *Ciulfina klassi* × female *Ciulfina klassi* (n = 20)

Heterospecific pairings:

Male *Ciulfina rentzi* × female *Ciulfina klassi* (n = 10),

Male *Ciulfina klassi* × female *Ciulfina rentzi* (n = 10),

There were more pairs in the conspecific pairing of *Ciulfina klassi* due to a larger number of mantids collected from the field. Pairs were placed together on the mating arena as described above. Copulation duration was recorded using a stopwatch.

Preparation of genitalia and geometric morphometrics

After copulation, males were euthanased by freezing and genitalia were dissected out and submerged in 10% KOH for one hour to dissolve the muscle and fat bodies. The two major phallomeres from the male genitalic complex (hypophallus and left epiphallus) were removed and placed in glycerol on glass slides. Other components of the genitalic complex were not used as they did not possess any structures that would be suitable for landmark-based geometric morphometrics. Digital images were taken of each genital phallomere using a Moticam 480[®] microscope-mounted camera. These images were then imported into the tpsDig2 (Rohlf, 2004) digitization program.

Landmark-based geometric morphometric analysis was used to quantify the variation in genital shape for both the hypophallus and the left epiphallus of *C. klassi*. Compared to traditional morphometrics which analyses variation in pre-determined linear measurements of morphological structures, landmark-based geometric morphometrics assigns landmarks to recognisably equivalent points. Analyses of total morphological variation between samples are then based on the relative positions of assigned landmarks, after the images are normalised for position, orientation and scale. For this study, five landmarks were recorded onto the dorsal view of the hypophallus (Figure 1) and seven landmarks were recorded onto a dorsal view of the left epiphallus (Figure 2) for each male. Landmarks were essentially type-two landmarks representing extremes of curvature around the shape of

these genital structures. Landmarks were superimposed onto images using the program tpsDig2 (Rohlf, 2004). Variation in the position of the X-Y coordinates of landmarks for all samples of both the hypophallus and left epiphallus were analysed using the program tpsRelw (Rohlf, 1997). This program normalised all specimens for position, orientation and scale, so as to remove 'non-shape variation' (Adams *et al*, 2004) and reduced the data to a series of relative warp scores (RWS) using a principal components analysis. tpsRelw also allowed us to visualise variation in the shape of each genital component as shape deformations of the thin-plate-spline (Zelditch *et al.*, 2004) so as to compare the genitalia of males that copulated for longer or shorter durations. Centroid size, which is the square root of the sum of squared distances of landmarks from the centroid, was calculated using tpsRelw (Rohlf, 1997) for both the hypophallus and left epiphallus and was used as a measure of genital size in further analyses. A detailed mathematical explanation of geometric morphometrics, along with general concepts and methods can be found in Rohlf and Marcus (1993), Adams *et al* (2004) and Zelditch *et al* (2004).

Statistical analyses

Data were checked for normal distribution and homogeneity of variance prior to analyses. We used a general linear model to analyse the effects of male mass, female mass, hypophallus morphology (centroid size and RWS 1-4) and left epiphallus morphology (centroid size and RWS 1-5) on copulation duration for *C. klassi*. RWS 1-4 for the hypophallus represented 100% of its morphological variation, while RWS 1-5 for the left epiphallus represented 96.5% of the morphological variation for this genital structure. RWS 1, 2 and 5 for the left epiphallus were removed from the final model due to collinearity.

A t-test was used to compare intraspecific copulation duration between *C. klassi* and *C. rentzi*. ANOVA was used to determine the effect of male and female species identity on copulation duration in cross-mating experiments between *C. klassi* and *C. rentzi*. These analyses were conducted using SPSS 11 for Mac OS X.

Results:

Genital morphology and copulation duration

Overall the generalized linear model was significant ($F_{9,15} = 11.06$; $p < 0.01$, $R^2 = 0.957$, see Table 1) explaining more than 95% of the variation in copulation duration. Analysis of the model indicated that male genital morphology significantly explained the variation in copulation duration for *C. klassi*. The two variables contributing significantly to the overall model were the centroid size ($F_{1,15} = 2.892$; $p < 0.05$) and relative warp score 3 ($F_{1,15} = 2.736$; $p < 0.05$) for the left epiphallus (Table 1). Analysis using tpsRelw (Rohlf, 1997) indicated that RWS 3 for the left epiphallus represented 15.6% of the overall morphological variation in this structure. Partial plots of left epiphallus size and shape (RWS 3) against copulation duration are presented in Figures 3 & 4. Variation in the left epiphallus shape of males that copulated for short and long durations were observable as deformations of the thin-plate-spline grid and are presented in Figure 3.

Male and female influence over copulation duration

Copulation duration of *Ciulfina rentzi* was significantly longer (two-tailed t-test: $t_{9.56} = -6.24$, $p < 0.0001$) than that of *Ciulfina klassi*. These results indicate a major difference in reproductive behaviour between the two species studied.

The corrected model for the interaction between copulation duration and male/female species identity was highly significant ($F_{3,45} = 33.58$; $p < 0.001$). Specifically, copulation duration was highly significantly influenced by male species identity ($F_{1,45} = 96.6$; $p < 0.001$) and significantly influenced by the interaction between male and female species identity ($F_{1,45} = 6.17$; $p < 0.05$). However the species identity of the female did not influence copulation duration ($F_{1,45} = 0.216$; $p = 0.645$). Mean copulation for intraspecific pairs and heterospecific crosses between *C. rentzi* and *C. klassi* are presented in Figure 5.

Discussion.

Genital morphology and copulation duration

Our results show a direct correlation between male genital morphology and copulation duration in *C. klassi*. Significant contributors to the linear model were left epiphallus size (centroid area) and relative warp score three describing left epiphallus shape. Generally, the noticeable genital shape difference involved a broadening of the most distal region of the left epiphallus in males who copulated for longer durations (Figure 3). The relationship between genital size and copulation duration was more easily interpreted: males possessing a larger left epiphallus copulated for longer durations. Other recent studies have shown the effect of sexual selection on genital morphology by demonstrating a relationship between male genital morphology and fertilization success. A direct influence of sexual selection on genital morphology has been shown for the water striders *Gerris lateralis* (Arnqvist & Danielsson, 1999) *Gerris lacustris* (Danielsson & Askenmo, 1999) and *Aquarius remigis* (Bertin & Fairbairn, 2005), the dung beetle *Onthophagus taurus* (House & Simmons, 2003; House & Simmons, 2005; House & Simmons, 2006), the seed bug *Lygaeus simulans* (Tadler, 1999) and the fly *Dryomyza anilis* (Otronen, 1998).

Although correlations with fertilization success provide the most unambiguous evidence for the effects of sexual selection in promiscuous species, more indirect measures can also be useful. Correlations between male traits and copulation duration (Michiels, 1992), sperm transfer (Svensson *et al.*, 1996) and female remating rate (Singh *et al.*, 2002) are often used to infer sexual selection on such traits. Copulation duration has been strongly correlated to fertilization success in many species [reviewed in (Simmons, 2001)] and the relationship between genital morphology and copulation duration we have described for *C. klassi* is a convenient indirect measure of the action of sexual selection on genital morphology in these mantids. However, it is important to consider the function of copulation duration in *Ciulfina*. Variation in copulation duration may represent variation in: (1) male ejaculate investment and the amount of sperm transferred (Wedell *et al.*, 2002), (2) the duration of copulatory mate-guarding (Carroll, 1991), and/or (3) the duration of copulatory courtship (Eberhard, 1994). Each of these possible functions however, suggests that the optimisation of copulation duration would benefit male reproductive success and would be under sexual selection (Simmons, 2001). The relationship shown between the genital morphology of male *C. klassi* and copulation duration therefore suggests that males possessing a larger, broader left epiphallus either (a) invest more into their spermatophore, (b) attach themselves for longer periods of copulatory mate-guarding, (c) stimulate females for longer durations through copulatory courtship or (d) require longer to successfully produce and deliver a spermatophore. Further experimental work is required to distinguish between these mechanisms and to determine the relationship between genital morphology, copulation duration and fertilization success.

Male control of copulation duration

Cross-mating experiments have revealed that male *Ciulfina* have a greater influence over copulation duration than females. Such cross-mating studies have previously been used to determine levels of male and female influence over copulation duration (Krebs, 1991). The most dominant influence over copulation duration was male species identity, although the interaction between male and female species identity was also significant as overall, heterospecific pairings were shorter than conspecific pairings. However, female identity did not contribute to the variation in copulation duration. This suggests that female *Ciulfina* have little control over copulation duration.

An alternative explanation of these patterns is that different levels of reproductive isolation between the species could produce these results, which is supported by the significant interaction between male and female species identity. However, this does not explain why female species identity does not have any influence over copulation duration. In fact, previous studies have shown that the costs of mismating with incompatible species are likely to be higher for females than for males (Sota & Kubota, 1998). Thus, if reproductive isolation were responsible for the differences in copulation duration between heterospecific and conspecific matings, we would expect that female identity would have the greater influence. Therefore, it is more likely that these differences are due to male effects rather than mismating effects.

With the understanding that males control copulation duration in *Ciulfina*, we can reinterpret the relationship between copulation duration and male genital morphology. Two potential explanations now seem relevant:

(1) Males possessing larger, broader left epiphalli are able to copulate for longer durations because their genital morphology allows them to remain attached to females for

longer and possibly deposit a larger ejaculate. This would support sexual selection via sperm competition as the mechanism for rapid genital evolution.

(2) Males possessing larger, broader left epiphalli are able to invest more time into copulatory courtship and are better stimulators. This would support sexual selection via female choice as the mechanism driving genitalic divergence.

Determining between these hypotheses may prove difficult. However, closer examination of patterns of sperm allocation and investigation into the functional morphology of *Ciulfina* genitalia, are potential paths to further elucidation of this intriguing system.

Acknowledgements:

We gratefully thank Anne Gaskett, Matt Bruce, Scott Holwell, Scott Ginn, James O'Hanlon and Matt Kovach for assistance with field collection and animal maintenance. Special thanks also go to Goran Arnqvist, Luke Finley and James Rohlf for morphometric advice, and to Ray Cameron and Peter Tung for assistance with digital imaging. Funding was provided by the Australian Research Council and Macquarie University.

References:

- Adams, D. C., Rohlf, F. J. & Slice, D. E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Ital. J. Zool.*, 71: 5-16
- Arnqvist, G. 1997. The evolution of animal genitalia: distinguishing between hypotheses by single species studies. *Biol. J. Linn. Soc.*, 60, 365-379.

- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature*, 393, 784-786.
- Arnqvist, G. & Danielsson, I. 1999. Copulatory behavior, genital morphology and male fertilization success in water striders. *Evolution*, 53, 147-156.
- Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. 2000. Sexual conflict promotes speciation in insects. *PNAS*, 97, 10460-10464.
- Arnqvist, G. & Rowe, L. 2005. *Sexual conflict*. Princeton: Princeton University Press.
- Bertin, A. & Fairbairn, D. J. 2005. One tool, many uses: precopulatory sexual selection on genital morphology in *Aquarius remigis*. *J. Evol. Biol.*, 18, 949-961.
- Carroll, S. P. 1991. The adaptive significance of mate guarding in the soapberry bug, *Jadera haematoloma* (Hemiptera: Rhopalidae). *J. Insect. Behav.*, 4, 509-530.
- Crudgington, H. S. & Siva-Jothy, M. T. 2000. Genital damage, kicking and early death - the battle of the sexes takes a sinister turn in the bean weevil. *Nature*, 407, 855-856.
- Danielsson, I. & Askenmo, C. 1999. Male genital traits and mating interval affect male fertilization success in the water strider *Gerris lacustris*. *Behav. Ecol. Sociobiol.*, 46, 149-156.
- Eberhard, W. G. 1985. *Sexual selection and animal genitalia*. Harvard University Press.
- Eberhard, W. G. 1993. Evaluating models of sexual selection - genitalia as a test-case. *Am. Nat.*, 142, 564-571.
- Eberhard, W. G. 1994. Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution*, 48, 711-733.
- Eberhard, W. G. 2004a. Male-female conflict and genitalia: failure to confirm predictions in insects and spiders. *Biol. Rev.*, 79, 121-186.

- Eberhard, W. G. 2004b. Rapid divergent evolution of sexual morphology: Comparative tests of antagonistic coevolution and traditional female choice. *Evolution*, 58, 1947-1970.
- Eberhard, W. G. 2006. Sexually antagonistic coevolution in insects is associated with only limited morphological diversity. *J. Evol. Biol.*, doi: 10.1111/j.1420-9101.2005.01057.x.
- Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature*, 403, 886-889.
- Hosken, D. J. & Stockley, P. 2004. Sexual selection and animal genitalia. *TREE*, 19, 87-93.
- House, C. M. & Simmons, L. W. 2003. Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc. R. Soc. Lond. B*, 270, 447-455.
- House, C. M. & Simmons, L. W. 2005. Relative influence of male and female genital morphology on paternity in the dung beetle *Onthophagus taurus*. *Behav. Ecol.* 16, 889-897.
- House, C. M. & Simmons, L. W. 2006. Offensive and defensive sperm competition roles in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabeidae). *Behav. Ecol. Sociobiol.* DOI 10.1007/s00265-005-0149-x
- Krebs, R. A. 1991. Function and genetics of long versus short copulations in the cactophilic fruit fly, *Drosophila mojavensis* (Diptera: Drosophilidae). *J. Insect Behav.*, 4, 1991.
- Michiels, N. K. 1992. Consequences and adaptive significance of variation in copulation duration in the dragonfly *Sympetrum danae*. *Behav. Ecol. Sociobiol.*, 429-435.

- Otronen, M. 1998. Male asymmetry and postcopulatory sexual selection in the fly *Dryomyza anilis*. *Behav. Ecol. Sociobiol.*, 42, 185-191.
- Rohlf, F. J. & Marcus, L. F. 1993. A revolution in morphometrics. *TREE* 8 (4) 129 -134.
- Rohlf, F. J. 1997. *tpsRelw - Relative warp analysis* (Windows) version 1.09. New York: Department of Ecology and Evolution, State University of New York.
- Rohlf, F. J. 2004. *TpsDig2*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.
- Simmons, L. W. 2001. *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press.
- Singh, S. R., Singh, B. N. & Hoenigsberg, H. F. 2002. Female remating, sperm competition and sexual selection in *Drosophila*. *Genet. Mol. Res.*, 1, 178-215.
- Sota, T. & Kubota, K. 1998. Genital lock-and-key as a selective agent against hybridization. *Evolution*, 52, 1507-1513.
- Svensson, B. G., Peterson, E. & Frisk, M. 1996. Nuptial gift size prolongs copulation duration in the dance fly *Empis borealis*. *Ecol. Entomol.*, 15, 225-229.
- Tadler, A. 1999. Selection of a conspicuous male genitalic trait in the seedbug *Lygaeus simulans*. *Proc. Roy. Soc. Lond. B*, 266, 1773-1777.
- Usami, T., Yokoyama, J., Kubota, K. & Kawata, M. 2006. Genital lock-and-key system and premating isolation by mate preference in carabid beetles (*Carabus* subgenus *Ohomopterus*). *Biol. J. Linn. Soc.*, 87, 145-154.
- Vepsäläinen, K. & Savolainen, R. 1995. Operational sex ratios and mating conflict between the sexes in the water strider *Gerris lacustris*. *Am. Nat.*, 146, 869-880.
- Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. *Science*, 203, 916-918.

- Wedell, N., Gage, M. J. G. & Parker, G. A. 2002. Sperm competition, male prudence and sperm-limited females. *TREE*, 17, 313-320.
- Zelditch, M. L., Swiderski, D. L., Sheets, H. D. & Fink, W. L. 2004. *Geometric morphometrics for biologists: a primer*. San Diego: Elsevier Academic Press.

Table 1. General linear model of the effects of male and female mass, male genital size (CS) and male genital shape (RWS) on copulation duration in *C. klassi*.

	<i>B</i>	<i>S. E.</i>	<i>t</i>	<i>P</i>
Intercept	-16037.8	9697.0	-1.654	.159
Male Mass	-6064.7	15501.1	-.391	.712
CS Hypophallus	7.7	24.4	.316	.765
RWS1 Hypophallus	-7195.7	6394.9	-1.125	.312
RWS2 Hypophallus	8155.3	6033.9	1.352	.234
RWS3 Hypophallus	-27135.3	12425.4	-2.184	.081
RWS4 Hypophallus	59789.4	41080.9	1.455	.205
CS Left epiphallus	41.8	14.4	2.892	.034
RWS3 Left epiphallus	71366.2	26087.2	2.736	.041
RWS4 Left epiphallus	18232.2	10385.4	1.756	.140
Female mass	3404.4	2426.3	1.403	.220

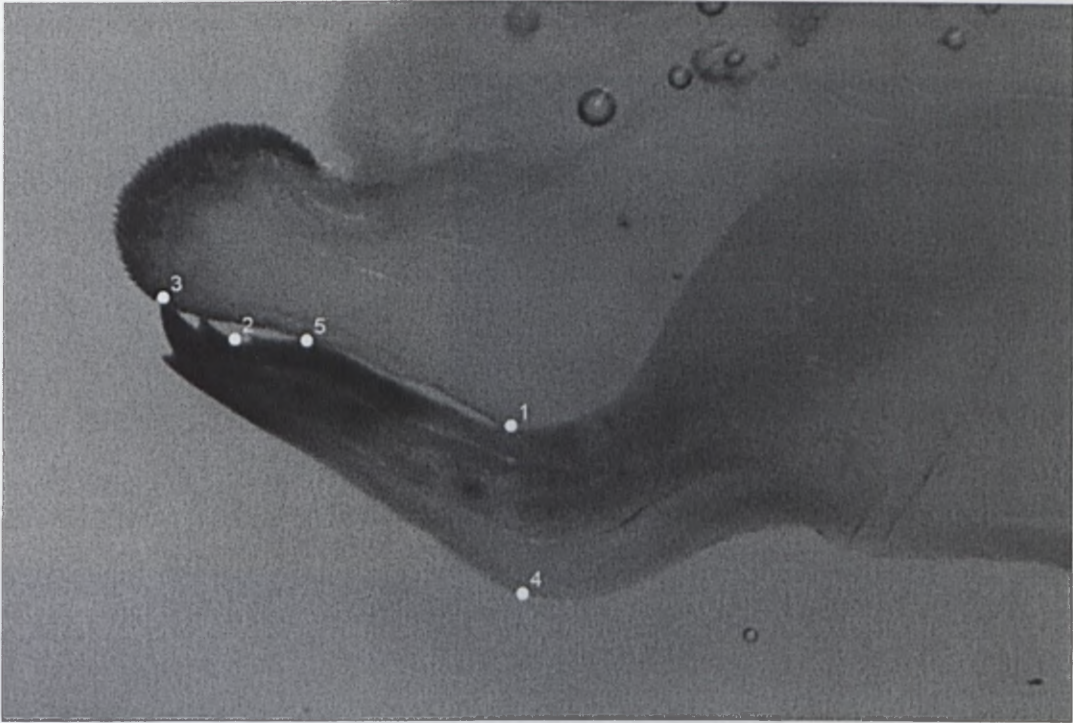


Figure 1. Position of landmarks 1-5 on the hypophallus.

Figure 2. Position of landmarks 1-5 on the left epiphallus.

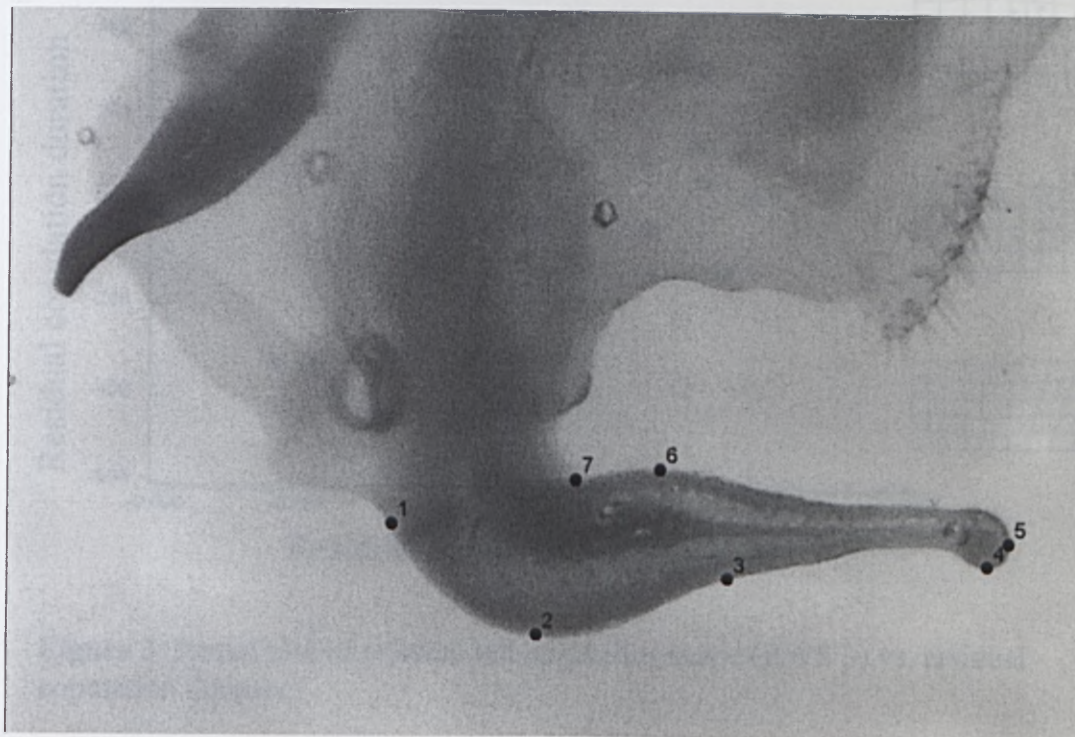


Figure 2. Position of landmarks 1-7 on the left epiphallus.

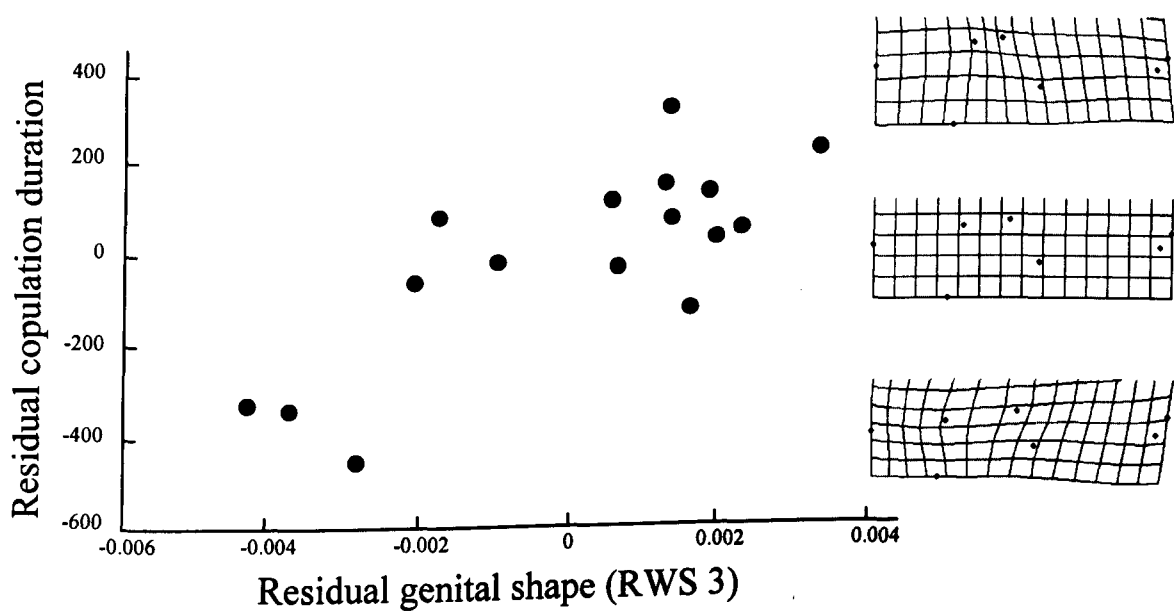


Figure 3. Partial plot of residual left epiphallus shape (RWS 3) vs. residual copulation duration.

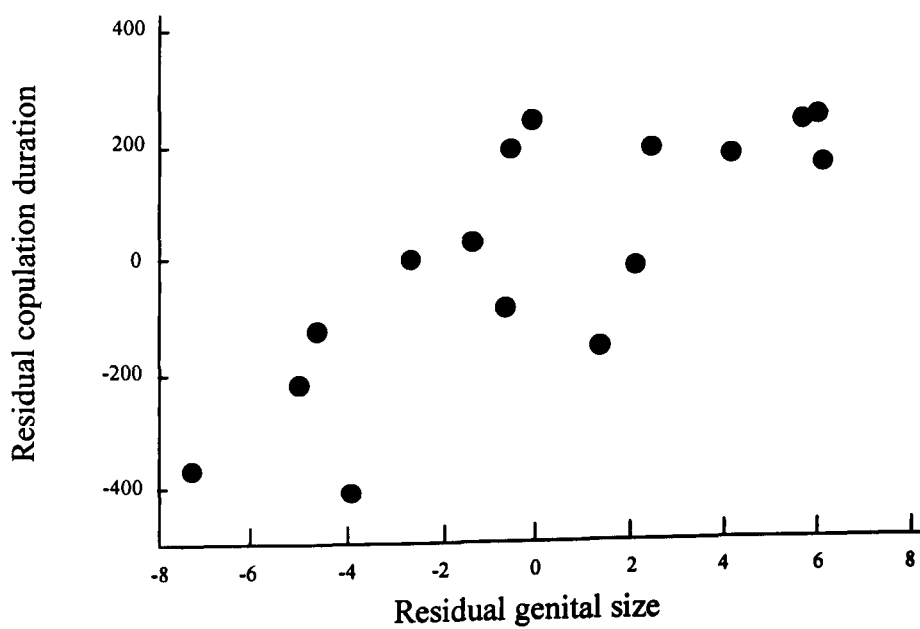


Figure 5. Residual left epiphallus size (centroid area) vs. residual copulation duration.

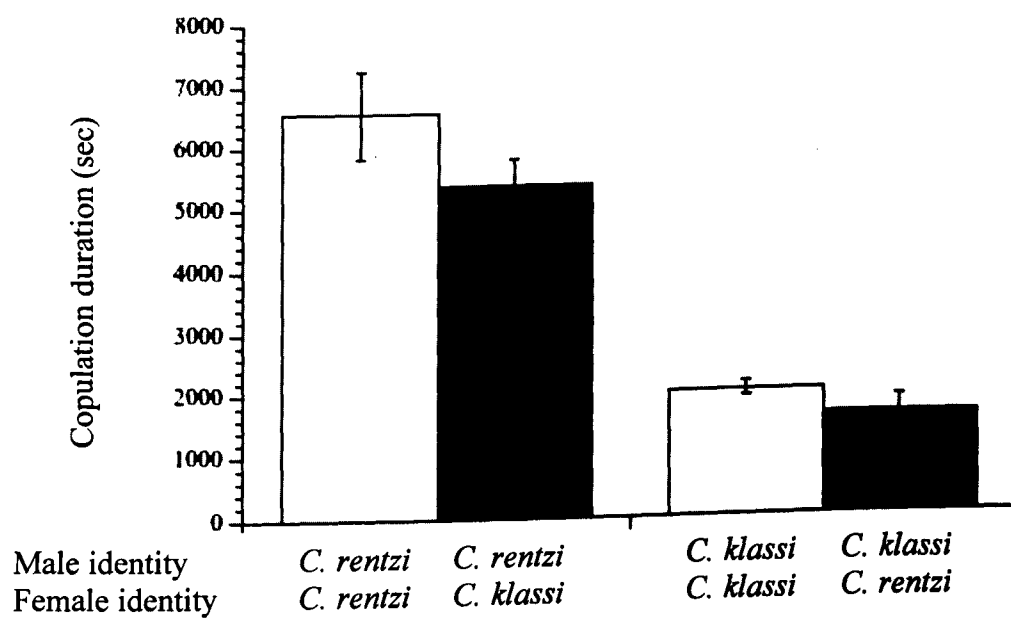


Figure 6. Copulation duration for *C. rentzi*, *C. klassi* and for heterospecific crosses between the two species. Columns represent means with standard errors.

Conclusions

The Ciulfina system

Perhaps the most significant finding of this research is the identification of the *Ciulfina* system as a model with which to investigate a number of currently important questions in behavioural ecology, animal morphology, ecology and evolutionary biology. Questions that can be addressed with further study of *Ciulfina* genitalia relate to the evolution and development of insect genital morphology, the significance of genital morphology for studies of postcopulatory sexual selection, and the maintenance of genital polymorphism.

Further study of *Ciulfina* mating behaviour may also be useful in furthering our understanding of the ecology of mate searching, the significance of spermatophore feeding and general mantid mating biology in the absence of sexual cannibalism. Finally, this system is likely to be a useful model with which to test current theories about the evolutionary ecological significance of parapatric distributions.

Genital morphology & evolution

The main area of evolutionary biology that this research contributes to is the ever-growing interest in the evolution of genitalia. The majority of recent literature on this topic (Arnqvist *et al.*, 1997; Arnqvist, 1998; Arnqvist & Danielsson, 1999; Cordoba-Aguilar, 1999; Danielsson & Askenmo, 1999; Tadler, 1999; Crudgington & Siva-Jothy, 2000; Arnqvist & Rowe, 2002; Stockley, 2002; Fairbairn *et al.*, 2003; House & Simmons, 2003; Sirot, 2003; Takami, 2003; Cordero Rivera *et al.*, 2004; Eberhard, 2004a; Eberhard, 2004b; Hosken & Stockley, 2004; Bertin & Fairbairn, 2005; Cordoba-Aguilar, 2005;

House & Simmons, 2005a; House & Simmons, 2005b) supports Eberhard (1985) and acknowledges the role of sexual selection in driving the rapid and divergent evolution of genitalic (particularly male) traits, and their extraordinary morphological elaboration. While some role for genitalia in species-isolation is recognised for some groups of taxa (Sota & Kubota, 1998; Usami *et al.*, 2006), it is now widely disregarded as the driving force in rapid genital evolution. Results from the *Ciulfina* system lend further support to the sexual selection hypothesis. The general level of geographic variation found in genital shape and size for the four species of *Ciulfina* studied typifies the patterns of divergence you would expect due to sexual selection. The species isolation hypothesis was rejected as an explanation for genital divergence in *Ciulfina* due to the variation between pairs of populations that did not differ in their risk of mating with heterospecifics. Previous studies of carabid beetles (Sota & Kubota, 1998; Usami *et al.*, 2006) and millipedes (Tanabe *et al.*, 2001) have compared the levels of premating and mechanical isolation, and the imposed costs of heterospecific copulations between pairs of species that coexisted sympatrically and pairs of species that had no distributional overlap (either allopatric or parapatric pairs). However, variation exists between the genital morphology of both allopatric pairs and sympatric pairs, suggesting that species-isolation has not driven the initial divergence of these groups, and that other non-genital mechanisms must be associated with reproductive isolation in these groups. Despite the usefulness of studies of geographic variation for our understanding of the mechanisms of genital divergence, very few studies have investigated the phenomenon. Those studies that have attempted to describe and quantify geographic variation have generally found it in diverse taxa from snakes (Inger & Marx, 1962) to snails (Madec & Guillar, 1994), mosquitoes (Hribar, 1994) and guppies (Kelly *et al.*, 2000). Unravelling the processes leading to geographic variation in genitalia may be an

important future avenue for studies of genital evolution. In this respect, *Ciulfina* is a good candidate for more intensive study of geographic genital variation.

Additionally, the relationship between male genital morphology and copulation duration in *C. klassi* is intriguing and certainly worthy of future attention. Studies of the relationship between genitalic traits and reproductive success (and processes that influence reproductive success) are currently leading the way in supporting the sexual selection hypothesis of genital evolution (Arnqvist & Danielsson, 1999a; Danielsson & Askenmo, 1999; House & Simmons, 2003; House & Simmons, 2006; Otronen, 1998; Tadler, 1999), and will be likely to play an important role in distinguishing between the various mechanisms of sexual selection that may be responsible (female choice, sperm competition and sexual conflict).

Polymorphisms & chirality

Along with generally addressing some of the major theories regarding genital evolution, a remarkable polymorphism in male genitalia has been described and investigated. The chiral dimorphism found in *C. baldersoni*, *C. rentzi* and (in one population) *C. biseriata*, is the first of its kind to be described. Genital polymorphisms are notably absent from most of the literature, but this may be due to biases in taxonomic procedures. In response to this, genital polymorphisms have now been described in some recent studies of spiders (Huber & Gonzalez, 2001), thrips (Mound *et al.*, 1998) and moths (Mutanen & Kaitala, 2006). Adding support to Huber's (2003) suggestion that genital polymorphism may be more common than is currently reported in the literature, *Ciulfina* praying mantids exhibit a genital dimorphism that is maintained in a number of closely-related species. Due to the

multiple occurrence of this polymorphism within the genus, and the presence of a species that is monomorphic (*C. klassi*), this group of insects presents us with a unique opportunity to grasp the significance of this phenomenon. The mirror-image morphology of *Ciulfina* genital traits suggests that the dimorphism may be selectively neutral. However, the inheritance of orientation and its significance for reproductive success in the context of female choice are avenues by which this can be determined. The chiral dimorphisms in gastropod shells (Schilthuizen & Davison, 2005; Asami *et al.*, 1998) have provided unique opportunities for understanding key evolutionary processes. This perplexing dimorphism in *Ciulfina* is likely to be just as rewarding.

Mate searching

The unique aspects of mate searching in *Ciulfina* make it an ideal group in which to study scramble competition, indirect mate choice and the effects of sexual selection on mate locating ability. In contrast to other mantids, which largely use airborne sex pheromones to bring mates together (Gemenio *et al.*, 2005; Maxwell, 1999; Perez, 2005; Hurd *et al.*, 2004; Prete *et al.*, 2000; Robinson & Robinson, 1979), male *Ciulfina* appears to predominantly use vision to locate females. As traits that would increase the performance of *Ciulfina* males in searching for and approaching females (i.e. visual acuity, stealth, locomotory ability) would also be influenced by natural selection, due to their role in predation, this group of insects would be an ideal system in which to investigate the roles of different forms of selection. The comparison of antennal morphology between two species of mantids with differing mate location patterns also revealed potential for further

comparative research in order to explore patterns of evolution in chemical sensory morphology and potentially their relationship with ecological variables.

Spermatophore feeding

The identification of *Ciulfina* as new group of organisms exhibiting spermatophore feeding provides a number of possibilities for further study. Spermatophore feeding among the Orthoptera has received a great deal of attention from behavioural ecologists as it allows an opportunity to investigate mechanisms of postcopulatory sexual selection (Fleischman & Sakaluk, 2004; Gwynne, 1988; Gwynne, 1997; Gwynne, 1985; Reinhold & von Helversen, 1997; Sakaluk, 1984; Sakaluk & Eggert, 1996; Simmons, 1986; Simmons, 2001; Simmons, 1987; Wedell, 1991; Wedell, 1993; Wedell & Arak, 1989). Whilst these important studies have elucidated much of what we understand about processes like cryptic female choice and role-reversal, the identification of *Ciulfina* as a new non-Orthopteran group in which to investigate spermatophore feeding, provides exciting opportunities with which to explore the generality of theory developed for the Orthoptera.

Parapatry

The geographic distributions of *Ciulfina* species abut one-another at parapatric boundaries. Although parapatry is commonly reported in numerous taxa, the mechanisms responsible for its maintenance are still uncertain. Very few studies have explored a number of possible explanations for parapatry within a system. The parapatric zone between the reptile ticks *Aponemma hydrosauri* and *Amblyomma limbatum* (Bull & Burzacott, 2001; Andrews *et al.*, 1982; Bull, 1991) remains the only system for which most theories have

been tested. Considering the distributions of taxa can only interact in a few ways (sympatry, allopatry and parapatry), our ignorance of the processes leading to and maintaining parapatry is truly perplexing. Three of the species featuring in this research (*C. rentzi*, *C. klassi* and *C. biseriata*) form parapatric boundaries with each other's distributions. Multiple tests and duplication of experimental approaches will be therefore possible between the three types of boundaries between these species (i.e. *C. rentzi*/*C. klassi*, *C. rentzi*/*C. biseriata* & *C. klassi*/*C. biseriata*). The genus *Ciulfina* therefore presents an ideal opportunity with which to test current theories regarding parapatry.

References.

- Andrews, R. H., Petney, T. N. & Bull, C. M. 1982. Reproductive interference between three parapatric species of reptile tick. *Oecologia*, **52**, 281-286.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature*, **393**, 784-786.
- Arnqvist, G. & Danielsson, I. 1999. Copulatory behavior, genital morphology and male fertilization success in water striders. *Evolution*, **53**, 147-156.
- Asami, T., Cowie, R. H. & Ohbayashi, K. 1998. Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. *Am. Nat.*, **152**, 225-236.
- Bertin, A. & Fairbairn, D. J. 2005. One tool, many uses: precopulatory sexual selection on genital morphology in *Aquarius remigis*. *J. Evol. Biol.*, **18**, 949-961.
- Bull, C. M. 1991. Ecology of parapatric distributions. *Annu. Rev. Ecol. Syst.*, **22**, 19-36.
- Bull, C. M. & Burzacott, D. 2001. Temporal and spatial dynamics of a parapatric boundary between two Australian reptile ticks. *Mol. Ecol.*, **10**, 639-648.

- Cordero Rivera, A., Andres, J. A., Cordoba-Aguilar, A. & Utzeri, C. 2004. Postmating sexual selection: allopatric evolution of sperm competition mechanisms and genital morphology in calopterygid damselflies (Insecta: Odonata). *Evolution*, **58**, 349-359.
- Cordoba-Aguilar, A. 1999. Male copulatory sensory stimulation induces female ejection of rival sperm in a damselfly. *Proc. Roy. Soc. Lond. B*, **266**, 779-784.
- Cordoba-Aguilar, A. 2005. Possible coevolution of male and female genital form and function in a calopterygid damselfly. *J. Evol. Biol.*, **18**, 132-137.
- Crudgington, H. S. & Siva-Jothy, M. T. 2000. Genital damage, kicking and early death - the battle of the sexes takes a sinister turn in the bean weevil. *Nature*, **407**, 855-856.
- Danielsson, I. & Askenmo, C. 1999. Male genital traits and mating interval affect male fertilization success in the water strider *Gerris lacustris*. *Behav. Ecol. Sociobiol.*, **46**, 149-156.
- Eberhard, W. G. 1985. *Sexual selection and animal genitalia*. Harvard University Press.
- Eberhard, W. G. 2004a. Male-female conflict and genitalia: failure to confirm predictions in insects and spiders. *Biol. Reviews*, **79**, 121-186.
- Eberhard, W. G. 2004b. Rapid divergent evolution of sexual morphology: Comparative tests of antagonistic coevolution and traditional female choice. *Evolution*, **58**, 1947-1970.
- Fairbairn, D. J., Vermette, R., Kapoor, N. N. & Zehiri, N. 2003. Functional morphology of sexually selected genitalia in the water strider *Aquarius remigis*. *Can. J. Zool.*, **81**, 400-413.
- Fleischman, R. R. & Sakuluk, S. K. 2004. No direct or indirect benefits to cryptic female choice in house crickets (*Acheta domesticus*). *Behav. Ecol.*, **15**, 793-798.

- Gemeno, C., Claramunt, J. & Dasca, J. 2005. Nocturnal calling behavior in mantids. *J. Insect. Behav.*, **18**, 389-403.
- Gwynne, D. T. 1985. Role-reversal in katydids: habitat influences reproductive behaviour (Orthoptera: Tettigoniidae, *Metaballus* sp.). *Behav. Ecol. & Sociobiol.*, **16**, 355-361.
- Gwynne, D. T. 1988. Courtship feeding and the fitness of female katydids (Orthoptera: Tettigoniidae). *Evolution*, **42**, 545-555.
- Gwynne, D. T. 1997. The evolution of edible sperm sacs and other forms of courtship feeding in crickets, katydids and their kin (Orthoptera: Ensifera). In: *The evolution of mating systems in insects and arachnids* (Ed. by Choe, J. C. & Crespi, B. J.), pp. 110-129. Cambridge: Cambridge University Press.
- Hosken, D. J. & Stockley, P. 2004. Sexual selection and animal genitalia. *TREE*, **19**, 87-93.
- House, C. M. & Simmons, L. W. 2003. Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc. R. Soc. Lond. B*, **270**, 447-455.
- House, C. M. & Simmons, L. W. 2005a. The evolution of male genitalia: patterns of genetic variation and covariation in the genital sclerites of the dung beetle *Onthophagus taurus*. *J. Evol. Biol.*, **18**, 1281-1292.
- House, C. M. & Simmons, L. W. 2005b. Relative influence of male and female genital morphology on paternity in the dung beetle *Onthophagus taurus*. *Behav. Ecol.*, **16**, 889-897.
- Hribar, L. J. 1994. Geographic variation of male genitalia of *Anopheles nuneztovari* (Diptera: Culicidae). *Mosquito Systematics*, **26**, 132-144.

- Huber, B. A. 2003. Rapid evolution and species-specificity of arthropod genitalia: fact or artifact? *ODE*, **3**, 63-71.
- Huber, B. A. & Gonzalez, A. P. 2001. Female genital dimorphism in a spider - (Araneae: Pholcidae). *J. Zool. Lond*, **255**, 301-304.
- Hurd, L. E., Prete, F. R., Jones, T. H., Singh, T. B., Co, J. E. & Portman, R. T. 2004. First identification of a putative sex pheromone in a praying mantid. *Journal of Chem. Ecol.*, **30**, 155-166.
- Inger, R. F. & Marx, H. 1962. Variation of hemipenis and cloaca in the colubrid snake *Calamaria lumbricoidea*. *Syst. Zool.*, **11**, 32-38.
- Kelly, C. D., Godin, J. G. J. & Abdallah, G. 2000. Geographical variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*). *Can. J. Zool.*, **78**, 1674-1680.
- Langerhans, R. B., Layman, C. A. & DeWitt, T. J. 2005. Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species. *PNAS*, **102**, 7618-7623.
- Madec, L. & Guillar, A. 1994. Geographic variation of distal genitalia in the landsnail *Helix aspersa* (Mollusca: Gastropoda). *J. Zool. Lond.*, **233**, 215-231.
- Maxwell, M. R. 1999. Mating behavior. In: *The praying mantids* (Ed. by Prete, F. R., Wells, H., Wells, P. H. & Hurd, L. E.), pp. 69-89. Baltimore: The John Hopkins University Press.
- Mound, L. A., Crespi, B. J. & Tucker, A. 1998. Polymorphism and kleptoparasitism in thrips (Thysanoptera: Phlaeothripidae) from woody galls on *Casuarina* trees. *Aust. J. Ent.*, 8-16.

- Mutanen, M. & Kaitala, A. 2006. Genital variation in a dimorphic moth *Selenia tetralunaria* (Lepidoptera, Geometridae). *Biol. J. Linn. Soc.*, **87**, 297-307.
- Otronen, M. 1998. Male asymmetry and postcopulatory sexual selection in the fly *Dryomyza anilis*. *Behav. Ecol. Sociobiol.*, **42**, 185-191.
- Perez, B. 2005. Calling behaviour in the female praying mantis, *Hierodula patellifera*. *Physiol. Entomol.*, **30**, 42-47.
- Prete, F. R., Hurd, L. E., Jones, T., Portman, R., Singh, T. B. & Co, J. E. 2000. Scent of a woman: the first identification and assay of a mantid pheromone. *Am. Zool.*, **40**, 1180-1180.
- Reinhold, K. & von Helversen, D. 1997. Sperm number, spermatophore weight and remating in the bushcricket *Poecilimon veluchianus*. *Ethology*, **103**, 12-18.
- Robinson, M. H. & Robinson, B. 1979. By dawn's early light: matitudinal mating and sex attractants in a neotropical mantid. *Science*, **205**, 825-827.
- Sakaluk, S. K. 1984. Male crickets feed females to ensure complete sperm transfer. *Science*, **223**, 609-610.
- Sakaluk, S. K. & Eggert, A. K. 1996. Female control of sperm transfer and intraspecific variation in sperm precedence: antecedents to the evolution of a courtship food gift. *Evolution*, **50**, 694-703.
- Schilthuizen, M. & Davison, A. 2005. The convoluted evolution of snail chirality. *Naturwissenschaften*, **92**, 504-515.
- Simmons, L. W. 1986. Female choice in the field cricket, *Gryllus bimaculatus* (DeGeer). *Anim. Behav.*, **34**, 1463-1470.
- Simmons, L. W. 1987. Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.*, **21**, 197-202.

- Simmons, L. W. 2001. *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press.
- Siro, L. K. 2003. The evolution of insect mating structures through sexual selection. *Florida Entomologist*, **86**, 124-133.
- Sota, T. & Kubota, K. 1998. Genital lock-and-key as a selective agent against hybridization. *Evolution*, **52**, 1507-1513.
- Stockley, P. 2002a. Sperm competition risk and male genital anatomy: comparative evidence for reduced duration of female sexual receptivity in primates with penile spines. *Evol. Ecol.*, **16**, 123-137.
- Tadler, A. 1999. Selection of a conspicuous male genitalic trait in the seedbug *Lygaeus simulans*. *Proc. Roy. Soc. Lond. B*, **266**, 1773-1777.
- Takami, Y. 2003. Experimental analysis of the effect of genital morphology on insemination success in the ground beetle *Carabus insulicola* (Coleoptera Carabidae). *Ethol. Ecol. Evol.*, **15**, 51-61.
- Tanabe, T., Katakura, H. & Mawatari, S. F. 2001. Morphological difference and reproductive isolation: morphometrics in the millipede *Parafontaria tonominea* and its allied forms. *Biol. J. Linn. Soc.*, **72**, 249-264.
- Usami, T., Yokoyama, J., Kubota, K. & Kawata, M. 2006. Genital lock-and-key system and premating isolation by mate preference in carabid beetles (*Carabus* subgenus *Ohomopterus*). *Biol. J. Linn. Soc.*, **87**, 145-154.
- Wedell, N. 1991. Sperm competition selects for nuptial feeding in a bushcricket. *Evolution*, **145**, 1975-1978.
- Wedell, N. 1993. Spermatophore size in bush-crickets - comparative evidence for Nuptial gifts as a sperm protection device. *Evolution*, **47**, 1203-1212.

Wedell, N. & Arak, A. 1989. The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). *Behav. Ecol. Sociobiol.*, **24**, 117-125.

Appendices

The following appendices represent work to which I contributed during the candidature of my PhD, and have subsequently been published.

Appendix One:

Hill, P. J. B., Holwell, G. I., Goth, A. & Herberstein, M. E. (2004). Preference for habitats with low structural complexity in the praying mantid *Ciulfina* sp. (Mantidae). *Acta Oecologica* **26** 1-7.

Appendix Two:

Griffiths, B. V., Holwell, G. I., Herberstein, M. E. & Elgar, M. A. (2003). Frequency, composition and variation in external food stores constructed by orb-web spiders: *Nephila edulis* and *Nephila plumipes* (Araneae: Araneoidea). *Australian Journal of Zoology* **51** 119-128.

Original article

Preference for habitats with low structural complexity in the praying mantid *Ciulfina* sp. (Mantidae)

Phoebe J.B. Hill ^a, Greg I. Holwell ^a, Ann Göth ^b, Marie E. Herberstein ^{a,*}

^a Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia

^b Department of Psychology, Macquarie University, North Ryde, NSW 2109, Australia

Received 20 May 2003; accepted 11 February 2004

Available online 16 April 2004

Abstract

Habitat selection by mobile animals is strongly influenced by habitat characteristics that allow for effective predator avoidance, increased foraging efficiency and reproductive success. Often predator avoidance takes priority; this can be achieved through choosing habitats with high structural complexity. By contrast, the praying mantid *Ciulfina* inhabits structurally simple tree trunk habitats in woodlands and rainforests of northern Queensland, Australia. Field observations and experiments revealed a strong preference for tree trunks with a smooth bark type and small circumference. Subsequent laboratory experiments supported these field results, and further indicated that the preference for such habitat structures may be associated with a faster running speed across the smooth textured bark and around trunks of a smaller circumference. *Ciulfina* run to escape predation and actively forage across the open tree trunk habitat. Thus selecting smooth trees with small circumference facilitates increased running speeds and thereby may increase foraging ability and predator avoidance.

© 2004 Elsevier SAS. All rights reserved.

Keywords: Habitat selection; Habitat complexity; Tree trunk; Predator avoidance; Running speed; Mantid; Bark; Australia

1. Introduction

The distribution of mobile animals is typically determined by a number of animate and inanimate habitat characteristics. Animate habitat characteristics are associated with the density of conspecifics, inter-specific competitors, prey and potential predators (Mangel, 1990; Martin and Salvador, 1995). Inanimate characteristics include light availability and physical habitat structures. Habitat structures have been defined as the shapes and surfaces of habitats with a particular color, texture and size (Eterovick and Figueira, 1997). These habitat characteristics influence how vulnerable animals are to predation or how efficient they are at foraging, thereby affecting growth, survival and fecundity (Dyer and Landis, 1996; Jones and Boulding, 1999; Morris, 1987, 1992; Ray and Stoner, 1995). Inter-specific competitors may reduce access to one or more limited resources such as food or space per se. Intense competition may result in niche partitioning between

species or even extinction of the less competitive species (see Amarasekare, 2003 for a review). Theoretically, an optimal habitat patch maximizes foraging efficiency while minimizing predation risk. Ultimately, the quality and availability of habitat patches determines the distribution of populations, and at a smaller scale, the dispersion patterns within populations (Lima, 1998).

The common pattern seen in habitat choice among mobile animals, and especially invertebrates, is the preference for high structural complexity such as dense vegetation (Greenstone, 1984; McNett and Rypstra, 2000; Perkins-Visser et al., 1996) or a high availability of crevices (Guidetti, 2000; Martin and Salvador, 1995). For example, complexity (i.e. vegetative structures) aids web construction in the spider *Argiope trifasciata*, and is the primary determinant of web site selection (McNett and Rypstra, 2000). The foraging efficiency of many animals also depends on the structural complexity of a habitat (Bradley, 1993; Heck and Crowder, 1991). Increased habitat complexity allows animals to forage for prey, whilst minimizing predation risk (Lima and Dill, 1990). Many mobile animals have to routinely choose between habitats that differ in their net energetic return rate and risk of mortality (Gilliam and Fraser, 1987). Most often, they

* Corresponding author.

E-mail address: mherbers@bio.mq.edu.au (M.E. Herberstein).

reduce their risk of predation at the cost of a diminished foraging rate (Lima, 1985; Lima et al., 1985; Holbrook and Schmitt, 1988; Utne and Aksnes, 1994).

The level of predation risk in a certain habitat depends on inanimate characteristics (Heck and Crowder, 1991), characteristics of the predator (Gotceitas and Colgan, 1989), and characteristics of the prey such as their behavior, crypsis or if they are defended by morphological or physiological traits (Lima and Dill, 1990; Swisher et al., 1998). Prey often select habitats with high structural complexity as it restricts the predator's movement and visual range, reducing predation risk (Anderson, 1984; Elkin and Baker, 2000; Savino and Stein, 1982, 1989). Prey may also alter their behavior so that they are more difficult to encounter, detect or capture (Lima, 1998). However, animals that choose habitats with high structural complexity sometimes suffer a decrease in their own ability to escape (Brownsmith, 1977; Crist et al., 1992; Lima, 1998; Schooley et al., 1996). Reduced mobility and visual range due to high complexity may also decrease foraging ability (Gilliam and Fraser, 1987; Holbrook and Schmitt, 1988; Holomuzki and Short, 1988) and mating opportunities (Sih, 1987). Therefore, species that do not rely on cover for predator avoidance may avoid habitats with high structural complexity (Lima, 1993). In summary, animals face a number of conflicting demands when selecting a habitat, but predator avoidance seems to be one of the key factors in shaping preferences (Decaester et al., 2002; Downes and Shine, 1998; Grand, 2002; Lima, 1998).

Among praying mantids (Mantodea), a preference for habitats of high structural complexity has been documented (Balderson, 1991; Rentz, 1996). The majority of praying mantids rely on their cryptic coloration and immobile stance to remain cryptic within dense vegetation (Balderson, 1991). This assists them both in ambushing prey and avoiding predation. Contrary to this common pattern, *Ciulfina* inhabits the structurally simple trunk habitat of certain trees (Rentz, 1996; Holwell, unpublished). *Ciulfina* are more active when foraging than related genera, and pursue prey over the entire lower trunk of their tree habitat (Balderson, 1991). When threatened, *Ciulfina* will also run quickly around the circumference of the tree to the other side of the trunk, rarely leaving the tree during a chase (Rentz, 1996). To the human observer, *Ciulfina* are cryptic and can generally only be detected when they move.

Structurally simple trunks offer little cover and expose *Ciulfina* to high risk of detection by visually-orientating predators. The aim of this study is to describe habitat choice in a *Ciulfina* species in detail and to discuss the costs and benefits of such habitat choice in terms of escape efficiency and prey capture.

2. Methods

2.1. Field study

The field study was conducted in September 2002 at the end of the dry season in a 6 ha parkland (21S, 149E) situated

in Mackay, central Queensland, Australia. The natural flora in the region has primarily been cleared for the sugar cane industry, leaving most remaining areas of bush, including the study site, as parklands with a mosaic of exotic and native flora.

2.2. Field counts

All of the accessible trees (approximately 90% of all trees) within the park were thoroughly searched for the presence of *Ciulfina*. Once an individual was detected, its sex and location (height) on the tree was recorded. It was not possible to sex juveniles and these were thus recorded as nymphs. All 217 trees used in this study were labeled according to whether or not they were occupied by a *Ciulfina*. Tree measurements included the circumference of the tree trunk at 1 m, where *Ciulfina* were commonly observed (Holwell, unpublished), and the distance to the nearest tree. Tree trunks were categorized as 'smooth' or 'rough' based on the surface texture of the bark. The trees with smooth bark ('smooth trees') were without crevices within the bark, while the roughly textured bark ('rough trees') comprised obvious crevices approximately 0.5–1 cm deep.

2.3. Translocation experiment

To determine whether individual mantids seek out a preferred bark type, a translocation experiment was conducted using a total of 39 adult *Ciulfina*. The number of *Ciulfina* initially found on rough trees was limited, and so only six of the 39 *Ciulfina* were removed from rough trees, and the remaining 33 were taken from smooth trees. Each mantid was marked using a bee tag (small, numbered plastic discs) and released onto a tree with the opposite bark type to the tree it originally occupied (i.e. rough to smooth or smooth to rough). Each tree in the study site was monitored daily and the absence or presence of released individuals was recorded.

2.4. Field habitat choice

To determine whether *Ciulfina* prefer trees with smooth bark over rough bark in the field, we conducted a choice experiment utilizing two naturally occurring trees (one smooth and one rough). The two trees were approximately of the same circumference (smooth, 46 cm; rough, 54 cm) and were positioned 220 cm apart. Each mantid was released 150 cm away in front and directly in between both trees. The behavior, latency to approach, and the final choice of tree were recorded.

2.5. Laboratory study—animal maintenance

A population of 40 adult mantids was collected from the study site in March 2002 and September 2002. Each mantid was housed individually in labeled, upturned plastic cups (10 × 7 cm) under room temperature, sprayed with water

daily and fed on vinegar flies, *Drosophila melanogaster*, and crickets, *Acheta domestica*, three times a week.

2.6. Habitat choice experiments

To test mantid choice between rough and smooth bark types in the laboratory, two semi-natural experimental 'tree trunks' were constructed based on bark types observed in the field. Two 30 cm tall solid timber cylinders were used. No modification was necessary to create the smooth experimental trunk. Securing bark taken from rough trees in the field to the trunk using non-toxic Clag® glue created the rough surface texture. Including modifications, both trunks were 7 cm in diameter.

Previous occupation of an experimental trunk by a mantid of either sex does not significantly affect subsequent habitat choice by other individuals (Hill, 2002). This allowed the same trunk structures to be used in choice experiments. Experimental trunks were placed 30 cm apart in an experimental arena (135 × 75 cm), which was surrounded by black cardboard (50 cm tall). Each mantid was released on the ground 30 cm in front and directly between the two cylinders, and the entire surface of the arena was scattered with leaf litter to create a more natural environment. From a laboratory population of 40 individuals, only one randomly selected mantid was used for each choice test, however, mantids were re-used in subsequent experiments testing other variables (see below). The position of the two cylinders was switched between trials. Light sources included the normal overhead lights of the laboratory and two lamps with Daylight Blue, 60 W, practitone Philips bulbs (Philips Electronics N.V., Holland) directed at each of the trunks from 50 cm away. Light conditions were identical in all experiments and thus should not be responsible for any differences in the mantids choice. A trial was considered successful if a trunk was chosen within 30 min.

To determine whether tree trunk circumference influences habitat selection in *Ciulfina*, three artificial trunks were constructed to represent a range of the circumference sizes found in the field: 155, 55, 15 cm (Fig. 1). Each artificial trunk was 45 cm tall and made from cardboard, mesh and non-toxic glue. To avoid the potential positional bias that may occur when using three different options in a choice test, and to maintain experimental consistency, each mantid was used in two separate choice tests: large (155 cm) versus medium (55 cm) and medium (55 cm) versus small (15 cm). Assignment was made random to control the effects of presentation order. The experimental set-up was identical to the surface texture choice experiment. A trial was considered successful when a trunk was chosen within 30 min.

2.7. Running speeds

We compared running speeds of *Ciulfina* on smooth and rough surfaces, by creating two semi-natural running tracks from a single cylinder of smooth barked timber cut down the

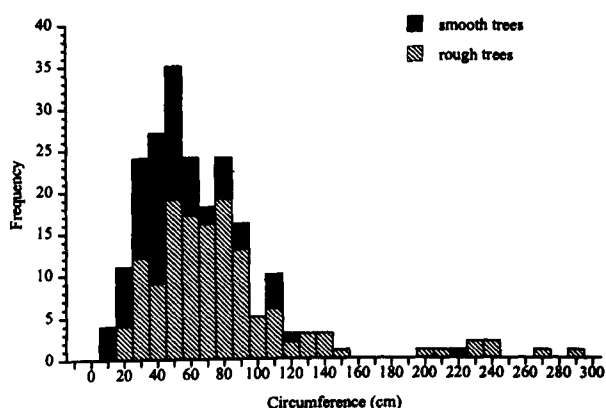


Fig. 1. The frequency distribution of trunk size (circumference, cm) of rough ($n = 137$) and smooth ($n = 80$) trees measured in the field.

middle (25 × 15 cm). This produced two identical horizontal running tracks when laid down on the flat surface in an arena sized 60 × 60 cm. One of the tracks was smooth, while the other track had rough bark pieces glued to the running surface. Each randomly chosen mantid was timed running on both tracks (rough and smooth); the order of presentation was random. Individual mantids ($n = 25$) were held at the start of the track in an unturned container (6 × 4 cm) with a fan (Eveready® DC 6 V) forcing wind over the track to provide a constant stimulus to elicit running. The stopwatch was started once the container was lifted and was stopped when the mantid reached the end of the running track. Lighting was the same as in the choice experiments.

We measured escape time around trunks of differing circumference, using artificial trunks of three circumference sizes (155, 55, 15 cm). Each randomly chosen mantid was tested on all three trunks in a random order. Each mantid was placed at the same point in the middle of the front face of the artificial trunk. An observer stood directly facing the front of the artificial trunk and simultaneously started the stopwatch when administering a single tap with a small paintbrush on the end of the mantid's abdomen. This prompted the mantid to run to the other side of the trunk. The stopwatch was stopped as soon as the mantid was out of the observer's view.

2.8. Statistical analyses

All continuous data were tested for normal distribution (Kolmogorov–Smirnov test) and inspected for equality of variance. Field and laboratory data were not normally distributed and we were unable to improve distribution with transformations. Therefore, the data were analyzed using non-parametric tests. The distribution of mantids on different tree types was analyzed using χ^2 -tests. Tree parameters (width, distance to nearest tree) were compared between rough and smooth trees using Mann–Whitney *U*-tests. All choice experiments and the relocation experiment were analyzed using binomial probability (<http://vassun.vassar.edu/~lowry/VassarStats.html>). Running speed on smooth, rough

and various circumference diameters was analyzed using Wilcoxon and Friedman tests, respectively.

3. Results

Of the 217 trees surveyed in the field, 80 were assigned to the category 'smooth bark'. These included the following species with their percent frequency: guava tree (*Psidium guajava*; 37%), flame tree (*Delonix regia*; 36%), mango tree (*Mangifera indica*; 13%), rusty pittosporum (*Pittosporum rhombifolium*; 5%), umbrella tree (*Schefflera actinophylla*; 5%) and yellow oleander (*Thevetia peruviana*; 4%). Trees with rough bark ($n = 137$) included the moreton bay ash (*Eucalyptus tessellata*; 70%) and the Clarkson's bloodwood (*Corymbia clarksoniana*; 30%). *Ciulfina* were found on all tree species within the categories of rough and smooth proportional to the availability of these trees in the field (χ^2 -test: smooth bark: $\chi^2 = 2.1$, $df = 5$, $P = 0.84$; rough bark: $\chi^2 = 1.9$, $df = 2$, $P = 0.59$). Of the 80 smooth barked trees available, 41.3% were occupied by *Ciulfina*, while mantids were only present on 4.4% of the available 137 rough barked trees (χ^2 -test: $\chi^2 = 46.6$, $df = 3$, $P < 0.01$; Fig. 2).

Smooth trees had a significantly smaller average circumference (Mann–Whitney U -test: $U = 2978$, $n_1 = 80$, $n_2 = 137$, $P < 0.01$) and a shorter average distance to the nearest tree compared with rough trees (Mann–Whitney U -test: $U = 4268$, $n_1 = 80$, $n_2 = 137$, $P < 0.01$; Table 1). Among smooth trees, neither the circumference (Mann–Whitney U -test: $U = 729.5$, $n_1 = 33$, $n_2 = 47$, $P = 0.65$; Table 1) nor the distance to the nearest tree was found to significantly affect

whether *Ciulfina* was present or absent (Mann–Whitney U -test: $U = 595.0$, $n_1 = 33$, $n_2 = 47$, $P = 0.103$; Table 1). Among rough trees, the distance to the nearest tree did not affect *Ciulfina* presence (Mann–Whitney U -test: $U = 331.5$, $n_1 = 6$, $n_2 = 131$, $P = 0.54$). However, the circumference was significantly smaller on rough trees occupied by mantids than on vacant trees (Mann–Whitney U -test: $U = 167.5$, $n_1 = 6$, $n_2 = 131$, $P = 0.02$; Table 1). *Ciulfina* were often discovered lying flat against the trunk facing the ground at an average height of 88 cm (\pm S.E. = 7.81 cm; $n = 39$).

3.1. Translocations

A total of 45 *Ciulfina* were tagged and translocated, however, only 10 individuals were recaptured. Five of six mantids (83.3%) transplanted from a rough tree to a smooth tree were recaptured, while only five of 39 (12.8%) mantids taken from a smooth tree to a rough tree were recaptured. All 10 mantids (100%) that were recaptured were found on a smooth tree (binomial test, $n = 10$, $P < 0.01$).

3.2. Behavioral observations

Only one *Ciulfina* was found per tree. Of the 39 *Ciulfina* found, 42% were adult males, 31% adult females, and 27% nymphs. Adult animals possess wings, but when under threat only one individual ever used its wings to escape. Furthermore, only one individual left the trunk habitat when being pursued. The majority of animals moved to the back of the tree trunk and ran either up or down.

3.3. Predators

In the field, insectivorous birds and lizards appeared to be the most common potential predators of *Ciulfina*. Blue-faced honeyeaters (*Entomyzon cyanotis*) were observed to use their claws to secure themselves to rough bark, while probing the bark with their beaks. Common tree skinks (*Cryptoblepharus virgatus*) pursued *Ciulfina* by actively chasing them around the circumference of rough and smooth barked trees.

3.4. Habitat choice

When released in front and directly between a smooth and rough tree in the field, 89% of *Ciulfina* chose the smooth bark type over the rough barked alternative (binomial test, $n = 18$, $P = 0.0006$). On average it took a mantid 643 s (\pm S.E. = 110)

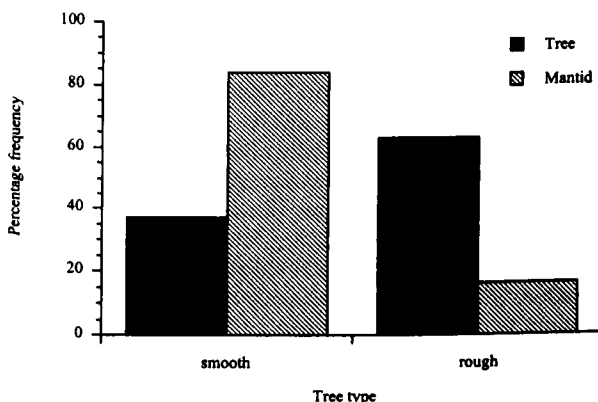


Fig. 2. The relative abundance of smooth ($n = 80$) and rough ($n = 137$) trees compared to the relative abundance of *Ciulfina* ($n = 38$) on these trees.

Table 1

Mean (\pm S.E.) circumference (cm) and distance (cm) to the nearest tree for rough and smooth trees when a mantid was present or absent. Sample size given in parentheses

	Smooth barked trees		Rough barked trees	
	Circumference	Distance	Circumference	Distance
Mantid present	46.91 \pm 5.04 ($n = 33$)	127.58 \pm 22.91 ($n = 33$)	45.33 \pm 5.09 ($n = 6$)	163.00 \pm 16.32 ($n = 6$)
Mantid absent	52.47 \pm 4.90 ($n = 47$)	139.17 \pm 14.44 ($n = 47$)	80.09 \pm 4.31 ($n = 131$)	170.01 \pm 11.17 ($n = 131$)
Total	50.17 \pm 3.54 ($n = 80$)	134.33 \pm 12.66 ($n = 80$)	78.57 \pm 4.17 ($n = 137$)	169.70 \pm 10.70 ($n = 137$)

to choose between the two bark types. Once released from the plastic container *Ciulfina* spent 1–5 min ‘peering’ back and forth between the two tree types before moving in either direction. They traveled to the tree in small sprints of 5–10 cm, with pauses of approximately 1 min during which they displayed side-to-side head movements in the direction of the chosen tree. We never observed any mantids switching between trunks once they have made their initial choice.

In laboratory choice tests with a rough and a smooth tree trunk, 85% of *Ciulfina* chose smooth surface texture over rough surface texture (binomial test, $n = 39$, $P < 0.01$). When given the choice of two artificial trunks with differing circumferences, of 30 *Ciulfina*, 24 chose the medium sized trunk significantly more often than the large trunk (binomial test, $n = 30$, $P < 0.01$). Furthermore, when selecting between the medium and the small sized trunk, of 30 *Ciulfina*, 21 chose the small trunk significantly more frequently than the medium sized trunk (binomial test, $n = 30$, $P < 0.01$).

3.5. Running speed experiments

Ciulfina reached the end of a running track significantly faster when the track was smooth (Wilcoxon test, $Z = -3.73$, $n = 25$, $P < 0.01$, Fig. 3a). When running on the smooth surface locomotion was typically a continuous sprint with no pausing, while locomotion was more staggered on the rough track. The running times of *Ciulfina* also varied depending on the circumference of the tree trunk: as circumference size increased, so did running time (Friedman test, $\chi^2 = 47.64$, $n = 25$, $P < 0.01$; Fig. 3b).

4. Discussion

This study shows that *Ciulfina* inhabit the structurally simple habitats of tree trunks, and exhibit a preference for narrower tree trunks with a relatively lower structural surface complexity. Such preference for a simple habitat is in contrast to the pattern typically seen among invertebrates (Andow, 1991; McNett and Rypstra, 2000; Perkins-Visser et al., 1996) and especially the Mantodea (Balderson, 1991; Rentz, 1996). Habitats of high complexity are commonly preferred because cover created by vegetative structures or crevices allows for efficient foraging and mating whilst decreasing the risk of predation (Lima and Dill, 1990). However, we observed that *Ciulfina* mostly run to escape predation. Therefore, selecting habitats with relatively high complexity, such as trees with rough bark may impede movement and slow their escape speed (Lima, 1993; Schooley et al., 1996).

On open tree trunks, *Ciulfina* are exposed to numerous predators such as the two observed in the field: the blue-faced honeyeater and common tree skink. With no structural cover on a trunk, *Ciulfina* must retreat from the visual field of predators by running to the opposite side of the trunk. Escape times were significantly faster on the smooth barked trees and, not surprisingly, on trunks with a smaller circumference.

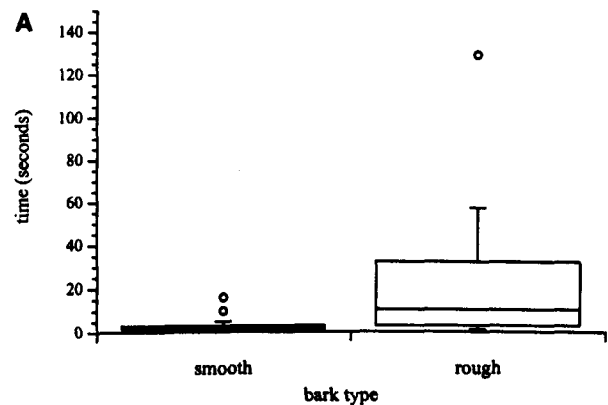


Fig. 3a. Running times of *Ciulfina* along a smooth and a rough horizontal running track (box boundaries present the 25th and 75th percentiles; the middle line is the 50th percentile. The whiskers bound the 10th and 90th percentiles with data outside these boundaries indicated by the open circles).

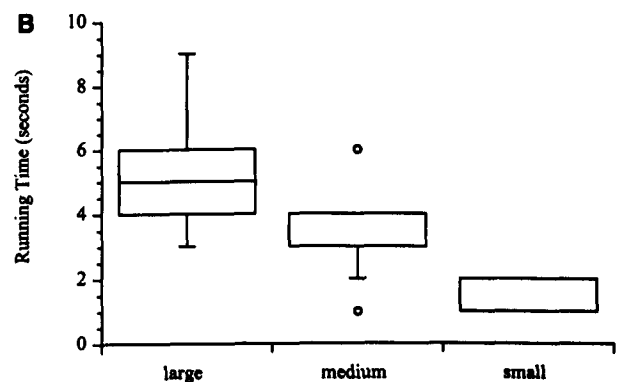


Fig. 3b. Running times of *Ciulfina* compared across three artificial trunks of differing circumference size (15, 55, 155 cm). Running time was measured as the time it took each *Ciulfina* to run from corresponding starting points to the back of the trunk where the mantid could no longer be seen (box boundaries present the 25th and 75th percentiles; the middle line is the 50th percentile. The whiskers bound the 10th and 90th percentiles with data outside these boundaries indicated by the open circles; for medium and small circumference size, the 50th and 25th percentile is the same value).

Therefore, selecting trees with a smooth bark texture and smaller circumference may lower the risk of predation by allowing greater escape speed.

Predatory behavior in mantids is mediated by vision, and recognition of an appropriate target depends on size, speed, orientation and contrast of the object with the background (Prete and Hamilton, 1999). Once prey is identified, mantids generally pursue their targets (Prete et al., 1993). Specifically, *Ciulfina* actively chase prey along the trunk of trees (Rentz, 1996). Smooth trees may allow clearer and more direct detection of prey than rough trees, as well as unencumbered pursuit once prey has been identified.

Interactions with conspecifics, including mate availability, have also been suggested as a key factor in habitat selection (Andow, 1991). In our study population, only one individual was found per tree, supporting previous observations that the presence of a conspecific discourages newcom-

ers from settling (Rentz, 1996; Rosenzweig, 1991; Sutherland, 1996). Two *Ciulfina* will occupy a single trunk when mating. However, as no mating was observed during the field study, mate choice is unlikely to have been a factor in habitat choice at the time when this study was conducted.

Predator avoidance often plays a predominant role in habitat selection (Dacaester et al., 2002; Elkin and Baker, 2000; Grand, 2002; Lima and Dill, 1990). In our system, predator avoidance and foraging success may lead to the selection of habitats of low structural complexity. These may provide lower risk of predation due to increased escape speed and higher foraging success through improved prey recognition and pursuit. Hence, although the preference for a structurally simple habitat in *Ciulfina* may be unusual, the reasons underlying the preference are universal ones.

Acknowledgements

We thank Alison Downing for identifying the trees, Phil Taylor for helpful comments on the manuscript, Mackay Council for permission to use the Annie Wood Park, and for supplying maps and additional information, two anonymous reviewers for their helpful comments.

References

- Amarasekare, P., 2003. Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters* 6, 1109–1122.
- Anderson, O., 1984. Optimal foraging by largemouth bass in structural environments. *Ecology* 65, 851–861.
- Andow, D.A., 1991. Vegetational diversity and arthropod response. *Annual Review of Entomology* 36, 561–586.
- Balderson, J., 1991. *The Insects of Australia*. Division of Entomology, CSIRO, Melbourne University Press, Carlton South, Victoria.
- Bradley, R.A., 1993. The influence of prey availability and habitat on activity patterns and abundance of *Argiope keyserlingi* (Araneae: Araneidae). *Journal of Arachnology* 21, 91–106.
- Brownsmith, C.B., 1977. Foraging rates of starlings in two habitats. *Condor* 79, 387–408.
- Crist, T.O., Guertin, D.S., Wiens, J.A., Milne, B.T., 1992. Animal movement in heterogeneous landscapes: an experiment with *Elodes* beetles in shortgrass prairie. *Functional Ecology* 6, 536–544.
- Dacaester, E., Meester, D.L., Dieter, E., 2002. In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences, USA* 99, 5481–5485.
- Downes, S., Shine, R., 1998. Heat, safety or solitude? Using habitat selection experiments to identify a lizard's priorities. *Animal Behaviour* 55, 1387–1396.
- Dyer, L.E., Landis, D.A., 1996. Effects of habitat, temperature and sugar availability on longevity of *Eriborus terebrans* (Hymenoptera: Ichneumonidae). *Environmental Entomology* 25, 1192–1201.
- Elkin, C.M., Baker, R.L., 2000. Lack of preference for low-predation-risk habitats in larval damselflies explained by costs of intraspecific interactions. *Animal Behaviour* 60, 511–521.
- Eterovick, P.C., Figueira, J.E., 1997. Cryptic colouration and choice of escape microhabitats by grasshoppers (*Orthoptera acrididae*). *Biological Journal of the Linnean Society* 61, 485–499.
- Gilliam, J.F., Fraser, D.F., 1987. Habitat selection under predation hazard: test of a model with foraging minnows. *Ecology* 68, 1856–1862.
- Gotceitas, V., Colgan, P., 1989. Selection between densities of artificial vegetation by young bluegills avoiding predation. *Transactions of the American Fisheries Society* 116, 40–49.
- Grand, T.C., 2002. Alternative forms of competition and predation dramatically affect habitat selection under foraging–predation–risk trade-offs. *Behavioural Ecology* 2, 280–290.
- Greenstone, M.H., 1984. Determinants of web spider species diversity: vegetational structural diversity vs. prey availability. *Oecologia* 62, 299–304.
- Guidetti, P., 2000. Differences among fish assemblages associated with nearshore *Posidonia oceanica* seagrass beds, rocky-algal reefs and unvegetated sand habitats in the Adriatic Sea. *Estuarine, Coastal and Shelf Science* 50, 515–529.
- Heck, K.L., Crowder, L.B., 1991. Habitat structure and predator prey interactions in vegetated aquatic systems. In: Bell, S.S., McCoy, E.D., Mushinsky, H.R. (Eds.), *Habitat Structure: The Physical Arrangement of Objects in Space*. Chapman & Hall, New York, pp. 281–299.
- Hill, P.J.B., 2002. Habitat selection in the tree-dwelling mantid, *Ciulfina* sp. Honours Thesis. Macquarie University.
- Holbrook, S.J., Schmitt, R.J., 1988. Effects of predation risk on foraging behaviour: mechanisms altering patch choice. *Journal of Experimental Marine Biology and Ecology* 121, 151–163.
- Holomuzki, J.R., Short, T.M., 1988. Habitat use and fish avoidance behaviours by the stream dwelling isopod *Liceus fontinalis*. *Oikos* 52, 79–86.
- Jones, K.M., Boulding, E.G., 1999. State-dependent habitat selection by an intertidal snail: the costs of selecting a physically stressful microhabitat. *Journal of Experimental Marine Biology and Ecology* 242, 149–177.
- Lima, S.L., 1985. Maximising feeding efficiency and minimising time exposed to predators: a trade-off in the black-capped chickadee. *Oecologia* 66, 60–67.
- Lima, S.L., 1993. Ecological and evolutionary perspectives on escape from predatory attack: a survey of North American birds. *Wilson Bulletin* 105, 1–47.
- Lima, S.L., 1998. Nonlethal effects in the ecology of predator–prey interactions. What are the ecological effects of anti-predator decision making? *BioScience* 48, 25–34.
- Lima, S.L., Dill, L.M., 1990. Behavioural decisions made under risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68, 619–640.
- Lima, S.L., Valone, T.J., Caraco, T., 1985. Foraging efficiency: predation risk trade-off in the grey squirrel. *Animal Behaviour* 33, 155–165.
- Mangel, M., 1990. A dynamic habitat selection game. *Mathematical Biosciences* 100, 241–248.
- Martin, J., Salvador, A., 1995. Microhabitat selection by the Iberian Rock lizard, *Lacerta monticola*: effects on density and spatial distribution of individuals. *Biological Conservation* 79, 303–307.
- McNett, J.B., Rypstra, A.L., 2000. Habitat selection in a large orb-weaving spider: vegetational complexity determines site selection and distribution. *Ecological Entomology* 25, 423–433.
- Morris, D.W., 1987. Ecological scale and habitat use. *Ecology* 68, 362–369.
- Morris, D.W., 1992. Scales and costs of habitat selection in heterogeneous landscapes. *Evolutionary Ecology* 6, 412–432.
- Perkins-Visser, E., Wolcott, T.G., Wolcott, D.L., 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus*). *Journal of Experimental Marine Biology and Ecology* 198, 155–173.
- Prete, F.R., Hamilton, K., 1999. Prey capture. In: Prete, F.R., Wells, H., Wells, P.H., Hurd, L.E. (Eds.), *The Preying Mantids*. Johns Hopkins University Press, Baltimore, MD, pp. 194–223.
- Prete, F.R., Placek, P.J., Wilson, M.A., Mahaffey, R.J., Nemcek, R.R., 1993. Stimulus speed and order of presentation effect the visually released predatory behaviors of the preying mantis, *Sphodromantis lineola* (Burr.). *Brain Behavior and Evolution* 42, 281–294.
- Ray, M., Stoner, A.W., 1995. Growth, survivorship, and habitat choice in newly settled seagrass gastropod, *Strombus gigas*. *Marine Ecology Progress Series A* 123, 83–94.

- Rentz, D., 1996. Grasshopper Country. CSIRO. Division of Entomology, UNSW Press, Canberra, Australia.
- Rosenzweig, M.L., 1991. Habitat selection and population interactions: the search for mechanism. *American Naturalist Supplement* 137, 5–28.
- Savino, J.F., Stein, R.A., 1982. Predator–prey interaction between large-mouth bass and bluegills as influenced by simulated submersed vegetation. *Transactions of the American Fisheries Society* 111, 255–266.
- Savino, J.F., Stein, R.A., 1989. Behavioural interactions between fish predators and their prey: effect of plant density. *Animal Behaviour* 37, 311–321.
- Schooley, R.L., Sharpe, P.B., Van Horne, B., 1996. Can shrub cover increase predation risk for a desert rodent? *Canadian Journal of Zoology* 74, 157–163.
- Sih, A., 1987. Predators and prey lifestyles: an evolutionary and ecological overview. In: Kerfoot, W.C., Sih, A. (Eds.), *Predation: Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, New Hampshire, pp. 203–222.
- Sutherland, W.J., 1996. *From Individual Behaviour to Population Ecology*. Oxford University Press, New York.
- Swisher, B.J., Soluk, D.A., Wahl, D.H., 1998. Non-additive predation in littoral habitats: influences of habitat complexity. *Oikos* 81, 30–37.
- Utne, A.C.W., Aksnes, D.L., 1994. An experimental study on the influence of feeding versus predation risk in the habitat choice of juvenile and adult two-spotted goby, *Gobiusculus flavescens* (Fabricus). *Journal of Experimental Marine Biology and Ecology* 179, 69–79.

**Frequency, composition and variation in
external food stores constructed by orb-web spiders:
Nephila edulis and *Nephila plumipes* (Araneae : Araneoidea)**

B. V. Griffiths^A, G. I. Holwell^B, M. E. Herberstein^{B,C} and M. A. Elgar^A

^ADepartment of Zoology, University of Melbourne, VIC 3010 Australia.

^BDepartment of Biological Sciences, Macquarie University, NSW 2109, Australia.

^CTo whom correspondence should be addressed. Email: m.herberstein@bio.mq.edu.au

Abstract

Few examples of food hoarding have been documented in spiders, yet two Australian species of orb-web spiders, *Nephila edulis* and *N. plumipes*, typically incorporate previously captured prey into the web. The effect of prey density and prey-encounter rate on the storage behaviour of adult female *N. edulis* was tested in the laboratory. Prey density had a significant effect on the propensity to construct external caches: when more food was available, food caches were larger than when the supply of prey was limited. Caching behaviour also differed with the rate of prey encounter, even though the total amount of food supplied was the same. When prey were encountered at constant rates, spiders allocated more food to external storage compared with random encounter rates. Finally, we tested the quality of different prey types for external or internal storage. *N. edulis* were fed with blow-flies or crickets, and these prey were stored in the web, discarded or totally consumed. Crickets were typically consumed or stored, while flies were more frequently discarded. Field observations of the storage behaviour in *N. edulis* and *N. plumipes* found surprising differences in the composition of the cache. While *N. plumipes* incorporated only animal material, *N. edulis* also utilised plant material, suggesting that the storage band in *N. edulis* has other, non-food-storing functions. Field experiments indicated that the presence or absence of external stores in the web of *N. plumipes* had no influence on mortality, weight gain, or the presence of *Argyrodes* kleptoparasites.

Introduction

Foraging has two major components, the acquisition of food and the maintenance of these acquired resources. The former consists of searching, handling and ingestion of food, and these resources can be maintained internally as fat deposits or externally as short- or long-term caches (Stephens and Krebs 1986). Storing food resources internally by eating them immediately has the advantage that the nutrients are secured as fat. On the other hand, most animals are physiologically limited in how much they can eat at any one time and how much fat they can store (Johnson *et al.* 1975). Furthermore, a higher body mass due to fat storage requires an elevated metabolic rate and decreases mobility, thereby increasing the risk of predation and lowering foraging efficiency (e.g. Witter and Cuthill 1993).

Maintaining external food storages is an alternative to internal fat stores and occurs among many animals (see Vander Wall 1990). External caches may reduce the risk of starvation by reducing the variation in food intake during times of low food availability (McNamara *et al.* 1990). Nevertheless there are disadvantages associated with food hoarding. Animals may have problems relocating the cache, the cache may be pilfered (Leaver and Daly 2001), and/or the food may be spoilt by mould, germs or parasites. Furthermore, searching for external stores involves higher travel costs and may increase exposure to predators and unfavourable conditions (McNamara *et al.* 1990). Clearly, the future value of stored food depends on nutrient and energy content and the likelihood of consumption later on (Andersson and Krebs 1978; Moreno *et al.* 1981; Kagel *et al.* 1986).

Food hoarding is widely documented in birds and mammals and has evolved several times independently (Andersson and Krebs 1978; Smith and Reichman 1984; Sherry 1985; Stevens and Krebs 1986; Vander Wall 1990; Leaver and Daly 2001). Among invertebrates, the classic examples of food hoarding are found among social insects such as honeybees and ants (see Vander Wall 1990).

In spiders, two types of hoarding behaviour have been described. Short-term storage involves wrapping the freshly captured prey in silk and leaving it in the web for later consumption (Eberhard 1967). Generally, spiders will ingest the wrapped prey at the end of their foraging period, typically after about 12–24 hours. Champion de Crespigny *et al.* (2001) documented a long-term storage mechanism (several days to weeks) in the orb-web spider *N. edulis* (Tetragnathidae). This spider, like other congeners, incorporates a densely packed conspicuous band of previously captured uneaten prey and prey remains in their web (Main 1976). *N. edulis* can maintain body mass in periods of low prey capture by ingesting the stored items, a strategy that is particularly evident for larger individuals that also tend to store more prey at the hub (Champion de Crespigny *et al.* 2001).

Spiders are extra-oral fluid feeders that can ingest enormous amounts of food relative to body mass due to the presence of gut diverticula (Foelix 1992). Thus they may not be limited by the amount of food they can ingest. Nevertheless, weight gain through internal food storage is still costly as it interferes with locomotion (Herberstein and Heiling 1999). Despite physiological and behavioural adaptations to maximise food intake, spiders in general, and the genus *Nephila* specifically, experience limitations in food supply over time (Wise 1993).

A disadvantage of hoarding behaviour in web-building spiders is that the secured prey items may be lost to kleptoparasites or through web damage (Vander Wall 1990; Elgar 1993). Spiders in the genus *Nephila* are often host to kleptoparasitic spiders (Elgar 1993). For example, various species of *Argyrodes* (Theridiidae) collect tiny prey items from host webs (Exline and Levi 1962; Liao *et al.* 1984), share food directly with the host (Vollrath 1979) or steal prey from the host (Rypstra 1981). Additionally, they may ingest the web silk, incurring high costs for the host (Tso and Severinghaus 1998). Thus, storing food in the web may attract larger numbers of kleptoparasites as they may access caches more easily. An additional cost specific to spiders is that desiccated prey remains have to be re-liquefied to extract nutrients. Under dry or limited condition, spiders may not be able to access these external caches.

The present study examines prey-hoarding behaviour in two species of orb-web spiders, *N. edulis* and *N. plumipes*. These two species are widely distributed throughout the tropical and temperate regions of Australia. Both species incorporate a storage band into their web that is usually attached to the barrier web near the hub. We investigated the composition of the food caches constructed by *N. edulis* and *N. plumipes* and the food-storing behaviour with respect to prey availability. Specifically, we predicted that when prey density is high or prey-encounter rates are unpredictable, these spiders will store more prey externally than when prey is scarce or encountered at constant rates. Finally, field experiments were conducted to observe the relationship between food stores, weight gain, rates of mortality and the activity of kleptoparasites in the webs of food-storing females. We predicted that removal of the external stores would reduce weight gain in spiders and would reduce the density of kleptoparasites in the web. Finally, if these bands function to reduce predation by concealing the spider, we would expect spiders in webs without storage bands to suffer higher rates of mortality than spiders that retained the storage bands.

Material and Methods

Animal maintenance

Females of *N. edulis* were collected from Euroa (Victoria) and Brisbane (Queensland) and maintained in the laboratory at the University of Melbourne. All spiders were housed individually in upturned plastic cups (100 mm × 73 mm). They were fed *ad libitum* with Australian sheep blowflies, *Lucilia cuprina* (Diptera, Calliphoridae), and sprayed with water every second day. For experiments, females of variable ages (adult, penultimate, immature) were placed in three-dimensional Perspex frames (580 mm × 580 mm × 150 mm), where they constructed typical orb webs. During the experiments, the spiders were fed with blowflies (mean ± s.e. = 0.017 ± 0.0062 g, *n* = 2631) or crickets (mean ± s.e. = 0.152 ± 0.02 g, *n* = 38). Only those individuals that built regular webs and that did not die during the experiment were included in the analyses. In the field, the prey items are typically stored outside the web in the barrier web. Spiders do not build barrier webs in the laboratory because the frames are insufficiently deep, and so the food was stored in the orb web, above the hub and arranged as a vertical band.

Natural history of the storage band

N. edulis were observed under natural conditions at Euroa during February 2001 and *N. plumipes* during March 2001 in West Pymble Park (Sydney). The contents of the storage bands were investigated by excising the entire bands from a random subset of 25 adult female *N. edulis* and 49 adult female *N. plumipes* on the last day of observations. The stores were returned to the laboratory, dried in an oven at 180°C for 30 min and examined for animal and plant material using a dissecting microscope. Animal and plant components were weighed separately.

Field observations and continuous observations of storage-band growth do not allow for the band to be excised and weighed. Thus we have used band length as a reliable surrogate variable for weight as these two factors are significantly correlated ($r = 0.797$, $n = 123$, $P < 0.001$; data taken from laboratory webs). This provides better comparative data by using one common size variable for all experiments and field observations.

Prey density and food hoarding in N. edulis

This experiment examined variation in storage behaviour by providing prey at different densities using a paired design. Spiders that receive prey at high densities are predicted to store more prey than spiders that receive prey at low densities. Individuals were randomly assigned to two experimental treatments, and weighed before they were introduced into the frames. Spiders in the 'low-prey' treatment received one fly per day, while spiders in the 'high-prey' treatment received three flies per day. After seven days, the storage band was measured (length) with callipers and the spiders were weighed on the same day, and returned to their frames for another seven days, during which spiders in the 'low-prey' treatment were fed excess flies to even out both treatments. The spiders were weighed again on the same day, any storage band present was removed and they were then provided with the alternate feeding treatment for another seven days. The storage bands and the spiders were measured on Day 8 of the alternate treatment. Thus, each spider experienced a low-density and a high-density feeding treatment. We used paired parametric and non-parametric tests to examine the difference in spider weight and size of food storage before and after the paired treatments.

Prey encounter rates and food hoarding in N. edulis

Here we tested the influence of varying prey-encounter rates on the storage behaviour of *N. edulis*. Accordingly, spiders that receive food at constant rates may ingest the food immediately and store less externally than those that encounter food at variable rates, where excess food may be stored externally. We designed two experiments that varied in the difference between constant and variable prey-encounter rates. In both experiments, the mean prey-encounter rate was the same in each treatment.

In the first experiment, spiders were provided with two flies per day for 15 days in the constant treatment, and six flies every third day for 15 days in the variable treatment. After 15 days, the treatments were reversed and continued for another 15 days. Spiders were weighed and the storage bands removed and their length measured before and after each 15-day treatment. We used paired *t*-tests to examine the effect of prey-encounter rate and spider weight on storage behaviour. In the second experiment, spiders in the constant treatment were provided with four flies every day for nine days and 12 flies or no flies at a random order (from Day 1 to Day 9: 12, 12, 0, 0, 12, 0, 0, 0, 0) in the variable treatment. These treatments were not reversed, as the spiders became highly satiated. Spiders were weighed the day before the treatment

commenced and the day after the treatments finished. The length of the storage bands was measured on Day 10, and analysed with *t*-tests.

Food storage and the size and species of prey in N. edulis

Here we investigated whether spiders store prey selectively according to their type and size. We identified three different fates for prey: stored in the web, discarded (dropped to the floor) or consumed entirely. Almost all prey offered were attacked by the spiders. Females were placed in clean frames and each individual was fed two prey types: a single blowfly or a single cricket in random order, with 24 h between the feeding events. The prey was weighed several hours before being fed to the spider and the fate of each item was recorded the following day. The prey remains (whether stored or discarded) were dried in an oven (180°C for 30 min) and weighed to calculate the proportion of prey ingested. The frequencies of flies and crickets that were stored, discarded or consumed were analysed using Chi-square tests.

Effects of storage-band removal on N. edulis and N. plumipes in the field

N. edulis was observed under natural conditions in Euroa during February 2001. The following parameters were recorded: number of kleptoparasites present, female weight and storage size (length). The influence of food storage on future storage behaviour was investigated by removing the storage band from the webs of a random subset of *N. edulis*. The control group suffered a similar disturbance to the web but without removal of the storage band. We measured the size (length) of the storage after seven days to estimate size increase in the storage band.

Adults of *N. plumipes* were observed under natural conditions during March 2001 in West Pymble Park. We marked 80 adult female *N. plumipes*, and allocated them to one of four treatments. Treatment 1 had the food storage and all kleptoparasites removed every day; Treatment 2 had only the kleptoparasites removed each day; Treatment 3 had the food storage removed each day but the kleptoparasites were not removed; and Treatment 4 retained the food storage and natural densities of kleptoparasites, but were exposed to similar treatment disturbance. These treatments were maintained for seven days and we monitored daily the number of kleptoparasites, the size of the food storage, and the prey-capture rates. All spiders were weighed on Day 7 and the vertical and horizontal web diameters were measured at the start and at the end of the experimental period. Web size was estimated by multiplying the horizontal radius by the vertical web radius.

Antipredator function of the storage band in N. plumipes

We conducted a removal experiment in the field during April/May 2001 in West Pymble Park. In total, 60 adult female *N. plumipes* were selected, and their tibia patella length (first leg) and abdomen width were measured using callipers. After being marked individually with bee tags, the spiders were randomly allocated to two treatments: spiders in the experimental treatment had the storage band removed, whereas spiders in the control treatment were similarly disturbed without removing the storage band. Each spider was monitored daily for four weeks and we noted if any individual moved web site, was found dead in the web or disappeared altogether. Any storage bands constructed subsequently by the removal group were excised. We predict that if these bands function to deter predators, spiders whose storage bands were removed would suffer higher rates of mortality than those that retained the bands.

Analysis

We used a Kolmogorov–Smirnov test for normal distribution. All data that were not normally distributed were log-transformed and, if appropriate, analysed using parametric tests. Data that were still non-normal after transformation were analysed using non-parametric tests. All data are expressed as mean \pm s.e.

Results

Natural history of the storage band

Analysis of the content of the storage bands (length = 12.5 ± 1.53 cm, $n = 28$) in the webs of *N. edulis* showed that approximately 35% of the stored material consisted of plant matter such as small sticks, leaves and seeds. The remaining 65% consisted of prey remains. The storage bands of *N. plumipes* (length = 3.36 ± 0.38 cm, $n = 57$) were different in shape and

composition from those of *N. edulis*. There were no plant parts incorporated and the pieces of prey were not as densely packed as in those of *N. edulis*.

Prey density and food hoarding in N. edulis

There was no significant difference in the initial weights between spiders that first experienced the low (0.51 ± 0.052 g) and the high (0.53 ± 0.048 g) prey-density treatments (paired *t*-test: $t_{19} = -0.82$, $P = 0.42$). However, spiders did not gain weight when they experienced low prey density (0.51 ± 0.051 g; paired *t*-test: $t_{19} = 0.33$, $P = 0.73$), but did when they received prey at high densities (0.63 ± 0.058 g; paired *t*-test: $t_{19} = -2.46$, $P = 0.02$). Furthermore, when spiders captured more prey they also constructed larger storage bands (1.13 ± 0.2 cm) than when prey occurred at low densities (0.53 ± 0.14 cm; Wilcoxon signed ranks test: $z = 2.80$, $P = 0.005$).

Prey encounter rates and food hoarding in N. edulis

The final weight of spiders did not differ between the constant (0.54 ± 0.038 g) and slightly variable (0.54 ± 0.034 g) prey supply (paired *t*-test: $t_{20} = -0.15$, $P = 0.88$) treatment. Similarly, the length of the storage bands did not vary with the rate of prey encounter (paired *t*-test: $t_{20} = 0.92$, $P = 0.37$) (Fig. 1) when the contrast was low. When the contrast was high, spiders that received prey at a constant rate stored more food in the webs than did individuals that received prey at highly variable rates (*t*-test, $t_{37} = 2.46$, $P = 0.02$) (Fig. 1). There was no significant difference in the final weight of spiders in the constant (0.55 ± 0.04 g) and highly variable (0.67 ± 0.05 g) prey-supply treatments (*t*-test, $t_{37} = -1.62$, $P = 0.11$).

Prey size and species and food hoarding in N. edulis

Blowflies were more likely to be discarded and not stored in the web, while crickets were either stored in the web or completely consumed ($\chi^2_2 = 13.14$, $P < 0.01$) (Fig. 2). Even though flies (0.031 ± 0.002 g) were much smaller than crickets (0.15 ± 0.03 g), spiders ingested a higher proportion of the crickets (ANOVA: $F_{1,43} = 8.66$, $P = 0.005$) (Table 1).

Effects of storage-band removal on N. edulis and N. plumipes in the field

There was a significant difference in the initial weight of *N. edulis* spiders between the removal (1.60 ± 0.15 g) and the control (1.17 ± 0.16 g) group (*t*-test: $t_{21} = 2.15$, $P = 0.04$)

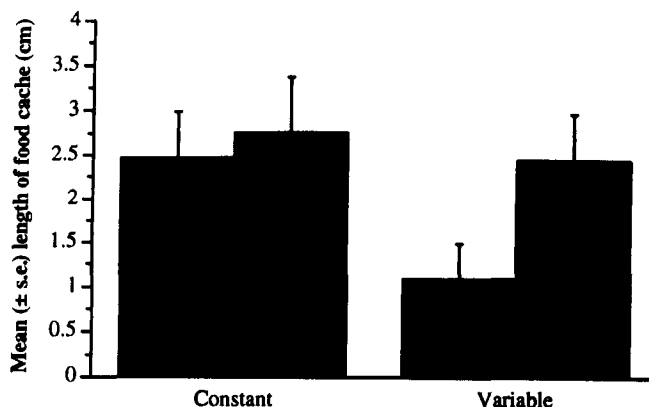


Fig. 1. Cache size in *N. edulis* under constant and variable feeding treatments. High contrast: black columns; low contrast: grey columns

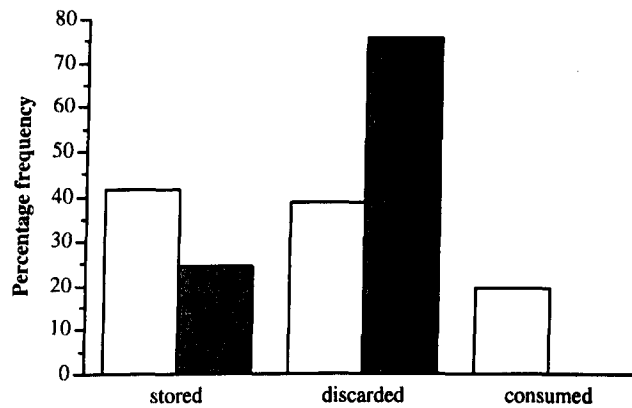


Fig. 2. Percentage of prey that was stored in the web of *N. edulis*, discarded or consumed. Crickets: white columns; flies: grey columns.

Table 1. The weight (g) of prey remains (flies or crickets) that were either stored in the web of *N. edulis* or discarded and dropped to the floor

Prey	Fate	Mean \pm s.e.	n
Fly	Stored	0.1 \pm 0.02 g	9
	Discarded	0.07 \pm 0.16 g	18
Cricket	Stored	0.04 \pm 0.02 g	12
	Discarded	0.02 \pm 0.02 g	8

despite random allocation of individuals to each treatment. Thus, initial weight was used as a covariate in subsequent analyses. The absence (weight gain: 0.24 ± 0.05 g) or presence (weight gain: 0.17 ± 0.04 g) of a food store did not affect weight gain (ANCOVA: $F_{1,22} = 1.75$, $P = 0.2$). However, spiders whose storage bands had been removed incorporated more material (4.95 ± 1.5 cm over 7 days) into a new band than did the control spiders (0.92 ± 0.3 cm over 7 days) (ANCOVA: $F_{1,22} = 7.59$, $P = 0.01$). Thus, in *N. edulis*, the length of the storage band after the experiment did not differ between the two groups (ANCOVA: $F_{1,24} = 0.001$, $P = 0.97$). The webs of *N. edulis* were host to few kleptoparasites (only 5 of 35 surveyed webs). By contrast, 53 of 62 webs of *N. plumipes* contained the kleptoparasite *Argyrodes antipodiana*. Thus we examined the effect of food storing on kleptoparasite activity only in *N. plumipes*.

Across all treatments, larger *N. plumipes* built larger webs ($r = 0.65$, $n = 49$, $P = 0.0001$), more prey was captured in larger webs ($r = 0.42$, $n = 49$, $P = 0.003$), and larger females stored more food than smaller females ($r = 0.35$, $n = 49$, $P = 0.014$). The initial weight of spiders differed significantly between treatments (ANOVA: $F_{3,48} = 5.10$, $P = 0.004$) (Table 2), and initial weight was used as a covariate in subsequent analyses. The weight of spiders at the end of the experiment did not differ between treatments (ANCOVA: $F_{3,48} = 2.40$, $P = 0.08$) (Table 2). In contrast to those of *N. edulis*, food stores in *N. plumipes* did not grow as rapidly in the removal group. Consequently, the length of the storage band at the end of the experiment differed significantly between the removal (1.14 ± 0.39 cm) and control (1.85 ± 0.37 cm) group (ANCOVA: $F_{1,62} = 23.40$, $P = 0.0001$). Weight gain of females over the seven experimental days (Fig. 3) was not influenced by the presence of the

Table 2. The mean (\pm s.e.) initial weight (g) of *N. plumipes* randomly allocated to four experimental treatments

Group	Cache	Kleptoparasites	Initial weight	Final weight	n
1	Removed	Removed	0.40 ± 0.09 g	0.53 ± 0.11 g	9
2	Retained	Removed	0.46 ± 0.1 g	0.62 ± 0.15 g	11
3	Removed	Retained	0.61 ± 0.09 g	0.65 ± 0.08 g	14
4	Retained	Retained	0.82 ± 0.08 g	0.70 ± 0.05 g	15

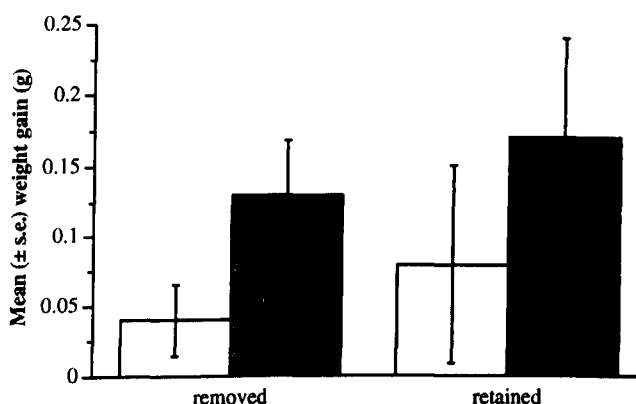


Fig. 3. Weight gain of *N. plumipes* where the food cache had been removed or retained. White columns: kleptoparasites removed; grey columns: kleptoparasites retained.

storage band (ANCOVA: $F_{1,48} = 0.32$, $P = 0.58$), the presence of kleptoparasites (ANCOVA: $F_{1,48} = 2.30$, $P = 0.14$) or the interaction of these two factors (ANCOVA: $F_{1,48} = 0.001$, $P = 0.98$).

Antipredator function of the storage band in *N. plumipes*

There was no significant size (tibia–patella length) difference between spiders in the control (1.17 ± 0.27 cm) and removal (1.16 ± 0.36 cm) treatments (t -test: $t_{38} = 0.22$, $P = 0.83$). Furthermore, the condition of spiders in both treatments (calculated as the standardised residuals generated by the regression of abdomen width on tibia–patella length) was similar (t -test: $t_{38} = -0.14$, $P = 0.89$). Contrary to prediction, spiders on webs without storage bands did not suffer higher rates of mortality: 15 of 30 individuals in the removal group and 15 of 30 individuals in the control group died or disappeared (Yates-corrected $\chi^2_1 = 0.07$, $P = 0.8$).

Discussion

Our experiments clearly showed that variation in hoarding behaviour in *Nephila* can be attributed to prey density, prey encounter rates and prey type. Nevertheless, removal experiments of external caches in the field were unable to detect changes in weight gain or an influence of kleptoparasites. Furthermore, spider mortality was unaffected by the absence or presence of the storage band.

Food hoarding is advantageous if it allows the animal to capitalise on temporarily abundant food resources and/or decreases foraging activities when costs, such as exposure

to predators, are high (Sherry 1985). Accordingly, we found that *N. edulis* stored more prey externally when prey was abundant than when it was limited. The established caches may be accessed when the animal has high energy requirements, such as during the final stages of an egg sac formation. Furthermore, the externally stored prey may allow the spider to process the prey at a later stage if they are physiologically limited by the amount of food they can ingest at any one time or if internal fat stores reduce mobility. Spiders are able to ingest a large amount of food at any time due to the presence of gut diverticula (Foelix 1992).

For most food-storing birds and mammals, a good spatial memory is often essential for retrieving the cache (e.g. Herz *et al.* 1994), but this is unlikely to limit cache relocation in *Nephila*, because the cache is located in the web. Moreover, the close proximity to the cache may also allow the spider to defend the cache from pilfering, a common cost of storing food externally (e.g. Leaver and Daly 2001).

Animals not only respond to food density, but also to variation associated with the food resource (Caraco 1981; Caraco and Gillespie 1986; Kalcenik and Bateson 1996; Herberstein *et al.* 2000b). Here, variation in prey encounter affected storage behaviour when the contrast between the treatments was high: spiders tended to store more food under constant prey supply than under variable rates. These patterns are consistent with those of other food-hoarders: for example, unpredictable food supply to starlings (*Sturnus vulgaris*) results in greater mass gain due to internal fat storage (Cuthill *et al.* 2000). Similarly, tufted titmice (*Parus bicolor*) do not increase their rate of food caching under unpredictable food regimes but rather increase their internal fat stores (Pravosudov and Grubb 1997). However, in these studies, food hoarding reflects the necessity to maintain high body temperature in cold nights without food (McNamara and Houston 1990; Pravosudov and Grubb 1997). In spiders, food hoarding is unlikely to be based on the same proximate mechanism. Thus the variation in caching in *N. edulis* may not reflect energy balance *per se* but the relative cost of cache loss, which may vary in different prey environments: when prey-encounter rates are constant, the cost of cache loss may be relatively low. In contrast, under unpredictable encounter rates loss of the external storage may be higher, if a period of food shortage follows. Under such prey conditions, the relative benefits of internal storage may be greater.

Food hoarding is adaptive if the individual can retrieve the cache under periods of food shortage or high energy requirements (Andersson and Krebs 1978). Removal of the cache did not affect weight gain in natural populations of *N. edulis* and *N. plumipes*. Perhaps our experiments were not sufficiently long to incorporate temporal changes in food availability, and hence detect the differences obtained in the laboratory experiments. Interestingly, removal of the cache did not affect the density of the kleptoparasite *A. antipodiana* in the webs of *N. plumipes*. Similarly, weight gain in *N. plumipes* was not influenced by the abundance of kleptoparasites. These data suggest that *A. antipodiana* do not significantly impact on the nutrients available in the cache, perhaps preferring to feed on those prey items ignored by the host spider (Cangialosi 1990; but see Whitehouse 1997; Tso and Severinghaus 1998). Nevertheless, it is tempting to infer that the relatively high abundance of kleptoparasites on the webs of *N. plumipes* (see also Elgar 1989) compared with *N. edulis* is responsible for the relatively large food cache that is typical of *N. edulis*. Such large food caches may attract extremely large numbers of kleptoparasites in *N. plumipes*.

The value of an external cache depends, in part, on the future value of the stored items. Food may improve during storage (e.g. ripen) or perish through microbial activity (Gendron and Reichman 1995). Consequently, not all food items are suitable for long-term caching (Reichman 1988). Nevertheless, by modifying food items, storability can be increased (e.g.

Fox 1982; Reichman *et al.* 1986). Our laboratory experiments similarly suggest that not all prey types are suitable for storage in *Nephila*. The remains of blowflies were discarded more frequently than stored and never consumed completely. In contrast, crickets were stored externally or consumed entirely, even though the crickets were a much larger meal than the flies. Thus, crickets may be more suitable for external and internal storage than flies. Alternatively, flies may contain more indigestible exoskeleton than crickets and are therefore never consumed entirely.

The storage bands of *N. edulis* in the field include about 35% plant material, while those of *N. plumipes* contain only animal material, even though plant material was available. This difference is intriguing and suggests that the storage band may serve other functions for *N. edulis*. For example, decomposing plant and animal material may attract insects to the web, thus increasing the spider's foraging success. However, the rate of decomposition appears to be very slow, and was not obvious throughout the study. Alternatively, the storage bands may deflect predators from the spider by acting as a decoy, thereby confusing the predator (Hingston 1927; Edmunds and Edmunds 1986; and see Herberstein *et al.* 2000a for a review). However, our field experiment found no mortality effect upon the removal of the storage band in *N. plumipes*.

Acknowledgments

We thank Therésa Jones, Jutta Schneider and Anne Gaskett for their helpful comments and discussion, and the Australian Research Council (Grant A19802502 and A19930103 to MAE) for financial support.

References

- Andersson, M., and Krebs, J. (1978). On the evolution of hoarding behaviour. *Animal Behaviour* **26**, 701–711.
- Cangialosi, K. R. (1990). Life cycle and behavior of the kleptoparasitic spider *Argyrodus ululans* (Araneae, Theridiidae). *Journal of Arachnology* **18**, 347–358.
- Caraco, T. (1981). Risk-sensitivity and foraging groups. *Ecology* **62**, 527–531.
- Caraco, T., and Gillespie, R. G. (1986). Risk-sensitivity: foraging in an ambush predator. *Ecology* **67**, 1180–1185.
- Champion de Crespigny, F. E., Herberstein, M. E., and Elgar, M. A. (2001). Food caching in orb-web spiders (Araneae: Araneoidea). *Naturwissenschaften* **88**, 42–45.
- Cuthill, I. C., Maddocks, S. A., Weall, C. V., and Jones, E. K. M. (2000). Body mass regulation in response to changes in feeding predictability and overnight energy expenditure. *Behavioral Ecology* **11**, 189–195.
- Edmunds, J., and Edmunds, M. (1986). The defensive mechanism of orb weavers (Araneae: Araneidae) in Ghana, West Africa. In 'Ninth International Congress of Arachnology, Panama 1983'. pp. 73–89. (Smithsonian Institution Press: Washington.)
- Elgar, M. A. (1989). Kleptoparasitism: a cost of aggregation for an orb-weaving spider. *Animal Behaviour* **37**, 1052–1054.
- Elgar, M. A. (1993). Inter-specific associations involving spiders: kleptoparasitism, mimicry, and mutualism. *Memoirs of the Queensland Museum* **33**, 411–430.
- Exline, H., and Levi, H. W. (1962). American spiders of the genus *Argyrodus* (Araneae, Theridiidae). *Bulletin of the Museum of Comparative Zoology, Harvard* **127**, 75–204.
- Fox, J. F. (1982). Adaptation of gray squirrel behavior to autumn germination by white oak acorns. *Evolution* **36**, 800–809.
- Gendron, R. P., and Reichman, O. J. (1995). Food perishability and inventory management: a comparison of three caching strategies. *American Naturalist* **145**, 948–968.
- Herberstein, M. E., and Heiling, A. M. (1999). Web asymmetry in spider orb-webs (Araneidae): a result of physical constraints? *Animal Behaviour* **58**, 1241–1246.

- Herberstein, M. E., Craig, C. L., Coddington, J. A., and Elgar, M. A. (2000a). The functional significance of silk decorations of orb-web spiders: a critical review of the empirical evidence. *Biological Reviews* 75, 649–669.
- Herberstein, M. E., Craig, C. L., and Elgar, M. A. (2000b). Foraging strategies and feeding regimes: web and decoration investment in *Argiope keyserlingi* Karsch (Araneae: Araneidae). *Evolutionary Ecology Research* 2, 69–80.
- Herz, R. S., Zanette, L., and Sherry, D. F. (1994). Spatial cues for cache retrieval by black-capped chickadees. *Animal Behaviour* 48, 343–351.
- Johnson, D. M., Akre, B. G., and Crowley, P. H. (1975). Modelling arthropod predation: wasteful killing by damselfly naiads. *Ecology* 56, 1081–1093.
- Kacelnik, A., and Bateson, M. (1996). Risky theories – the effects of variance on foraging decisions. *American Zoologist* 36, 402–434.
- Kagel, J. H., Green, L., and Caraco, T. (1986). When foragers discount the future: constraint or adaptation? *Animal Behaviour* 34, 271–283.
- Leaver, L. A., and Daly, M. (2001). Food caching and differential cache pilferage: a field study of coexistence of sympatric kangaroo rats and pocket mice. *Oecologia* 128, 577–584.
- Liao, C., Chen, M., and Song, D. (1984). On morphological characteristics and biology of the silk spider *Nephila imperialis* (Araneae: Araneidae). *Acta Zoologica Sinica* 30, 67–71.
- Main, B. (1976). Spiders. (Collins, Sydney.)
- McNamara, J. M., Houston, A. I., and Krebs, J. R. (1990). Why hoard? The economics of food storing in tits, *Parus* spp. *Behavioural Ecology* 1, 12–23.
- Moreno, J., Lundberg, A., and Carlson, A. (1981). Hoarding of individual nuthatches (*Sitta europaea*) and marsh tits (*Parus palustris*). *Holarctic Ecology* 4, 263–269.
- Pravosudov, V. V., and Grubb, T. C. (1997). Management of fat reserves and food caches in tufted titmice (*Parus bicolor*) in relation to unpredictable food supply. *Behavioural Ecology* 8, 332–339.
- Reichman, O. J. (1988). Caching behaviour by the eastern woodrat, *Neotoma floridana*, in relation to food perishability. *Animal Behaviour* 36, 1525–1532.
- Reichman, O. J., Fattaey, A., and Fattaey, K. (1986). Management of sterile and mouldy seeds by a desert rodent. *Animal Behaviour* 34, 221–225.
- Rypstra, A. L. (1981). The effect of kleptoparasitism on prey consumption and web relocation in a Peruvian population of the spider *Nephila clavipes*. *Oikos* 37, 179–182.
- Sherry, D. F. (1985). Food storage in birds and mammals. *Advances in the Study of Behavior* 15, 153–188.
- Smith, C. C., and Reichman, O. J. (1984). The evolution of food caching in birds and mammals. *Annual Review of Ecology and Systematics* 15, 329–351.
- Stephens, D. W., and Krebs, J. R. (1986). 'Foraging Theory.' (Princeton University Press: Princeton, NJ.)
- Tso, I. M., and Severinghaus, L. L. (1998). Silk stealing by *Argyrodes lanyuensis* (Araneae: Theridiidae): a unique form of kleptoparasitism. *Animal Behaviour* 56, 219–225.
- Vander Wall, S. B. (1990). 'Food Hoarding in Animals.' (The University of Chicago Press: Chicago.)
- Vollrath, F. (1979). Behavior of the kleptoparasitic spider *Argyrodes elevatus* (Araneae, Theridiidae). *Animal Behaviour* 27, 515–521.
- Whitehouse, M. E. A. (1997). The benefits of stealing from a predator: foraging rates, predation risk, and intraspecific aggression in the kleptoparasitic spider *Argyrodes antipodiana*. *Behavioral Ecology* 8, 663–667.
- Wise, D. H. (1993). 'Spiders in Ecological Webs.' (Cambridge University Press: Cambridge.)
- Witter, M. S., and Cuthill, I. C. (1993). The ecological costs of avian fat storage. *Philosophical Transactions of the Royal Society of London B* 340, 73–92.